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THE FAUNA OF NORTH AMERICA,

ITS ORIGIN AND UNIQUE COMPOSITION

A symposium organized for the
XVI International Congress of Zoology

by

Ernst Mayr

Museum of Comparative Zoology
Cambridge, Massachusetts

THE FAUNA OF NORTH AMERICA, ITS ORIGIN AND UNIQUE COMPOSITION.

Ernst Mayr, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts.

One of the major objectives of this symposium is to acquaint zoologists from other countries with the peculiarities of the American fauna and to try explaining how this fauna evolved. A visitor from Europe will encounter familiar organisms like deer, hares, squirrels, thrushes and finches, as well as strange types like porcupines, opossums, and armadillos among mammals, or tanagers, troupials, and turkeys among birds. A look at a world map tells us why the North American fauna is so mixed. We see that the North American continent is widely separated by oceans from Europe and tropical Asia but tenuously connected with adjacent continents, in the north with Asia across Bering Straits and in the south with South America across the Isthmus of Panama.

The obvious questions to be asked about the existing North American fauna are:

(1) what faunal elements came from Eurasia across Bering Straits?,

(2) what other elements came from South America across the Isthmus of Panama?, and finally,

(3) what elements are restricted to North America or at least show by their pattern of distribution that they might have had their center of origin or of major radiation in North America. In other words, what is the typically autochthonous North American fauna? Various speakers in this symposium will consider these questions for the particular organisms with which they are most familiar. I, myself, will limit my discussions to mammals and birds.

The faunal history of no other group of organisms is as well understood as that of mammals. G. G. Simpson has carefully analyzed the faunal exchanges between North America and Asia across Bering Straits (Simpson 1947) and in a later paper (Simpson 1950) the exchanges between North and South America. In this short survey, we can merely summarize some of his findings. Mammals are more valuable in zoogeography than birds for two reasons. They do not cross water gaps as easily as birds do and consequently reflect the separation of continents far more accurately. Also, they have left a relatively good fossil record so that one can make an independent check on inferences derived from patterns of current distribution. For instance, the fossil record fully substantiates that camels formerly occurred in North America, which was inferred because the contemporary relict distribution of camelids in the Old World and in South America requires a former continuity across North America. Many of the South American families and suborders of mammals are clearly derived from North American types (see

below), and the fossil record in either or both continents often permits determining fairly accurately the approximate time of colonization (Table 1). Birds are far less suitable for zoogeographic studies because the fossil record is less adequate, the radiation of higher taxa earlier, and the ability to cross water gaps much greater. Nevertheless, the inferences which one can draw from the pattern of current distributions is remarkably consistent with the basic pattern of mammal distribution. It is believed therefore that the inferences derived from the distribution of birds have a considerable degree of probability.

Let us now look at what we know about the answers to our three questions.

Bering Straits Immigrants

Simpson (1947) has shown clearly (Fig. 1) that there was free faunal exchange across Bering Straits bridge in the Late Eocene and in the Pleistocene, and fairly active exchange in the Early Eocene, in the Early Oligocene and in the Late Miocene. There has been very little faunal movement during the Middle Eocene, the Middle and Late Oligocene, the Early Miocene and the Early Pliocene. Movement has been in both directions. In birds it appears that more elements have moved from Asia into North America than reverse. Among the latter are only the Tetraonidae (grouse family) (probably), the Emberizidae (buntings), as well as some quite recent forms such as the wren (Troglodytes), the jay (Garrulus), and perhaps the Cinclidae (dippers) and the Bombycillidae (waxwings). None of the South American groups has succeeded in pushing across North America and Bering Straits into Eurasia no matter how successfully they radiated in North America. It is known that the climate in the Bering Straits region was considerably warmer in early Tertiary than it was in the Pleistocene and post-Pleistocene period, nevertheless, at least as far back as the Cretaceous, it was probably never milder than warm temperate. Movement across Bering Straits was probably impeded throughout by the existence of seasons. No matter how warm the climate, there must always have been an Arctic night during the winter, because there is no evidence for any drastic change in the ecliptic. We shall come back to this problem.

The Autochthonous North American Element

Unlike New Zealand or Australia, the North American continent has never been totally isolated. Being connected with both Asia and South America either by isthmic bridges or through stepping stones, it has exchanged faunal elements with these continents throughout the entire Tertiary. This fact notwithstanding, North America has served as a center of origin or at least of adaptive radiation for a large number of species groups, genera, subfamilies, and even families and higher taxa,

which can be considered genuinely North American elements, an autochthonous North American fauna. The composition of this North American fauna has been determined by Dunn (1931) for reptiles and amphibians, by Simpson (1947) for mammals, and by Mayr (1946, 1963) for birds.

In order to understand the nature of this fauna, we must consider the geography, vegetation, and climate of Tertiary North America.

In the Early Tertiary, the upfolding of the western mountains had hardly begun, there were no great deserts in the Southwest and in Mexico, indeed a faunal exchange between Central America and the regions to the north of it had few if any obstacles. Subtropical forests extended in the Eocene as far north as 55° N., the Mississippi embayment was definitely tropical, and so were the Upper Eocene floras of Oregon and Washington. There was then at that time a huge tropical-subtropical North America with a tropical North American fauna and flora. Central America extended southward perhaps as far as Honduras, forming a southern peninsula of North America, not separated from the remainder of the continent either by physical or climatic barriers. There is no evidence that its fauna differed from that of the remainder of North America except for latitudinal effects. When in these discussions I speak of the North American fauna, I always refer to this combined fauna. Only after the climatic deterioration had pushed the northern edge of the tropical zone southward into Mexico did it become legitimate to distinguish a Central American and a North American fauna.

The Fate of the Tropical North American Fauna

When we look at a map of the now existing vegetation zones of the Americas, we find a continuous, solid zone of tropical vegetation extending from Mexico in the north across the Isthmus of Panama to southern Brazil. The fauna within this large area has a considerable degree of homogeneity. This observation forces us to ask: what happened to the flourishing tropical North American fauna? We now suspect that the answer to this question is twofold. Part of this fauna radiated into other habitats and life zones while still separated from its South American counterpart. Indeed, the arid North American faunas as well as the subtropical ones are largely derived from it. That part of this fauna, however, which stayed tropical was pushed further and further south in the Late Tertiary and was finally completely swamped by the South American tropical fauna when the latter invaded Central America after the closing of the Panama Gap. The neo-tropical fauna of Central America, which at first sight seems so uniform, is actually the product of the mixing of two Tertiary faunas, that of tropical North America and that of tropical South America.

The Panamanian Bridge

In the above discussion of the autochthonous North American

fauna, we have already stressed the importance of the connection with South America. We now must give more detail on the nature of this faunal exchange. The ocean gap between North and South America lasted for a very long time. It probably existed already far back in the Cretaceous and did not close until the latest Pliocene, some two to six million years ago. Crossing the gap was facilitated by a number of islands that served as stepping stones for transoceanic colonists. The closing occurred apparently primarily from the north southward, so that by Late Miocene (? Early Pliocene) terrestrial North American mammals extended as far south as Panama.

This water gap was a highly efficient barrier for mammals. As Simpson (1950) has shown, only a few elements such as the ancestors of the South American monkeys, rodents, and raccoons moved south during the Tertiary and only few moved north. The gap was not always equally efficient, for there was more faunal exchange, apparently, in the Eocene than later. Those elements that reached Wouth America from the north experienced tremendous radiations and accounted for the rich South American fauna.

The picture for birds is different (Mayr 1963). Here, too, we have the development of a rich autochthonous fauna in South America (Table 2) characterized particularly by the enormous radiation of the suboscines, the South American passerine birds. In contra-distinction to the mammals, however, a rather active faunal exchange across the stepping stones of the Panama Gap continued throughout the Tertiary. On the basis of relationships we can postulate that the hummingbirds (Trochilidae) and the tyrant flycatchers (Tyrannidae) are ultimately South American elements, and that the tanagers (Thraupidae), the blackbirds (Icteridae) and the American finches (Emberizinae and Cardinalinae) are ultimately North American birds, because these nine-primaried oscines appear most closely related to the Old World flycatchers (Muscicapidae). Yet all these families, whether ultimately derived from North or South America, have moved back and forth so frequently and actively and have formed new centers of radiation wherever they got to, that it is best to designate these elements as Pan-American. The three just-mentioned families designated as "ultimately North American," for instance, are now far richer in genera and species in South America than in North America. As the gap narrowed throughout the later Tertiary, the faunal exchange became increasingly more active and, as we mentioned above, the final closing of the gap led to a complete faunal fusion.

This analysis confirms Dunn's assertion (1922) that a purely static regional approach is misleading. The regional zoogeographer includes all of the tropical and subtropical parts of Central America in the neotropical region, thereby concealing the interesting history of this fauna.

Our analysis has also revealed why the students of mammals usually attach North America as the Nearctic to Eurasia, combining

the two in the Holarctic Region. The long continued active exchange across the Bering Straits bridge and the high efficiency of the Panama Gap make such a classification quite legitimate. With birds, the climatic barrier of Bering Straits was a greater barrier than the water gap at Panama. Consequently, North America south of the Arctic region has as much or even more in common with South America than it has with Asia. Furthermore, the ability of birds to migrate has given them an opportunity to escape annually the northern winter and to recolonize the area each spring. As a result, we have even in relatively northern latitudes a considerable number of such ultimately tropical elements as hummingbirds, tanagers, warblers, and tyrant flycatchers. North America itself has been such a important center of faunal diversification for birds, it has been such an important home of autochthonous elements, and its faunal exchange with Asia and South America has been essentially so equal that it is far more acceptable to consider North America an independent zoogeographic region than to fuse it into the Holarctic. It would be interesting to know whether there are other groups of animals which differ from mammals the way birds do.

Open Problems

In concluding this discussion, let me specify some of the major problems of North American zoogeography as they appear to me on the basis of a study of mammal and bird faunas.

(1) What elements from the order down to the species can be considered as essentially autochthonous North American elements? It is obvious that the answers may be different at different categorical levels. For instance, the crow family (Corvidae) is clearly of Old World origin. Yet, one subdivision of this family, the jays (Cyanocitta and relatives), have had a secondary center of radiation in North America, particularly in tropical North America. An analysis is most meaningful and rewarding on the level bordered by the genus at the lower and by the family at the upper limit. It is very difficult to make meaningful statements as to the center of origin of orders, particularly for birds.

(2) What elements of our fauna have moved easily across dispersal barriers (either Bering Straits or the Panama Gap) and what other elements were rather efficiently confined to North America by these two intermittent water gaps, one of them (Bering Straits) reinforced by a climatic barrier? It is noteworthy that families such as the Parulidae, the Icteridae, the Tyrannidae, and the Trochilidae, which more or less freely crossed the Panamanian Gap, were almost or entirely unable to cross Bering Straits into Eurasia.

(3) What is the history of the "pan-tropical element?" As pantropical, I designate an element (Mayr 1946) which is found in the American and in the Old World tropics, apparently centering in the tropics of southeastern Asia. This element somehow crossed into tropical North America in early Tertiary or earlier, and has sub-

sequently radiated into tropical South America. Although we assume that the original invasion took place across the Bering Straits bridge, we are puzzled about the climatic difficulties. Regardless of minor shifts of the poles, we must assume that owing to the ecliptic, there was always an arctic winter night. The polar climate in Cretaceous and Early Tertiary was temperate rather than arctic, owing to the better circulation of the air and to the much warmer oceans. Nevertheless, it seems that there was no opportunity for tropical and perhaps even subtropical conditions further north than southern Canada. All of our present knowledge indicates that the Bering Straits bridge has not offered, since the Cretaceous, conditions suitable to make it a highway for tropical elements. How, then, did the trogons (Trogonidae), barbets (Capitonidae), parrots (Psittacidae), and such tropical water birds as the sun grebes (Heliornithidae), the jacanas (Jacanidae), and painted snipes (Rostratulidae) cross over into America? There is an implication that the outline of the continents, the distance between continents, the concentricity of the floristic zones around the poles, and other geographic features were sufficiently different in Late Cretaceous and Early Tertiary from what they are today to have permitted such an exchange of tropical elements.

(4) What is the history of the eremian (Sonoran) element in the North American fauna and indeed of all other ecologically specialized elements? The preliminary answer is quite clear, and supported by the evidence known from the Australian and Asiatic deserts. The fauna of specialized habitats is not due to independent origin but rather due to the continuous adaptive radiation from more mesic habitats. Eremian faunas being due to successive colonization have a highly unequal age.

Conclusion

The faunal history of North America is clear in its essential outline, yet we are still in the beginning of our research as far as detail is concerned. Not only the four questions which I have posed last are still largely unanswered, but more broadly we are still quite unable to say how great the differences in faunal structure are between different types of animals, how many types of faunal history we have in addition to the two here described for birds and mammals, and what generalizations one can make concerning the relation between the age of the taxa and their distribution pattern, the dispersal facility of taxa and their distribution pattern, and between their ecological requirements and distribution pattern. After 200 years of research, North American zoogeography is an area as rich as ever in intellectual challenge. I hope that the speakers who follow me on the program will provide some of the answers to our questions and, to make our program even more successful, pose some new and even more searching questions.

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Table 1

Mammalian colonizations of South America
by North American immigrants

<u>Across watergaps</u>

1. late Cretaceous - Paleocene
   - Marsupials
   - Condylarths and derived Ungulates (Pyrotheres, Astrapotheres, Xenungulates, Litopterns, etc.): possibly several colonizations
   - Notoungulates
   - Edentates
2. late Eocene - Oligocene
   - Caviomorph rodents
   - Primates
3. late Miocene or early Pliocene
   - Carnivores (one group of procyonids)

<u>Over land</u>

4. late Pliocene and Quaternary
   - North American rodents (squirrels, cricetids)
   - Carnivores (dogs, bears, weasels, cats)
   - Ungulates (peccaries, deer, tapirs, camels, horses)
   - Proboscidians
   - Lagomorphs (rabbits) (one genus with two species)
   - Insectivores (shrews) (one species, marginally)

Bats omitted from this tabulation

Table 2

Some autochthonous South American bird families
(families with less than 6 species omitted)

Tinamidae	Formicariidae
Bucconidae	Conopophagidae
Galbulidae	Rhinocryptidae
Ramphastidae	Cotingidae
Dendrocolaptidae	Pipridae
Furnariidae	

Table 3

Eurasian avian immigrants into North America

Oldest component	Most recent component
Gruidae (?Old World)	Tytonidae (Tyto)
Strigidae (?Old World)	Alaudidae (Eremophila)
Columbidae	Hirundinidae (pt.)
Cuculidae	Certhiidae (Certhia)
Corvidae (pt.)	Turdidae (pt.)
Turdidae (pt.)	Sylviidae (Regulus, Phylloscopus)
Odontophorinae	Laniidae
	Fringillidae (pt.)

More recent component

Alcedinidae
Corvidae (pt.)
Paridae
Sittidae
Timaliidae (Chamaea)
Motacillidae
Fringillidae (Carduelinae)
Sylviidae (Polioptilinae)

DEVELOPMENT OF THE HERPETOFAUNA

Jay M. Savage, University of Southern California, Los Angeles 7, California

The present distribution of the amphibians and reptiles of America, north of Colombia, is the product of a long-term developmental process beginning in Tertiary and involving a complex interaction between physiography, geology, climate, vegetation and the evolving herpetofaunal elements. The occurrence of particular species in any region is only explainable in terms of their present distribution, ecologic associates and limits, and the geodynamic, paleoecologic and evolutionary events resulting in current biogeographic patterns. The principal herpetofaunas of North and Middle America may be organized into a static system (Tab. 1), with each of the 20 provinces equivalent to a major fauna. The system must be considered extremely tentative for the neotropical areas.

The principal historical elements contributing to these herpetofaunas are outlined (Tab. 2) and their geofloral and climatic associations indicated. Correlation with the known facts of geofloral, climatic and geologic changes during Cenozoic makes it possible to trace the history of the units and the contributions each has made to present day herpetofaunas.

At the beginning of Tertiary the North American herpetofaunal elements were two in number. The ancestors of the Old Northern Element existed in warm and cool temperate areas in what is now Canada, Alaska, and the extreme northern United States. To the south in subtropical and tropical situations a generalized Tropical American unit occurred. Submergence of the connection between North and South America in early Tertiary set the stage for the independent development of the South American and Middle American Elements, to the south and north, respectively, of the water barrier, in continuing tropical and subtropical conditions. A third derivative of the generalized Tropical American unit, the Young Northern Element, evolved with the developing Madro-Tertiary Geoflora in marginal subtropical areas where the impact of Cenozoic trends toward increasing acidity were effective in mid-Tertiary. Certain warm-temperate representatives of the Old Northern Element become associated with the developing Middle American Element to form the Central American Complex sometime previous to Miocene, and the two stocks have evolved together in Middle America since that time. The other complexes of the Old Northern Element of cooler and more humid regions have moved southward as the result of cooling trends through Cenozoic but have not invaded the rapidly developed arid regions of Young Northern dominance. Each of the fragments from the original Old Northern Element has undergone independent subsequent evolution as the cumulative effects of temperature depression, increasing aridity and mountain building in late Cenozoic have isolated them.

The emergence of a connection between lower Central America and South America in Pliocene allowed invasion of Middle America by members of the South American Element. More recently representatives of the Holoarctic Element have crossed the Bering Strait and now are found in cool temperate situations, but this Element makes only a minor contribution to the herpetofaunal assemblage.

TABLE 1

NEARCTIC REGION

I. BOREAL NORTH AMERICAN SUBREGION

A. Boreal Province

II. EASTERN NORTH AMERICAN SUBREGION

A. Eastern Forest Province

B. Austroriparian Province

III. WESTERN MONTANE SUBREGION

A. Rocky Mountain Province

B. Pacific Border Province

IV. AMERICAN HIGHLANDS SUBREGION

A. Western Sierra Madrean Province

B. Eastern Sierra Madrean Province

C. Californian Province

D. Desert and Plains Province

NEOTROPICAL (MIDDLE AMERICAN) REGION

I. ATLANTIC LOWLAND SUBREGION

A. Veracruzian Province

B. Campechean Province

C. Caribbean Province

D. Panamanian Province

II. PACIFIC LOWLAND SUBREGION

A. West Mexican Province

B. Nicaraguan Province

III. CENTRAL AMERICAN HIGHLAND SUBREGION

A. Guatemalan Highland Province

B. Zacapan-Comayaguan Province

C. Honduranean Upland Province

IV. COSTA RICAN HIGHLAND SUBREGION

A. Talamancan Province

B. Cordillera Central Province

TABLE 2 -- HISTORICAL ELEMENTS OF NORTH AMERICAN HERPETOFAUNAS

UNITS	GEOFLORAL ASSOCIATION	CLIMATE	ORIGIN	EXAMPLES
A. South American Element	Neotropical Tertiary	Tropical	From generalized Tropical American unit	*Atelopus* *Erythrolamprus* *Caiman*
B. Old Northern Element	Arcto-Tertiary		North temperate areas	
1. Eastern American Complex		Cool temperate		*Cryptobranchus* *Gyrinophilus*
2. Western American Complex		Cool temperate		*Dicamptodon* *Ascaphus*
3. Central American Complex		Warm temperate, Subtropical		*Pseudoeurycea* *Staurotypus*
4. Southeastern American Complex		Warm temperate		*Siren* *Graptemys*
C. Middle American Element	Neotropical Tertiary	Tropical, subtropical	From generalized Tropical American unit	*Basciliscus* *Xenosaurus*
D. Young Northern Element	Madro-Tertiary		From generalized Tropical American unit	
1. Madrean Complex		Subhumid		*Gerrhonotus*
2. Desert and Plains Complex		Semiarid, arid		*Crotaphytus* *Phyllorhynchus*
E. Holarctic Element	Arcto-Tertiary	Cool temperate	Recent invaders from Eurasia	*Bufo boreas* *Rana sylvatica*

Arranged in approximate order from most ancient to most recent in America.

THE FRESH-WATER FISH FAUNA OF NORTH AMERICA. George S. Myers, Stanford University, Stanford, California.

1. Introduction.

As Dr. Mayr has stated in the opening paper of this symposium, two principal objectives are to acquaint zoologists from other countries with the American fauna, and to attempt some explanation of how the fauna was evolved or derived. I include in North America all of Central America and the West Indies.

2. What are Fresh-water Fishes?

Most fishes live in the sea. Only about one fifth, or fewer, are confined to fresh water. Of the latter, the majority are sharply restricted to fresh-water (primary or obligatory fresh-water fishes) by deep-seated and apparently ancient difficulties of osmotic regulation in sea water. Such fishes are unable to use the sea as a highway and can disperse across a land mass only through such extremely slow physiographic processes as stream capture and the development of periodically flooded lowland areas. Another, smaller segment (secondary fresh-water fishes) is composed of fishes which usually behave distributionally much like primary ones, but which have the observed ability to withstand sea water of more or less normal salinity for shorter or longer periods. Beyond these, there is a relatively small number of fishes which can, in various ways, survive in both sea water and fresh water. There are vicarious fresh-water fishes, consisting of non-diadromous, fresh-water representatives of primarily marine families, such as Lota, Cottus, Siniperca, Percichthys, and a number of fresh-water Atherinidae, Sciaenidae, Hemiramphidae, etc. Then there are diadromous fishes, which migrate regularly between the fresh-water and marine habitats, usually for spawning, such as Salmo salar, Oncorhynchus, Petromyzon, Anguilla, Alosa, and others. There are also a few sporadic fresh-water fishes which seem to move freely between fresh and salt water (Gasterosteus, Pristis, etc.) in a sporadic way, and finally, certain probably diadromous representatives of marine groups which appear to establish themselves in tropical fresh waters in the absence or paucity of a primary fresh-water fauna (complementary fresh-water fishes). All of the non-primary, non-secondary fresh-water fishes are sometimes lumped under the term peripheral.

The primary and secondary groupings consist mostly of entire families of fishes. For example, most of the numerous families of the teleostean order Ostariophysi (characids, carps, suckers, loaches, catfishes), which consists of nearly 5,000 species, are primary fresh-water fishes. Only two of these ostariophysan families, the catfish (siluroid) families Ariidae and Plotosidae, contain a large

proportion of species which live and breed in the sea, and can fairly be called marine families. A few species of the large ostariophysan family Cyprinidae (2,000 species) can withstand brackish water of rather high salinity. None of them breed in the sea, only one (the Japanese Tribolodon hakonensis) is known regularly to enter sea water of normal salinity, and the vast majority are strictly limited to fresh water. The South American siluroid family Aspredinidae has one subfamily (four species) found only in the Guiana Mangrove Province (Orinoco delta to Ceara) and confined to tidal and brackish or salt water. But nearly all characids, gymnotids, cyprinids, cobitids, homalopterids, and siluroids are primary fresh-water fishes.

3. The Fresh Waters and Their Fish Faunas.

North America extends from north of the Arctic Circle to within 7° of the equator. Approximately its western third, from the far north to the far south, is mountainous (the Cordilleran Chain). As a result, the Pacific rivers are usually much shorter, the Pacific slopes more precipitous, and the Pacific lowlands much less extensive than their Atlantic counterparts. The western Cordilleran regions have gone through extensive orogenic disturbances during the Cenozoic, which the regions east of them generally have not. In addition, Pleistocene glaciation covered northeastern North America with an ice-sheet reaching at times south of the Great Lakes, but leaving much of the northwestern part of the continent free of any but local glaciers. Since that time, progressive dessication has reduced the inter-mountain Cordilleran Great Basin and much of the rest of the southwestern United States and northwestern Mexico to desert. All of these things have had important effects upon the fishes, much more, in fact, than upon other vertebrates. The streams of the Pacific slope have mostly been cut off for a long time from the main lines of fish evolution going on in the more abundant rivers of the Atlantic slope. Moreover, orogenic, physiographic, and climatic changes in the West have caused much extinction without giving time for the evolution of the richer fish faunas characteristic of more stable continental regions to the eastward. Thus by far the greater part of North American fresh-water fishes, including most of the so-called typical or unique North American elements, occur only east of the Rocky Mountains and Sierra Madre of Mexico, leaving the intermontaine and Pacific drainages with greatly reduced, atypical faunas, including mostly endemic species and often endemic genera of greater or lesser age.

Greenland has no fresh-water fishes. Throughout all of Alaska and nearly all of Canada, the fresh-water fish fauna is a depauperate one. Even as far south as the Great Lakes and the St. Lawrence basin, the fish fauna is an impoverished one, quite as much so as the depauperate fresh-water

fish faunas of Europe, North Africa, the Near East and Siberia. Holarctic genera and even species (Salmo, Salvelinus, Thymallus, Stenodus, Coregonus, Esox, Dallia, Perca, Lota) are conspicuous, in addition to a relatively few species of carps (Cyprinidae), suckers (Catostomidae), catfishes (Ictaluridae), sunfishes and black-basses (Centrarchidae), miller's thumbs (Cottidae), etc.

It is only when we come to the Missouri, Mississippi, Ohio and more easterly drainages south of the Great Lakes and St. Lawrence that we meet the true richness of the Atlantic slope fish fauna. For example, the state of Ohio, which includes parts of both the Great Lakes and the Ohio River drainages, and was in large part glaciated in the Pleistocene, now supports approximately 170 species of fresh-water fishes. France, with over five times the area of Ohio, has less than one-third as many species. Faunas as large as, or larger, than that of Ohio exist in several of the Mississippi Valley states, as well as in the eastern states of Pennsylvania, Virginia, or North Carolina. Southward, the fauna becomes even richer for certain areas, but the totals diminish somewhat in such westerly plains states as Iowa and Kansas, and in the drier regions of Texas and New Mexico.

Along the Atlantic seaboard, from the Potomac River (on which Washington, D.C. is located) south to Georgia, there is considerable endemism among the smaller fishes of the coastal rivers draining the east side of the old, eroded Appalachian Mountains, this endemism being least apparent in the lower reaches of the rivers in the sandy, pineland of the largely drowned old Atlantic Coastal Plain, and greater among the species confined to the upper reaches of the same rivers. There is likewise much endemism in the smaller and some of the large fishes of the tributaries of the great central Mississippi River. In the Blue Ridge section of the Appalachian Mountains, in the western part of Virginia and North Carolina, the headwater streams of the separate Atlantic coastal rivers (Potomac, James, Roanoke, Yadkin), and of the two large easterly tributaries of the Mississippi (Ohio and Tennessee Rivers) interdigitate in a complex way. In this ancient highland area, one crosses many low ridges, between them descending to small streams each with a different assortment of often brilliantly colored endemic minnows (small Cyprinidae) and darters (small Percidae). No more striking picture of the effect of geography upon the evolution of fluviatile fishes is known to me.

In northern Mexico, North American elements are still dominant, but the first outliers of the Neotropical fresh-water fishes become noticeable in the Rio Grande, where one species of Characidae and one of Cichlidae occur. A few Nearctic elements continue southward beyond Mexico, but

most are confined to the Mexican Plateau. In the tropical Mexican lowlands, Neotropical elements predominate north almost to Texas. South to Mexico, the fish fauna is almost completely Neotropical, but a really large assortment of South American fish groups is not evident until Panama is reached.

The West Indian islands have no native primary fresh-water fishes at all except for Trinidad, close to the Venezuelan mainland. Instead, there is an exceptionally impoverished fauna of secondary fresh-water fishes on Cuba, Hispaniola and Jamaica, while Puerto Rico and the Lesser Antilles mostly have only complementary and sporadic fresh-water types, some of the former being diadromous.

4. Taxonomic Groups

I now list the main taxonomic groups of the North American fauna. Primary groups bear an asterisk (*).

Lampreys: Fourteen or fifteen species of Petromyzon, Ichthyomyzon, and Lampetra occur, some in almost every major drainage (except the Colorado), and they extend south to the Mexican Plateau. All are migratory but most exist only in fresh-water.

Primitive "ganoids": Diadromous sturgeons (Acipenseridae) occur in both coasts but stop short of the Mexican boundary. An Acipenser occurs in the Great Lakes and two Scaphirhynchus in the Mississippi. The plankton-feeding paddlefish (Polyodon*), allied to the Yangtse Psephurus, occurs in larger rivers of the Mississippi system. Five Garpikes (Lepisosteus) are widely distributed east of the Rocky Mountains; another is confined to Cuba, and one or two exist in Central America south to Costa Rica. The bowfin (Amia calva*) is found east of the Rocky Mountains throughout most of the United States, but is absent from the Atlantic slope of the Appalachians north of Carolina.

Herringlike families: The two living hiodontids*(Hiodon) occur throughout most of the Mississippi Basin and northward to Hudson Bay. Several diadromous (Alosa) and a few vicarious Clupeidae exist east of the Rocky Mountains and south to Nicaragua. Salmonidae, some resident, vicarious forms, some diadromous, are common from the Arctic to the central tier of states. One or two trout barely reach Mexico in the West.

Haplomi: Four species of Esox* occur east of the Rocky Mountains in Canada and the northern United States. One is apparently identical with Esox lucius of Europe; two of the species reach Florida. The Umbridae*, represented in Eurasia by Umbra krameri of Eastern Europe and Dallia in Eastern Siberia, have four American representatives. Dallia pectoralis occurs in northern Alaska, Novumbra hubbsi in Washington State, Umbra limi in the Mississippi Valley and U. pygmaea in the Atlantic coastal plain.

Ostariophysi: This order (5,000 species) comprises the bulk of fresh-water fishes on all continents except Australia. The characids (Characidae*) are the most generalized family, confined to Africa (23 genera, about 150 species) and tropical America (between 175 and 190 genera and about 875 known species). Of these, only 21 genera and about 50 species occur in Central America but most of these are in Panama only. Only six genera and about eight species are present in Nicaragua, and two genera and three species in Mexico. One of the latter reaches the United States in Texas and New Mexico. The electric eels (Gymnotidae*; 19 genera, about 40 species) are all tropical American. North of Panama, only one species of Gymnotus exists, from Nicaragua to Guatemala. The carps (Cyprinidae*; about 200 genera and nearly 2000 species) occur naturally in all continents except South America and Australia. There are about 40 genera and 250 species in North America. A number are present in the Mexican highlands, but none reaches southern Mexico. The suckers (Catostomidae*; 14 genera and about 80 species) are all North American except for a monotypic Chinese genus and a far northern American species which has reached eastern Siberia. Two species reach southern Mexico and Guatemala. Ameiurid catfishes (Ictaluridae*; 6 genera, 35 species) are all North American; one reaches Guatemala. Pimelodid catfishes (Pimelodidae*; about 35 genera and over 200 species) are all tropical American. One generalized South American genus, Rhamdia, has about 25 recognized species from Panama to southern Mexico. Several other families of South American catfishes reach no farther north than Panama.

Cyprinodonts: Killifishes (Cyprinodontidae, a circumtropical secondary family, have about 13 North American genera and 45 North American species, of which 35 are found north of Mexico. The live-bearers (Poeciliidae; generic situation fluid, about 200 species) are all American but primarily Central American (and West Indian), with an overflow into the southern U.S. and South America. Another small viviparous family (Goodeidae) is endemic in Central Mexico. The North American blind cave-fishes (Amblyopsidae*; 5 species) are present only in the central and eastern states; they may not be cyprinodonts.

Sticklebacks: Five species, only two primarily fresh-water, are found in the northern states and northward.

Salmopercae: Two species of Percopsidae* are known, one on the Pacific slope in the Columbia River, the other in the North Central states and Canada. One living species of Aphredoderidae*, exists in the central and eastern states.

Percoids: Black basses and sunfishes (Centrarchidae*; 11 genera, 30 species) are endemic in North America. Only one occurs naturally west of the Rocky Mountains, and a few reach northern Mexico. Percidae* are not present west of

the Rocky Mountains but reach northern Mexico. There is one species of Perca close to the two Eurasian ones, two of Stizostedion (=Lucioperca), and a series of about 100 small species (Etheostoma, Percina, etc.) called darters. The Cichlidae (about 88 genera, nearly 800 species) are of secondary type, occurring in Southern Inida, Madagascar, Syria, Africa, Cuba, Hispaniola, and tropical America. Only one reaches the U.S. border. There are 23 genera and about 225 species in America of which almost 100 (mostly of the genus Cichlasoma) are Central American, where the group (like the livebearers) has speciated in an extraordinary way in a near-vacuum of primary fresh-water fish stocks.

Sculpins: Miller's thumbs (Cottus) are present throughout the northern states and Canada.

In addition, a few vicarious cods (Lota), drumfishes (Sciaenidae), silversides (Atherinidae), etc., occur.

5. Derivation.

The "ganoids" have too ancient a distribution for easy elucidation. The hiodontids, ictalurids, cave-fishes, Salmopercae and Centrarchidae appear to be old-Cenozoic endemics in North America. The origin of all of them is obscure. Esox, the umbrids, Lota and the Percidae probably originated in Eurasia (where there is a long fossil record for some of them) and arrived in America over a Bering land-bridge in the middle to late Cenozoic. Cyprinidae (and probably Catostomidae) almost certainly arrived from Asia via such a bridge in the Miocene.

There is absolutely no fossil or recent evidence of South American groups in North America, or the reverse, more than is demonstrated by the living fauna. Apparently, no Nearctic group has ever penetrated farther south than the ancient garpikes (Costa Rica). No Neotropical group seems ever to have gotten beyond the Rio Grande. From the evidence of the living fishes, Central America seems never to have had a primary fresh-water ostariophysan fauna previous to the advent of the few invaders from the North and South now beginning to intermingle in Guatemala and Mexico. Primary fresh-water fishes desperse very slowly!

CHARACTERISTICS AND HISTORY OF THE NORTH AMERICAN FAUNA: LEPIDOPTERA. Eugene Munroe, Entomology Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Canada.

DEFINITION OF REGION. Most lepidopterists have restricted the North American faunal region to that part of the continent lying north of the Mexico - U.S. border. A faunistically more natural definition would take in the plateau region of Mexico and its bordering mountains. Though this is satisfactory for the study of existing fauna, it excludes elements that were important in the Cenezoic history of North America, but that are now confined to the tropical parts of the New World. If the tropical parts of Central America are included, we take in many faunistic elements that ranged into North America proper during the Cenozoic and we carry the geographic boundary south to a region that was marked by a sea barrier between North and South America during much of that era. This last definition will be adopted here, but more detailed consideration will be given to temperate than to tropical elements of the fauna.

FAUNISTIC AREAS. For Lepidoptera the most significant faunistic division in America north of Panama is that between temperate and tropical regions. The temperate fauna, though not without tropical affinities, consists overwhelmingly of species and genera that are either characteristically North American or, more often, that are related in varying degree to Palaearctic forms. In the tropics, on the other hand, species and genera of demonstrable northern affinity are in a small minority. Most groups are either endemic, often at the tribe, subfamily or family level, or else are related, usually at the major generic or the tribal level, to predominantly tropical groups of the Old World. The Lepidoptera of tropical Central America are on the whole closely related to those of tropical South America, especially to those of the Andean part of the continent. The two areas are, justly from the standpoint of modern fauna, united by zoogeographers as a single Neotropical region.

Temperate faunistic provinces, so far as Lepidoptera are concerned, may be divided as follows:-(1) Arctic-Alpine, with Arctic and Cordilleran subdivisions; (2) Boreal, including Boreal forest and Subarctic forest-tundra transition; (3) Eastern Temperate Forest, divisible according to latitude and altitude, and also into glaciated and nonglaciated portions; (4) Cordilleran Pacific Forest, likewise divisible according to latitude and altitude, according to proximity to the coast, and, in its southern part, into Rocky Mountain, Sierra-Nevada and Coastal main sections, supplemented by numerous isolated segments; (5) Mexican Temperate Forest; (6) Central Plains and Parklands; and (7) Southwestern Eremic Province. These are broader than the customary divisions, but each has considerable unity, and for specific purposes subdivision is possible.

Tropical faunistic provinces have not been so well studied. I know of no good tabulation of the distribution of Tropical Mexican and Central American Lepidoptera; indeed, much of the area has been very sketchily collected. Accordingly I will not try to divide the Tropical region systematically. An important distinction is that between the Floridan and the Central American-Mexican fau-

nas; there are evidently important differences between the eastern and western lowland faunas of Mexico, and between these and the southern Mexican tropical fauna, which has much in common with that of Guatemala and Costa Rica, much less in common with that of Panama at the level of the Canal Zone. The Central American high mountains, collected extensively by Schaus, have little in common with the Mexican Temperate Forest, but instead are largely populated with endemic species apparently derived from the forests below.

CHARACTERISTICS OF TEMPERATE FAUNISTIC PROVINCES. The Arctic-Alpine Province is characterized by a tundra or rock-barren environment, with the total absence of trees; certain special areas, such as parts of Greenland and Alaska, lack trees, but have a biota otherwise similar to that of treed areas. Such regions are best referred to the Boreal Province. In the Arctic Subprovince the Lepidoptera are few and restricted to characteristic groups in a few families. The species have physiological and behaviour modifications for Arctic life. A majority of the species are circumpolar or Amphiberingian. Those which are not have close specific relatives or at least congeners in the Old World. The species do not usually range southward into the Boreal Province, but they commonly are represented by very closely related or even sibling species in that Province. The Cordilleran Subprovince shares many species with the Arctic Subprovince, but it has also species of its own, some evidently derived from lower zones.

The Boreal Province is much richer in species than the Arctic-Alpine Province. It has strong affinities with the Palaearctic Taiga zone. Many of the species are shared, others have close relatives in Eurasia. There is also, however, considerable sharing of species with adjacent provinces to the south. There is endemism in this province, partly localized, partly more general.

The Eastern Temperate Forest Province is in turn much richer in species and has a much higher proportion of endemics than the Boreal Province. The forests of this province are largely broad-leaved, though with important coniferous elements, especially pines and Cupressaceae. The Lepidoptera show a corresponding affinity with those of the broad-leaved forest regions of Europe and of China and Japan. Many genera or groups are represented in all three regions, with or without some species in the intervening Boreal, Cordilleran-Pacific and Taiga provinces. Others are represented in only two of the three regions. These constitute about 10 per cent of the species with Old World affinities. More frequent in this group are those represented in Eastern North America and China and/or Japan, with or without occurrence in Western North America. Less frequent are those occurring in Europe and eastern America but not in Japan and China. The Eastern Temperate Forest becomes richer in Lepidoptera species southward, but the increase is by no means proportional to the increase in species of plants, and there are few endemic developments comparable to those seen in Trichoptera or aquatic molluscs in the southern Appalachian region.

The Cordilleran-Pacific Forest Province varies from the pine-fir subalpine forests of British Columbia to the open ponderosa-pine forests that border the drier interior plains and pinon-juniper scrub, and to the tall, dense, cedar-spruce-hemlock or redwood forests of the moist coastal zone. These and other variants form more or less well-defined subprovinces, with differing lepidopterous faunas. There is an overall unity in the Lepidoptera of this region, however. This is caused partly by the general dominance of conifers, with limited association of a few genera of broad-leaved trees in the understory, and absence of many of the common eastern tree genera or species.

The sharing of plant genera and species with the Boreal Forest Region leads to sharing of many lepidopterous genera or species. These resemblances are offset by differences. Many characteristic Cordilleran groups do not enter the Boreal Forest, and there is often specific or subspecific differentiation between populations of Boreal and Cordilleran forests. The contrast with the adjacent non-forested provinces is much sharper and may take effect abruptly within a space of a few yards.

The Mexican Temperate Forest Province combines characteristics of both Eastern and Cordilleran forest provinces with many endemic features. The nature and the geographic differentiation of the lepidopterous fauna are not well known. Characteristic northern genera of both eastern and western groups have been collected in this region. Species of archaic Central American type also occur.

The Central Plains Province has a more limited and less strongly characterized lepidopterous fauna than the adjacent forested provinces. A large proportion of the species are shared with the Eastern Forest Province, representing the open-field element of that province. Other species extend to the Western Eremic Province, or are related to species that occur there. There are also, however, many characteristic forms, including a large development of grass- and forb-feeding genera, some species of northern affinity, and some of southern origin.

The Western Eremic Province extends from the Great Basin and the Mojave Desert to the southern extremity of the Mexican Plateau. It is zoned in both north-south and east-west directions. Some of the zonation is caused by geographic barriers, some by ecological differences. Although there are wide differences in richness and composition of lepidopterous fauna over this area, the general facies is rather uniform and many species or genera occur throughout. In many ways the fauna is reminiscent of that of Old World eremic areas. Many characteristic groups are well represented in both, though usually not by directly related genera. Some widely distributed genera have comparable expansions in both, a few are represented in both with wide intervening disjunctions. A second element is composed of characteristically American genera or groups, whether of northern or of southern relationship, or whether virtually endemic in the area. To these are added many widespread and tolerant species which range into the area either from the south or from the north.

CHARACTERISTICS OF TROPICAL AND SUBTROPICAL NORTH AMERICAN FAUNA. The close resemblance of the tropical and subtropical fauna of North America to the South American tropical fauna has already been noted. This is dictated by the large representation of species and groups that are either characteristically South American or that at least are fairly evenly divided between Central and South America. Nonetheless there are many endemic elements, some of which at least may be remnants of an old North Neotropical fauna. These elements show a number of affinities with the fauna of the Greater Antilles.

OTHER CHARACTERISTICS OF EXISTING FAUNA. Range Types. A number of ranges are coextensive with the main provinces. More restricted ranges occur in most provinces. In the Arctic Subprovince, Beringian, western, high-Arctic, low-Arctic and eastern range types are recognizable. A large number of restricted Alpine range types occur in different parts of the Cordillera. In the Boreal Province there are several Gulf of St. Lawrence endemics, and a number of species generally restricted to eastern or western areas; Beringian ranges also occur. Species of the Eastern Temperate Forest tend to be restricted latitudinally; a few are confined to the Atlantic coast or to parts of the Appalachian chain. In the Cordilleran-Pacific Forest Region many species are restricted to northern, southern or central areas. A common type of range extends from southern British Columbia to Arizona, New Mexico and southern California. Other species have extended north-south ranges in the interior or along the coast. The latter Vancouveran, range type is less frequent than in Coleoptera or many groups of plants. The Sierra Nevada, the Coast Range of California, and the Rocky Mts. are areas with numerous endemics. The mountains of southern Arizona and New Mexico show less endemism than would be expected from their isolation. The Central Plains Province shows considerable latitudinal zonation. There is also a zonation correlated with rainfall from east to west. In the Southwestern Eremic Province many species have restricted ranges, e.g., in the U.S.A., to southern California, to southwestern or south-central Arizona, to western Texas or to extreme southern Texas. These ranges mostly continue southward into Mexico, but their detailed limits there are poorly known. The southern part of the Mexican Plateau has many endemic species, often belonging to the same groups as species in the southwestern U.S.A. The Mexican Temperate Forest has been insufficiently studied. In the Tropical-Subtropical region there are many restricted ranges, but again the details are poorly known. A number of species occur in North America only in southern Florida. These are mostly West Indian, but there are apparently a few endemics. In Central America the details of ranges have not been very thoroughly studied, but there are striking differences between Barro Colorado I. on the one hand and Guatemala and Chiapas on the other. The western Mexican provinces, e.g., Guerrero and Nayarit, have many striking endemics.

Ranges extending over more than one province are frequent in Lepidoptera. The boundary between Arctic and Boreal Forest provinces is an exception, with few species crossing it significantly. Particularly common combined ranges are: Eastern Forest-

Boreal, Eastern Forest-Central Plains, Boreal Forest-Cordilleran-Pacific. Wide transcontinental ranges are also frequent; these are often zoned latitudinally. A number of the widely ranging species are migratory, spreading temporarily from southern centres.

Geographic Differentiation. As to be expected in so large an area, North American species and species groups often show geographic differentiation. Several authors have shown that geographic variation of Nearctic species is often clinal, with the distribution of different genes and characters unco-ordinated. More definite differentiation also occurs, however, especially of Beringian segregates, between Canadian and Cordilleran populations and in latitudinal zones in the eastern part of the continent. It is common for species or species groups with little geographic variation in the east to break up into a bewildering array of partly geographic segregates in the Cordilleran-Pacific province. Corresponding differentiation in Lepidoptera of the Appalachian region is uncommon, though there may be rich species representation of groups favoured by the deciduous forest environment.

Centres of Endemism. A number of centres of endemism have already been indicated in the foregoing paragraphs. Whether such centres should also be considered centres of evolution is a separate question. In the north the Beringian centre is particularly striking. It is characterized by geographic segregates of widely ranging species and also by the presence of species not found elsewhere in North America. The Boreal Province as a whole has a moderate number of endemic species. A few are confined to eastern or western parts of the Province, more are generally distributed. The number of transcontinental species decreases sharply south of the Boreal Province. The Eastern Temperate Forest Province has a high proportion of endemic species. These are not strongly concentrated in the southern part of the region; many of the species are widespread, others occupy more restricted transverse tracts, doubtless determined by temperature. A number of additional species have almost similar ranges except that they extend westward to the Rocky Mountains across part of the Great Plains Province. The Great Plains Province is in contrast poor in endemics. The Cordilleran-Pacific Province contains many endemics, with a great variety of range patterns. The majority of species are widespread but there are clusters of endemics in the Sierra Nevada, in the Rocky Mountains and along the Pacific Coast. The Mexican Temperate Forest Province is known to contain a considerable number of endemics, but the details of their distribution are poorly known. The Southwestern Eremic Province is likewise rich in endemics. Many of these are distributed over much or all of it, others are confined to restricted areas, e.g., the Californian deserts, western Arizona and adjacent Mexico, Western New Mexico, Eastern Arizona and Sonora, western Texas and Coahuila, and the southern part of the plateau of Mexico. The amount and localization of endemism in the Tropical Region of North America is not easy to estimate, because of our poor knowledge of the detailed ranges of most species.

HISTORICAL INTERPRETATION. Evidence for the history of the North American lepidopterous fauna is incomplete and largely indirect.

It is of four main types: fossils, knowledge of past physical and biotic environments, parallels with the ranges of other groups, and the distribution of existing Lepidoptera.

Fossil Lepidoptera are few and fragmentary. The best known are the butterflies described by Scudder from the Oligocene of Florissant, Colo. These include ten species, mostly poorly preserved. One species belongs to a group now tropical, a second to the widespread but local and archaic genus Libythea, the others are of uncertain faunistic affinity of modern type. Most of the species belong to the highly developed Nymphalinae and Satyrinae.

Cenozoic Environments must be considered under two heads: Pleistocene and pre-Pleistocene. Pleistocene times witnessed at least four major glaciations that covered most of Canada and much of the northern U.S.A. Unglaciated refugia persisted in the Beringian region, probably in the high Arctic, and probably in the Newfoundland-Nova Scotia region. Special endemics persist in each of these areas, other species must have spread into the deglaciated region from these bases. Relict mountain and bog faunas show, however, that much of the Arctic and Boreal fauna must have survived south of the ice-sheet and followed it northward. The faunas of Mt. Washington and Mt. Katahdin, for example, are of Labradorian type, and Arctic and Subarctic relicts are found in the Lake Nipigon region. In the Southwest there were extensive pluvial stages, perhaps corresponding to glacial stages in the north. These probably permitted mixing of the faunas now isolated in the mountains of southern Arizona and New Mexico, explaining their relative lack of diversity. The survival of numerous subtropical but few tropical endemics in Florida indicates appreciable but not extreme Pleistocene cooling.

The history of the pre-Pleistocene Cenozoic is one of cooling of the whole continent, of desiccation of the interior accompanying the rise of the Cordillera, and of southward growth of land in the Mississippi embayment and on the Peninsula of Florida. Early Cenozoic temperate forests were of very modern type, but included mixtures of genera now characteristically eastern or western, or extinct in the New World. Tropical or subtropical flora and fauna extended far north of where they do now. There were periodic land connections across the Bering Strait, and the water barrier was never an insuperable one to flying organisms. During most of the early and middle Cenozoic South America was separated by a sea barrier from Central America, which, considering the northward extension of tropical and subtropical environments, implies the existence of substantially independent North Neotropical and South Neotropical faunas. Elements of the North Neotropical fauna are still recognizable in Mexico, Central America and the West Indies.

Parallels with Other Groups. The distributions of lepidopterous groups often show obvious parallels to those of plant or vertebrate groups whose fossil history is well known. This is best seen in the case of discontinuous ranges. Species now confined to the Eastern Temperate Forest, but with close relatives in the European and/or Sino-Japanese deciduous forests can be regarded

as relicts of the well-known Arcto-Tertiary forest of Holarctis. The independent development of vertebrate faunas in South America in the Cenozoic period of isolation finds its reflection in the vast autochthonous development of many Neotropical lepidopterous groups. A number of these contribute to the modern North American fauna. Camel-like (Palaearctic and Neotropical), tapir-like (Oriental and Neotropical) and gecko-like (Indo-Australian-Ethiopian-Neotropical) distributions indicate groups that probably formed part of earlier North American faunas.

Distribution of Existing Groups. It is impossible to do more than cite a few special examples. The *Argynnis* type of distribution, with a small number of eastern species expanded into a large complex in the west, is a recurring pattern in different groups. In this genus all North American stocks belong to a single subgenus, closely related to only one of the numerous Old World stocks of *Argynnis* s.l. The differentiation of Old World stocks is stronger and presumably earlier than that in North America. Absolute dating of this sequence is impossible, but relative dating can be attempted from geographical and phyletic considerations. Purely North American histories can also be established, e.g. in the *Papilio glaucus* group or in the genus *Hyalophora*. Sometimes existing distributions suggest false conclusions, as in the genus *Catocala*, which has many endemic species in the Eastern Temperate Forest. This rich representation is probably the result at least in part of restriction of species from the Arcto-Tertiary forest, rather than of a purely local differentiation.

THE CHARACTERISTICS AND HISTORY OF THE NORTH AMERICAN FAUNA: LONGHORNED BEETLES. E. Gorton Linsley, University of California, Berkeley, California.

The origins of the New World fauna of longhorned beetles (Cerambycidae) are presumably lost in the history of the Paleozoic Era. The modern aspect of the fauna, as we know it in terms of tribes and genera, was probably attained in the Cretaceous Period, but its evolutionary and distributional patterns reflect geological and climatic events of the Cenozoic Era. The principal identifiable end products of this history are the following:

Holarctic Fauna

Since Cerambycidae are primarily associated with woody plants, none are found in tundra beyond tree-line, and thus none would be classed as circumpolar in the sense of Benson (1962). However, at least eight species occur in northern regions of both hemispheres and may be regarded as Pan-Boreal Holarctic elements. These include a single species each in Tragosoma, Asemum, Arhopalus, Rhagium, Acmaeops, Pachyta, Judolia and Saperda. Most of these are associated with conifers, and range far north in the taiga forests of Fennoscandia, Siberia, Alaska and Canada in patterns reflecting post-glacial dispersal. They also range southward in the subalpine forests of Europe, Asia and North America. On this continent they are found in the Canadian and Hudsonian (and to a lesser extent Transition) zones of Merriam. At least four species - Tragosoma depsarium, Asemum striatum, Rhagium inquisitor, Judolia sexmaculata - range into the Chiricahua Mountains of southeastern Arizona and possibly into the Sierra Madre Occidental of Mexico.

Nearctic Faunas

Trans-Boreal Fauna. This fauna, comprising forms which range across the continent in northernmost forested latitudes, is made up almost entirely of endemic species in Holarctic genera. Twenty-four species in this category are known in such genera as Tetropium, Callidium, Semanotus, Pronocera, Phymatodes, Xylotrechus, Clytus, Toxotus, Pidonia, Grammoptera, Leptura, Monochamus, Acanthocinus and Pogonocherus, in addition to the genera containing Holarctic species, each of which includes additional species endemic to the Palaearctic or Nearctic regions, or both. An exception is Neoclytus muricatulus, one of two known conifer-infesting species in a genus which has more than thirty species in South America, seven each in Central America, the Antilles, and Mexico, and 23 species in the United States associated with non-coniferous trees. The Trans-Boreal fauna, like the Holarctic to which it has close affinity, exhibits relatively recent, post-glacial spread.

Alleghenian Fauna. Among the Cerambycidae, this fauna, which centers in the Appalachian and Ozark plateaus but includes species with a wide distribution over eastern North America,

represents the largest endemic fauna of North America. Its affinities are northern, but in many respects its relationships appear to be closer with the fauna of the Japano-Manchurian region than with the Vancouveran fauna of western North America. Unlike the recent holarctic elements which are attached mainly to spruces and other boreal conifers and to northern type deciduous trees, the Alleghenian species are mostly found on hardwoods and other elements derived from the Arcto-Tertiary Geoflora and exhibit a marked preference for warm, humid summers, little tolerance for cold and high altitudes. Few of its species have successfully penetrated the Canadian and Hudsonian Life Zones, but a number of related forms occur in montane Mexico. To account for the eastern temperate element in the flora of Mexico, Martin and Harrell (1959) suggest that the Arcto-Tertiary mesophytes reached the montane Mexican forest by the mid-Cenozoic at the latest,

TABLE I. CONTEMPORARY GEOGRAPHIC RANGES OF TEMPERATE AND ARID REGION NORTH AMERICAN CERAMBYCID FAUNAS

MAXIMUM ASSIGNABLE RANGE	NUMBERS ASSIGNABLE		PERCENTAGES	
	GENERA	SPECIES	GENERA	SPECIES
Holarctic	34	8	21.66	1.01
Nearctic	10	24	6.37	3.01
Alleghenian	30	264	19.11	33.12
Vancouveran	16	147	10.19	18.44
Rocky Mountain	0	39	00.00	4.89
Great Basin	2	27	1.27	3.39
Californian	18	45	11.46	5.65
Sonoran	47	243	29.94	30.49
TOTALS	157	797	100.00	100.00

rather than in late Pliocene-Pleistocene time as has been proposed by others. The Cerambycidae associated with these plants are not yet well enough known to judge whether or not they would support this conclusion.

Among the old, relict endemics found in the Alleghenian fauna, one of the most interesting is the primitive monotypic genus Scaphinus, which represents one of the two living genera of its subfamily. Its only relative is Spondylis, with a single species in the Palaearctic region, another in the Vancouveran, a third in the high mountains of the Mexican plateau. Other endemic genera with relatives in eastern Asia or southern Europe are: Cyrtophorus and Microclytus (the only New World Anaglyptini), Zammodes, Tylonotus, Physocnemum, Cyrtinus, Psenocerus, Hetoemis, and Dorcaschema. At least one genus, Michythisoma, is isolated in its subfamily without close phylogenetic or geographical relatives.

Endemic Alleghenian species fall in many genera not found elsewhere on this continent. There is a single species each of Ropalopus, Hesperophanes, and Clytanthus, genera highly developed

in the Palaearctuc Region. Other species, numerous in the Alleghenian, represent Holarctic genera absent, or nearly so, from the Vancouveran. Saperda, with two recently dispersed (postglacial) poplar-feeding species occurring from coast to coast, has fifteen endemic species in the Alleghenian Fauna, one in the Vancouveran attached to Salix. Similarly, Oberea has eighteen Alleghenian endemics, one, also attached to Salix, in the Vancouveran.

The recent Alleghenian fauna is not entirely composed of northern elements, but bears some ecotonal relations with southern faunas. Apparently a few tropical American derivatives become established at an early enough date to persist as endemics (e.g. Glycobius, Sarosesthes, Bellamira, Charisalia, and Strangalia). Several tropical American genera such as Neoclytus, Leptostylus, Lepturges and Leiopus, also give evidence of having come into the fauna at an early date.

Vancouveran Fauna. In its most restricted form this fauna occurs along the western coast of North America from the Aleutian Islands to central California; in more dilute form in the Bitter Root and northern Rocky Mountains, the Cascades, the Sierra Nevada, the San Bernardino, the San Jacinto, the San Pedro Martir Mountains in the far west, and counterpart ranges in Arizona, New Mexico, western Texas, and the northern mainland of Mexico. Genera of this fauna are dominant in the western Transition Zone of Merriam and, in general, show closer affinities with those of Europe and Eastern Asia than with their Alleghenian counterpart in eastern North America. Thus we find in the Vancouveran the endemic Opsimus and Dicentrus (only New World Saphanini), Synaphaeta (only New World genus of Mesosini), and Brothylus, Leptalia, Ulochaetes, Hybodera, and Lophopogonius, with related genera in the Old World. Several other genera share species with both the Vancouveran and Palaearctic faunas, but not with the Alleghenian. These include Plectrura (one species in Japano-Manchurian region, one in Vancouveran), Spondylis (one species Palaearctic, one Vancouveran), Megasemum (one species in Japano-Manchurian, one in Vancouveran), Callimellum (eight species in southern Europe and Asia Minor, two in Vancouveran), Cortodera (numerous species in both Europe and Vancouveran), Leptorhabdium (two or three species in southern Europe, one in Vancouveran), and Rosalia (one species in Japano-Manchurian, two in southern China, one in Europe, one in Vancouveran). Other genera endemic in the Vancouveran fauna are Xylocrius (two species), Poecilobrium (one species), Eumichthus (one species), Ortholeptura (three species), Piodes (one species), Pyrotrichus (one species), and Paratimia (one species), this last possibly related to Proatimia from southwest China.

Rocky Mountain Fauna. Authors not familiar with the fauna of the Rocky Mountains have occasionally attributed to this region a greater significance in contemporary distribution than is warranted by a study of the Cerambycidae, and many other groups of

insects. There are presently no known endemic genera known for the region, but nearly forty species have distributions which center there, most of which have northern Holarctic affinities. The fauna is primarily Vancouveran in the broad sense, with an intrusion of the Great Basin on the western slopes, the Sonoran-Mexican on the south, and a few Alleghenian elements on the east. It has the least clearly defined cerambycid fauna of any major topographic division of the continent.

Great Basin Fauna. The intermountain area between the Rocky Mountains and the Sierra Nevada-Cascade axis has two endemic monotypic genera, one a flightless form associated with shrubby compositae, the other attached to juniper. In addition, twenty-seven other species appear to be confined to this area. Some of these, such as the species of Crossidius, are highly polytypic, consisting of a large number of distinctive "insular" populations attached to discontinuously distributed host plants among the fall-blooming woody Astercae of such genera as Chrysothamnus, Haplopappus, and Gutierezia (Linsley and Chemsak, 1961).

Californian Fauna. This fauna, in its restricted sense, occupies the area from the middle west coast of Baja California to Monterey County and from the San Pedro Martir to the Tehachapi. It is characterized by many endemic groups of insects and corresponds generally to the center of the broad-sclerophyll vegetation of Cooper. In the broad sense it includes some elements now found in the western Sierra Nevada foothills and eastern Coast Ranges bordering the Central Valley of California and is sparsely represented in the Great Basin montane and mountains of southeastern Arizona. A typical Californian genus is Ipochus, a wingless group of closely related species or subspecies which occupies an area from the middle of Baja California north to Santa Cruz, California, including Guadalupe and Catalina islands, and is also represented in the Huachuca Mountains. Other groups with southern (but not Sonoran) affinities, apparently assignable as endemics of the Californian in the broad sense, include the monotypic genera Megobrium, Eudistenia, Meganoplium, Paranoplium, Hesperanoplium, Neobellamira, Triodoclytus, and Sternidocinus (associated primarily with oaks and chaparral shrubs); Vandykea, which is attached to Sargent Cypress; Eucrossus and Haplidus, occurring primarily with pinyon pine; and the polytypic genus Poliaenus, with one species limited to Fremontia (a chaparral type), others to pinyon and digger pines (woodland types).

Mexican Montane Fauna. The temperate montane fauna of Mexico is not well known for Cerambycidae. However, its nature can be judged to some extent by its northern extensions in the Chiricahua and Huachuca Mountains of southeastern Arizona. Of the one-hundred and thirty-two species reported from the Chiricahua Mountains, 43 per cent can be classed as Mexican Montane, in the Huachuca Mountains the per cent is 45. Presumably the proportion increases to the south, tapering off in the vicinity of Tehuantepee. Typical

elements of this fauna as it crosses the southern border of the United States include Euryptera, Elytroleptus, Stenosphenus, Aneflomorpha, Anoplocurius, Rhopalophora, and Estola. Several of these include species which have reached eastern or southeastern United States.

Sonoran Fauna. This fauna, so named because it is often found in the Lower Sonoran Life Zone of Merriam, includes Cerambycidae which are predominantly southern in affinity. Their near relatives are largely found in the Mexican and Central American tropics. Many of the species are associated with desert trees, thorn shrubs and Cactaceae of Madro-Tertiary derivation. The fauna centers in the central and northwestern plateaus of Mexico and is well represented in the arid areas of west Texas, New Mexico, Arizona, southeastern California and eastern Baja California. Its close affinities with the tropical American fauna are indicated by the Stenaspini, with nearly 20 genera and more than 60 species limited to Mexico, six other genes and twenty-four species extending southward in the lowland tropics to Central America and northern South America, and nearly a dozen genera restricted to South America. About half of the Mexican genera and species are found on the arid plateau, an additional eleven

TABLE II. CONTEMPORARY GEOGRAPHICAL RANGES OF TROPICAL AND SUBTROPICAL AMERICAN CERAMBYCIDAE

MAXIMUM ASSIGNABLE RANGE	NUMBERS ASSIGNABLE		PERCENTAGES	
	GENERA	SPECIES	GENERA	SPECIES
Pan-Tropical	1	0	0.13	00.00
Pan-American	110	23	14.21	.44
South American	414	3,171	53.48	60.19
S.Am. + Panama	27	83	3.49	1.58
C.Am. + Colombia	17	63	2.20	1.20
Central American	93	841	12.02	15.96
C.Am. to So.U.S.	7	28	.90	.53
Mexican	64	589	8.27	11.18
Mexico + S.E.U.S.	3	23	.39	.44
Austroriparian S.E.U.S.	0	17	00.00	.32
Floridan	2	29	.26	.55
Florida + Antilles	4	21	.52	.40
Antillean	32	380	4.13	7.21
TOTALS	774	5,268	100.00	100.00

genera and twenty-six species are shared with desert areas of southwestern United States. Of these southern forms, the genus Crossidius has penetrated farthest northward, extending beyond the Sonoran desert into the Great Basin as far north as southern Alberta. Typical genera of the Sonoran fauna, other than those of the Stenaspini, include Aneflus, Osmidus, Moneilema, Peritapnia, Coenopeus, Glaucotes, Styloxus and Methia, although the

latter has a species in Florida, three in the Antilles, and two in South America.

Tropical and Subtropical Faunas

The South American cerambycid fauna is far larger and more diverse than that of North America. At its apex are found ancient austral elements, some of which have relatives in South Africa and/or Australia or New Zealand. Many occur on southern hemisphere plants such as Nothofagus and Auricaria. The affinities of some are with boreal groups of the northern hemisphere but their relationships are remote.

Of greater immediate significance is the vast fauna of tropical South America, with more than 400 endemic genera and 3,000 endemic species, reflecting its Tertiary isolation. Nevertheless, 110 genera and 23 species have Pan-American tropical distributions.

Central American Tropical Fauna. Between the Isthmus of Panama and Tehuantepec, nearly 100 genera and more than 800 species of Cerambycidae occur. The region shares an additional 17 of its genera and 83 of its species with the neighboring Colombia and receives in exchange 27 South American genera and 83 such species.

Mexican Tropical Fauna. Sixty-four genera and 589 species are believed to be limited largely to the lowland tropics of Mexico north of Tehuantepec especially on the coastal plains. Twenty-three of the species reach the United States border at Brownsville, Texas, and join with a similar number of Austroriparian species to form the largest tropical and subtropical cerambycid fauna in the United States.

Floridan Tropical Fauna. As would be expected, the tropical fauna of southern Florida and the keys is largely West Indian, and includes such species as Stenodontes chevrolati, Strongylaspis corticaria, Derancistrus scabrosus, Eburia stigma, Elaphidion irroratum, Elaphidionodes albomaculatum, E. mutatum, Anelaphus subtropicus, A. inermis, Neso Stizocera floridana, Curtomerus flavus, C. fasciatus, Euryscelis suturalis, Neoclytus longipes, N. cordifer, Leptostylus argentatus, L. bahamicus, L. dorsalis, Lagocheirus araneiformis, Spalacopsis suturalis, and S. suffusa.

Austroriparian Subtropical Fauna. The humid eastern counterpart of the Sonoran Fauna of Western United States, centers mainly in the coastal plain bordering the Gulf of Mexico and in more dilute form on the Atlantic Coastal Plain. In its more restricted form it is represented by about 17 endemic cerambycid species, all falling in genera characteristic of the American tropics. These include Archodontes, Stenodontes, Strangalia, Euryptera, Ophistomis, Smodicum, Eburia, Elaphidion, Chion, Psyrassa, Heterachthes, Ibidion, Plinthocoelium, Euderces, Rhopalophora, Ancylocera, Agallisus, Leptostylus, Leiopus, Lepturges, Eupogonius, Oncideres, Ecyrus and Hippopsis.

MAMMALIAN EVOLUTION ON THE SOUTHERN CONTINENTS

A symposium organized for the
XVI International Congress of Zoology

by

Allen Keast

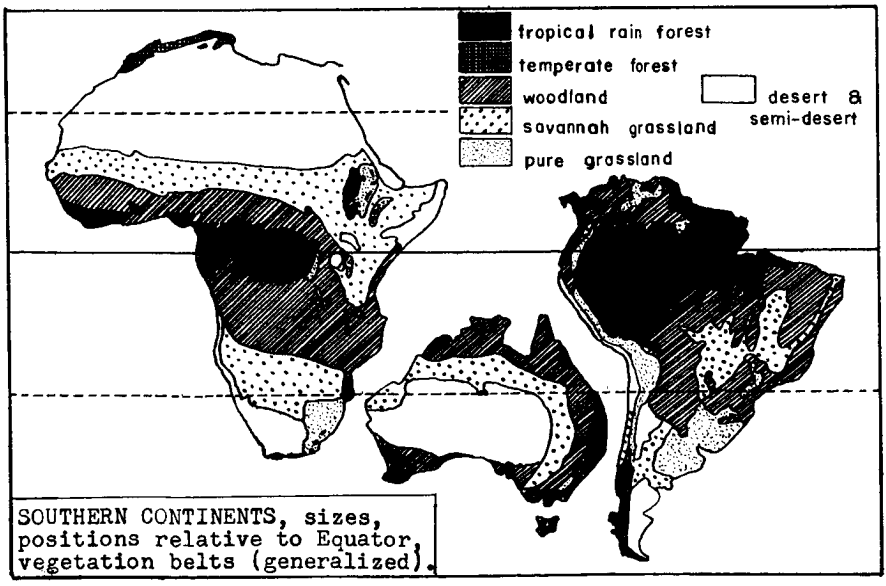
Department of Biology
Queen's University
Kingston, Ontario

MAMMALIAN EVOLUTION ON THE SOUTHERN CONTINENTS

INTRODUCTION TO SPECIAL SYMPOSIUM. Allen Keast, Queen's University, Kingston, Ontario.

This Symposium aims at a comparison of the dynamics of mammalian evolution on three, broadly comparable, continents: S. America, Africa and Australia. Each was isolated during the critical periods of mammalian evolution and radiation. This was complete for Australia, relatively complete for S. America, and intermittent for Africa. Periods of colonization can be dated approximately. The timing of climatic shifts, and hence movements of vegetation belts, presumably corresponded throughout the Cenozoic. In contrast with the Northern Hemisphere there was little Pleistocene glaciation (exceptions: Andes, higher mountains, Africa and Australia).

The three continents differ strikingly, however, in the initial mammalian raw material with which each was supplied. Thus, S. America received both marsupials and placentals from the late Mesozoic "World Continent" fauna, and Australia marsupials only (plus monotremes ?). Fossil marsupials are unknown from Africa. Thereafter, marked differences manifested themselves in the groups that radiated and became dominant on each continent, in the number of "major faunas" produced, histories of colonization, interchange with adjacent land-masses, and extinction. Thus, S. America can be regarded as having had 3 strata, an original early Tertiary endemic one, a later one in which "island hoppers" became prominant and a third (from N. America, middle Pleistocene) following the formation of the Panamanian isthmus. Africa, historically isolated by the Tethys Sea, was intermittently in direct contact with Eurasia during the Tertiary (with interchange of faunas). New fossil discoveries, however, reiterate the basic importance of major autochthonous elements throughout. The diverse Australian fauna, whose early radiation remains unknown, developed within the confines of the continent. Colonizing rodents and bats arrived during the Miocene (or early Pliocene).



SOUTHERN CONTINENTS, sizes, positions relative to Equator, vegetation belts (generalized).

2

The faunas of the southern continents pose many challenging historical questions: nature of successions, impacts of new immigrants, effects of climatic shifts, factors causing changes in dominance, gigantism, reasons for extinctions. Lastly, how do the contemporary faunas compare, numerically, and in groups represented? To what extent is success and dominance due, alternatively, to contemporary environmental and historical factors?

The Southern Continents as Environments for Mammals

The areas of the 3 continents are as follows: Africa, 11,700,000 square miles; S. America, 7,000,000; Australia, 2,950,000. Africa straddles the Equator and both Tropics. Apart from its contemporary significance this must have been an important historical factor in insulating the fauna against climatic shifts. S. America extends much further south, but there is a large tropical segment. Nearly 2/3 of Australia is below the Tropic of Capricorn. It has a subtropical to subtemperate climate.

Physiographically, S. America is dominated by the Guianan and Brazillian highlands (ancient igneous and metamorphosed rocks) and the Andean cordillera, rugged and folded, mainly Miocene but with strong Quaternary upthrusts. The Andes average 7,000 ft (higher peaks exceeding 20,000), hence altitudinal zonation is very marked. Plains cover 2/3 of S. America. Africa, a comparatively massive and stable continent in which Precambrian rocks are prominant and the degradational history a long one, has mainly an elevated rolling plateau surface. Faulting and folding is limited to certain areas. The higher peaks (Kilimanjaro, 19,000 ft; Kenya, 17,000) are recent volcanics. Australia, too, has an extensive outcropping of Precambrian strata and is a worn continent of low to moderate relief (highest peak, Koskiusko, 7,000 ft). The Great Divide paralleling the eastern coastline, mostly stems from the Miocene. Subsequent erosion and elevation has occurred.

The generalized vegetation maps (from UNESCO map of Africa, CSIRO's The Australian Environment, 1961, and the Oxford Atlas, 1951, respectively) stress the contemporary prominance of rain forest in S. America, savannah and grassland in Africa, and the central aridity (1/3 of area) of Australia.

Histories of Contact with Other Continents

(i) Continental Drift and Transoceanic Land Bridge Theories: The great botanist J.D. Hooker (1856) was the first to draw attention to marked floral similarities between the southern continents and suggested this might be explained by a former land connexion through Antarctica. By 1934, some 350 papers had been devoted to the Wegener theory (Wittman). Geologists strongly endorsing continental drift include Du Toit (1944) and Cary (1958) - see also other contributors to the Hobart Symposium.* Australian botanists have long been in the habit of using the terms "Andean" and "Antarctic" to refer to important floral segments. Of recent writers the phytogeographer Burbidge (1960) is uncommitted with respect to the theory, whilst to Gordon (1949) and Skottsberg (1956) it is inadequate to explain Pacific plant distributions. Evans (1958) stresses the close relationships of many Australian and S. American insects, including fresh-water and flightless forms. Vertebrate zoologists, at one time deeply impressed by the concept (e.g. Harrison, 1926), now largely follow Simpson (1939) whose extensive review concludes: "There is no known biotic fact that demands an Antarctic land-migration route for its explanation".

Palaeomagnetism, with its findings that the magnetic, and hence

*Wilson (1963) gives a popular account.

true, poles have shifted position during the Earth's history, and newer discoveries of fossil pollens in Antarctica, raise intriguing prospects. Nothofagus and Proteaceous pollens are abundant in Upper Cretaceous deposits in Graham Land (Cranwell, 1961), "as rich as anything in New Zealand", whilst undated material occurs at McMurdo Sound (pers.comm.). These, plus the "amazing assemblage of early conifers and members of the Proteaceae known from Kerguelen", believed to be "older than mid-Tertiary" (pers.comm.) suggest warmer conditions or movement of these lands southward.

Mammalogists retain an open mind with respect to drift. Fundamental considerations, as noted previously, still apply: historically the mammal faunas of the 3 continents have little, or nothing (Africa), in common; distributional facts are explained by northern colonization; and mammals essentially postdate the likely time of drifting. The Wegener theory has little relevance to the present Symposium.

(ii) Spatial Relationships with Adjacent Continents: The Isthmus of Panama is composed of Cretaceous rocks, below shallow water late-Eocene marine clastics occur, followed by Oligocene and Miocene formations; there is "nothing to indicate that the isthmus itself existed pre-Cretaceous" (King, 1961). The map of Eardley (1951) shows the extensive nature of the Tertiary deposits in central America. Mayr's reconstruction (1946) indicates 4 extended Tertiary water gaps and marked insularity between the continents. Africa was long isolated from Eurasia by the Tethys Sea, "Cretaceous possibly to the mid Eocene" (Umbgrove, 1930), though periods of temporary connection permitted faunal flow (Cooke). Africa today is partially divided internally by the desert Saharan belt. Australia has been isolated from Asia since at least the beginning of the Tertiary. The eastern Indonesian islands lie in an area of high tectonic instability. There are deep troughs through the region, including one east of Timor. Raven (1935) has quantitatively shown the striking differences in mammal faunas on either side of Wallace's Line, marking the sharpest zone of change in the world. Also, Mayr (1944) reviews avifaunal differences; see map of continental limits and discussion of narrowness of barrier. Australia and New Guinea (and Tasmania) lie on the same continental shelf and have been repeatedly joined at times of lowered sea-level: their mammal faunas are similar.

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* Cranwell - see also Nature 184: 1782; 186: 700. Some material possibly lower Tertiary.

THE RECENT MAMMALS OF SOUTH AMERICA. Philip Herskovitz, Chicago Natural History Museum, Chicago, Illinois.

South America is treated here as a faunal area identical with the Neotropical Region of zoogeographers. The Region extends from the isthmus of Tehuantepec, southern Mexico, to Cape Horn, Chile. It also embraces the continental islands as well as the oceanic islands of the Bahamas, West Indies, Galapagos and Falklands. Most of southern Mexico is a transition zone between the Neotropical Region and the Nearctic Subregion, or the North American portion of the Holarctic Region. The transition zone between the analogous zoogeographic Regions of early Tertiary probably extended from what is now the isthmus of Tehuantepec to what is now the Atrato River in northwestern Colombia. The nature and affinities of the mammals presently living in this area are South American.

The Neotropical Region and Nearctic Subregion are approximately equal in size but the mammalian fauna of the former is overwhelmingly richer in terms of all hierarchies from order to species, as shown below.

	Orders	Families	Genera	Species
Neotropical	12	46	271	826
Nearctic	8	28	115	442

Mammals taken into account in this tabulation are the terrestrial, volant and fresh water species. Whales, manatees, pinnipeds and sea otters are, with the few exceptions noted below, primarily marine and excluded from consideration here. The Neotropical mammalian orders include all those represented in Nearctica plus the Primates with two families and about 68 species, the Cetacea with two families and a fresh water species each (Inia geoffrensis, Sotalia fluviatilis), the Sirenia with the only living fresh water species of manatee (Trichechus inunguis) and the Perissodactyla with the three species of New World tapirs. The long periods of isolation of the southern continent from the northern is attested by the high proportion, about 82 percent, of endemic genera. In past geological ages, the southern continent received its fauna from the northern. In Recent times, however, the greater faunal flow is in the opposite direction. Twenty-two Neotropical genera are advancing through the Nearctic Region while only four Nearctic genera are penetrating Neotropica.

The most diversified order of Neotropical mammals is the Rodentia with approximately 16 families, 98 genera and 354 species, or 43 percent of the total. The Chiroptera follow with 27 percent of the species, then Primates with eight percent and Marsupialia with seven percent. The four orders comprise 85 percent of all Neotropical species. The largest of them is the capybara, but the vast majority of the species of the four orders are mouse- to ordinary rat- or squirrel-sized. In Nearctica, the richest orders of mammals are rodents with 58 percent of all species, insectivores with 13 percent and bats with 12 percent for a total of 83 percent. Here again the largest species is a rodent, the beaver, and the great bulk of the species range in size from that of the extremely small pygmy shrew to that of a gray squirrel.

Evidently, small size characterizes the bulk of the mammalian fauna whether northern or southern. On the other hand, the largest Neotropical mammals, the tapirs, and the tallest, the guanaco, are puny compared to the North American bison and moose, respectively. The wapiti, caribou, and some exceptionally large races of mule deer and white tail deer are other extremes in size among North American ungulates. The Nearctic bears are also larger than any South American carnivore. Otherwise, most larger northern and southern species can be evenly matched, species for species. If the mass for all species could be determined, the figure for each fauna would probably be about the same.

The greater variety of species in the Neotropical Region may be largely correlated with the greater variety, abundance, and year round availability of food. For example, bats of the tropical rain forests, primitively insect eaters, have also become omnivorous and specialized fruit, nectar, meat, fish or blood eaters. The northern bats, on the other hand, remained, with few exceptions, insect eaters but acquired, instead, the ability to hibernate

or migrate when food became scarce or disappeared.

Constant availability of sustaining amounts of food and cover, particularly in the tropical and subtropical latitudes of Neotropica, promotes year round or continuous reproduction. Reproductive periodicity where it occurs may be primarily dependent on the duration of gestation as in domestic animals, rather than correlated with season as in most wild living Nearctic species. Litter size of any one Neotropical form is not less than that of its generic group counterpart of the Nearctic Region. The combination of conditions conducive to year round activity and reproduction in Neotropica results in larger populations and, generally, more individuals per species than obtains in Nearctica. As a rule, however, the individuals of one or two mammalian species in any one locality outnumber those of all other mammalian species combined. The dominant species are usually small rodents but in certain situations may be bats, marsupials or a combination of these taxa. In general, the more diverse a taxon, the greater the number of its individual members.

Despite seemingly favorable living conditons, periodic fluctuations in numbers of Neotropical mammals are necessary and may be as drastic as any experienced by their respective Nearctic counterparts. Most violent fluctuations occur among smaller mammals particularly rodents. Rainy seasons with unusual abundance of food may result in unusual population highs while exceptionally dry or protracted dry seasons may lead to extreme lows. Periodic fruiting of certain common, widespread plants, particularly bamboos, are known to promote population explosions among cricetine rodents. With rapid or sudden disappearance of the nutritious harvest, the outbreak, or ratada, collapses to a low population level.

Hibernation or aestivation as a regular seasonal function has not been demonstrated in any Neotropical mammal. Nevertheless cold induced lethargy with lowered body temperatures has been recorded for some species of mouse opossums, sloths and the common marmoset. A few species of Patagonian cricetine rodents have been said to become torpid during cold spells.

Seasonal variations in pelage is expressed in weight, i.e., length and thickness, in color, and in a combination of both characters. Where warm summers are replaced by cold winters as in the southern part of South America, summer pelage is replaced by a heavier winter pelage. Where mean daily temperatures fluctuate little throughout the year, warm lowland races maintain a permanent "summer" pelage while cold highland races persist in permanent "fall" or "winter" pelage. On the other hand, dry season pelage in any constant temperature zone is, as a rule, replaced by a darker rainy season pelage of approximately equal weight. Conversly, rainy season pelage is replaced by a paler dry season pelage independently of extraneous bleaching or thinning factors. There are remarkable parallelisms between local constancy and seasonal variation in the nature of the pelage of wide ranging species. For example, the white-tailed deer of the lowlands of South America is practically indistinguishable in color and weight of pelage from its summer pelage relative of the northern United States. Likewise, the year round pelage of the race of white-tailed deer living between 9000-13,000 feet in the Andes is quite like the winter pelage race of the northern United States and Canada. Antler shedding among Neotropical deer is uncommon and not known to be correlated with seasons.

Discordance is the predominant note in geographic variation of body bulk or extremity size in Neotropical mammals. When Allen and Bergmann propounded generalizations bearing on these characters practically nothing was known of geographic variation in Neotropical and Paleotropical mammals and too little of it elsewhere

Mass movements of land animals over long distances are unknown among living species of Neotropical mammals. Many bats are migratory but only those of the genus Lasiurus with a range extending from Canada to Chile and Argentina are suspected of migrating between the two continents or anywhere within the Neotropical Region.

The principle topographic features of continental South America, not to be confused with the zoopeographic subregions and provinces described beyond, are the Andes and Pacific

and Caribbean coastal plains, the Guiana and Brazilian highlands, the Patagonian plateau and the interhighland basins of the Amazon, Orinoco and Paraná rivers. These basins were part of a mediterranean sea since uplifted and filled with deposits from the highlands. The basins retain their connections at the headwaters and the greater part of them are still swampy and subjected to periodic inundations.

The living mammals of the Patagonian plateau and southern Andes are descendants of the early Tertiary marsupial, edentate, and caviomorph rodent fauna and progressive elements of Pliocene-Pleistocene carnivores, cricetine rodents and artiodactyls. The bats are pervasive. A total of 70-80 genera of mammals are present in the Patagonian plateau with over half of them endemic. The older mammals of the Brazilian highlands (a part of the Brazilian zoogeographic subregion described below) were derived from the Patagonian highlands and the Andes. Many of these elements radiated into new niches. Together with the later influx of carnivores, cricetine rodents, lagomorphs and ungulates, these are now over 150 genera of mammals in the Brazilian highlands of which more than one-third are endemic. Primates, abundant in Brazil, existed in Patagonia during the Miocene and late Oligocene. Tapirs and rabbits, also present in Brazil since Pleistocene, evidently never reached Patagonia. All mammals of the Guianan highlands were derived from the surrounding lowlands during and since the last glacial period. With one dubious exception, none of the genera are endemic. The Andean highland was and remains the principle route for distribution of mammals throughout South America. Its present tropical zone fauna is the same as that of the Pacific and Caribbean coasts and upper Amazon-Orinoco-Paraná basin. Its temperate zone fauna was first spread along the base and foothills of the Andes but with the onset of a warmer climate it followed glacial wakes upward to higher altitudes or southward to higher latitudes. All major land mammal hierarchies are present in the Andes but endemic genera are relatively few with most of them isolated in the temperate zone forests of the northern half of the chain. The present Amazon-Orinoco-Paraná basin fauna is composed almost entirely of tropical zone elements derived from the Andean, Brazilian and Patagonian highlands and the Caribbean coast. Its most peculiar members are the riverine manatee and two fresh water dolphins. These aquatic species almost certainly evolved in situ from ancestors trapped in the ancient mediterranean basin. Bats are widespread but the greatest diversification exists in the northwestern portion of the Neotropical Region.

The most important ecological factors in adaptive radiation, speciation, and extinction among recent Neotropical mammals are 1. Establishment of new temperate zone habitats in the wake of retreating glaciers. 2. Establishment of new tropical zone habitats by the uplift and sedimentation of the ancient mediterranean basin into the present interconnecting Amazon-Orinoco-Paraná river basins. 3. Establishment of parallel but isolated habitats on opposite sides of the Andean mountain chains. 4. Formation of numerous insular and penisular arboreal habitats by dissection of the great river basins. 5. Incentives to occupation of new palustrine, aquatic and arboreal niches by the periodic flooding of the great river basins and other lowlands. 6. Destruction of natural habitats by man.

The Neotropical Region as a whole is divided into three subregions. The Brazilian Subregion includes the Middle American Province, Colombia, Venezuela, the Guianas, Brazil, Paraguay, the originally forested parts of Ecuador, Peru and Bolivia, northern Corrientes and Misiones in Argentina and the islands of Trinidad, Tobago, Margarita, Dutch West Indies and Galapagos. Forests dominate the Middle American Province and the Amazonian, Guianan, Colombian and Pacific coastal river basins. However, broad bands or large patches of grasslands or other types of low early seral stages of vegetation may occur on the mountain divides, along the banks of large streams and coastal fringes. Elsewhere, notably in eastern and southern Brazil, and the interior of the Guianas and Venezuela, there are extensive tracts of savannas and scrublands.

The Patagonian Subregion includes the remainder of continental South America and the Falkland Islands. Most of the Subregion is dominated by temperate zone savannas, scrublands and deserts while the southern portion of the Chilean Province is largely timbered.

The West Indian Subregion consists of the chain of oceanic islands extending from the

Bahamas and Cuba in the northwest to the Grenadas in the southeast. Its vegetation is diverse and its mammalian fauna a heterogeneous assemblage of waifs and misguided bats nearly all of which originated on the Neotropical mainland. The special historical and zoogeographic problems of the West Indian Subregion are primarily of specific and generic grade and not discussed here.

The dominant types of the Brazilian Subregion are sylvan, i.e., primarily adapted for living in forests. Structurally, they are near the generalized or ancestral sylvan forms and except for the non-insectivorous bats, their molars retain the primitive tritubercular or quadritubercular design with low crowns and raised cusps, interconnecting ridges and styles. Almost all mammals of the Patagonian Subregion are pastoral, i.e., primarily specialized for living in savannas, scrublands, open swamplands and deserts. They are demonstrably or hypothetically derived from sylvan stock from which they differ notably in their highly specialized locomotory organs and alimentary systems. Exclusively aquatic forms represented by the river manatee and the two kinds of fresh water dolphins are classified as fluviatile. These riverine species are restricted to the Brazilian Subregion.

Characteristic Neotropical sylvan forms are the arboreal marsupials, all primates and squirrels, tree dwelling cricetine rodents, the kinkajou and olingo. Semi-arboreal sylvan types include other cricetines and marsupials, the raccoon, spectacled bear and coatimundi and tayra. All Neotropical bats are primarily sylvan. Shrews and their ecological equivalents, the tiny short-tailed opossums and caenolestids, are rain forest ground dwellers. Another group of sylvan types including small cricetine rodents, (notably Thomasomys and Oryzomys), the raccoon (mentioned before), peccaries and tapirs may shelter in forest fringes and feed in bordering savannas and swamps. The aquatic and semi-aquatic group of quadrupedal sylvan mammals such as the yapock, water rice rat and fresh water otters, also shelter in forest or fringe cover and emerge to feed and travel in streams and lakes. Generalized types such as the common opossum (also mentioned before), a few species of cricetine rodents (e.g. pygmy rice rats), certain species of dogs, cats, mustelids, many kinds of bats and the common vampire (Desmodus), move freely from sylvan to pastoral habits and some may even adopt the latter habitat to the exclusion of the former. Primary sylvan mammals are not fossorial.

Living pastoral species are either progressive offshoots of sylvan ancestors which are comparatively newcomers in South America or they are highly specialized descendants of forms which were already pastoral at the time or prior to their arrival in Middle or South America during the early Tertiary.

Virtually all pastoral cricetine rodents belong to the first or progressive category. The difference between them and their nearest sylvan relatives is often less than generic. Indeed the adaptive radiation of cricetine rodents in the Neotropical Region has been explosive and is still so much in progress that it is not uncommon to find ancestral stocks living contemporaneously with their specialized or progressive descendants.

The Brazilian Subregion provided pastoral cricetines with many newly established and unoccupied niches. To fill them some cricetines became aquatic (Holochilus), others shrew-like (Oxymycterus), mole-like (Blarinomys), otter-like (Icthyomys). Still others just became or remained mouse-like. Adaptive radiation in the Patagonian Subregion led chiefly to vole, lemming and hamster-like forms but the evolution of others paralleled that of hares (Reithrodon), pocket gophers (Notiomys), tree rats (Phyllotis griseoflavus), conies (Neotomys), Old World gerbils (Eligmodontia). Here too, some cricetines persisted in retaining or resuming the characteristic mouse- or rat-like form. In most cases, however, divergence of Neotropical pastoral cricetines from their respective sylvan ancestors is not great and secondarily acquired adaptive characters for sylvan life are practically nil.

Other progressive pastoral forms include deer and camelids (lamas, vicugnas). The tiny spike antlered deer (brockets, pudus) are as much at home in forest as in bordering grassland. They constitute a remote link between the ancestral sylvan stock and the larger branch-antlered deer of the wooded savannas.

The second or specialized category of pastoral species forms the bulk of the Patagonian fauna and a large part of the Brazilian fauna. These old pastoral forms are remotely connected with the generalized or ancestral stock and their derivations from sylvan ancestors is problematical. Their most striking specializations are in the limbs, tail (often greatly reduced or absent) and molars which may be of the high, flat-crowned grinding type or they may be peg-like or entirely absent. All Neotropical edentates, i.e., Xenarthra, comprising armadillos, sloths and anteaters, are pastoral descendants of pastoral ancestors known from the early Tertiary of North America. The Neotropical species of rabbits, pocket gophers (Geomyidae), spiny pocket mice (Heteromyidae) and Caviomorpha (guinea pigs, porcupines, agutis, spiny rats, chinchilla, etc.) have been pastoral as far back in time as their history can be traced.

Edentates are well represented in the Patagonian Subregion and the scrublands of southern and eastern Brazil. Most armadillos, however, move freely between pastoral and sylvan habitats. Giant and common anteaters likewise do not discriminate between habitats whether pastoral or sylvan. Both types of anteaters are diggers and climbers, the common form climbing expertly with the aid of its prehensile tail. A third form, the diminutive termite-eating silky anteater, is completely arboreal with its prehensile tail, hooked claws, opposable foot pads and swivel jointed ankle bones.

Sloths are also highly specialized arboreal edentates. They prefer tall timber and feed on the leaves of the trees in which they live. Their grappling claws differ little in basic design from those of the grappling, ripping and digging claws of anteaters and armadillos. The long powerful tail of most armadillos and all anteaters are basically similar but sloths are practically tailless. Obsolescence of the tail in tree sloths is a process that began with ground sloths. These browsers first relied on sitting tripodally then standing bipedally and finally in becoming taller for reaching leaves growing ever higher. Their long, powerful arms and hooked claws helped bring nearer the larger branches with more leaves. The ancestor of tree sloths used its small size and hooked claws simply for climbing into the trees themselves for feeding. In a changing environment with the boundaries of tall tropical rain forests contracting into the equatorial flood plain, tree born sloths survived but the ground sloths did not.

The Caviomorphs diverged farther and in more directions than any other group of New World mammals living or extinct. They occupy all the more obvious types of pastoral and sylvan niches and many more which are little understood and even unknown. They vary in size from small ground squirrel-like octodonts to the giant of rodents, the capybara. The most fossorial caviomorph is the pocket gopher-like tuco tuco. The supremely adapted aquatic species is the muskrat-like coypu. The Patagonian hare strongly resembles its true leporid name-sake. The group includes the familiar chinchillas, viscachas and several kinds of cavies or guinea pigs. The three-toed agutis are the equivalent of the pygmy antelopes of the tropical forests. The paca is another small ungulate-like rodent with a uniquely inflated cheek bone which serves as a resonating chamber. The most diversified caviomorph family is the Echimyidae with 30 or 40 species of ground to tree dwelling forest forms. The arboreal species, like the dactylomyines are secondarily adapted for grazing and nesting in the canopies of tall grasses and bamboos. The ancestral form may well have climbed aloft to escape periodic flooding of its ground level pasture. The rat-like terrestrial echimyids of the genus Proechimys are the basic meat supply for all large predators. Their spiny pelage is too supple for defense but the tail, like that of a lizard can be cleanly detached from the body by a sharp tug thus conferring some survival value. Fecundity, however, appears to be the best defense of this ubiquitous and extremely abundant forest floor rodent.

Porcupines, short-tailed and long prehensile-tailed, are among the most intersting of caviomorphs. The short-tailed species are small comparatively short spined and rather primitive in structure. The prehensile-tailed forms are the most specialized members of the family. Their spines are long, stiff and erectile. Their palmar and plantar pads have developed into opposable grasping organs. Their tail, judged by structure and function, was secondarily acquired after the first had disappeared or become vestigial. Untrammeled

by ancestral genetic drag, the new tail curls in an unorthodox or reverse direction compared with the orthogenetically evolved prehensile tail of other mammals.

Neotropical species of rabbits, gophers and pocket mice evolved in the Central American Province and only two species of rabbits and one or two of pocket mice spread into South America perhaps during Pleistocene. Primarily pastoral in habitat, many races and local populations of the forms in question survived entrapment by successional forests.

The following is a realistic tabulation of the number of taxa of each order of New World mammals found in the Neotropical Region (first row) compared with the number in the Nearctic Region (second row). Sources of data include Hall and Kelson, 1959 (The Mammals of North America), Cabrera, 1958-1961 (Catálogo de los mamíferos de America del Sur) and published and unpublished taxonomic revisions to date.

Numerical Rank	Order	Families	Genera	Species	Percent of Species Total
1	RODENTIA	16	98	354	42
1	"	8	44	256	58
2	CHIROPTERA	9	85	227	30
3	"	5	25	53	12
3	PRIMATES	2	16	68	8
-	"	0	0	0	0
4	MARSUPIALIA	2	14	60	7
7	"	1	2	2	0.5
5	CARNIVORA	5	26	47	5
4	"	5	20	39	9
6	EDENTATA	3	14	26	3
8	"	1	1	1	0.2
7	INSECTIVORA	2	3	19	2
2	"	2	10	57	13
8	ARTIODACTYLA	3	10	17	2
6	"	4	10	11	2
9	PERISSODACTYLA	1	1	3	0.4
-	"	0	0	0	0
10	LAGOMORPHA	1	1	2	0.2
5	"	2	4	23	5
11	CETACEA	2	2	2	0.2
-	"	0	0	0	0
12	SIRENIA	1	1	1	0.1
-	"	0	0	0	0
12	NEOTROPICAL	46	271	826	100
8	NEARCTIC	28	115	442	100

AFRICAN MAMMALS IN THE FOSSIL RECORD

H. B. S. COOKE
Department of Geology, Dalhousie University,
Halifax N.S., Canada*

INTRODUCTION

Although the wild life of Africa constitutes a characteristic assemblage which warrants separation of the Ethiopian region as a zoogeographic entity, the African fauna does not differ as radically from that of the Holarctic region as do those of South America and Australia. It is clear that the genetic isolation of the latter continents at the end of the Mesozoic allowed their early inhabitants great freedom for development along lines unaffected by the march of events elsewhere. The presence of African types of mammal in the fossil record of Eurasia, and their subsequent extinction there, has led to the widespread belief that Africa possesses its present character primarily because it has served as a "refuge" for the survival of archaic forms of life. W. D. Matthews' classic study "Climate and evolution" (1915) did much to foster a vision of Asia as the major centre from which the various groups dispersed; the views of such workers as Pilgrim (1941) in favour of Africa as an important centre of evolution and source for diffusion into the Palearctic region have received scant approval or acceptance. Further fossil discoveries are needed before the extent of the interchanges between the Palearctic and Ethiopian regions can be fully evaluated, but in the past few years new evidence serves to emphasize the essentially indigenous nature of the living and extinct mammalian faunas.

THE TERTIARY MAMMALS

During the Permian and Triassic periods, southern Africa supported a large population of diverse mammal-like reptiles, the Therapsida, which bridged the morphological gap between primitive reptile and early mammal, including the possible acquisition of warm blood, hair and glands (Brink 1957). Van Valen (1960) and others have actually suggested that the Therapsids should be included in the Mammalia but Simpson (1961) has advanced cogent reasons for retaining the existing nomenclature. Although it is thus very possible that Africa could be the original birthplace of the placental mammals,

* formerly University of the Witwatersrand, Johannesburg, South Africa

there is no palaeontological evidence to support (or refute) such a view. Dinosaur fossils are known from a number of areas but the only post-Triassic Mesozoic mammals so far found belong to a peculiar pantothere from an upper Jurassic horizon in the dinosaur-rich Tendaguru beds of Tanganyika (Dietrich 1927, Simpson 1928).

Fossil mammals of Tertiary age are known only from a small number of sites in Africa, notably from Kenya and Egypt. The significant localities are listed in Table I and relative ages are indicated. The total so far recorded from all the pre-Pleistocene sites combined is only 130 genera (excluding marine forms) and under 200 distinct species. Figure 1 gives an analysis of the composition of these faunas, expressed in terms of the percentages of genera represented in each major order, in order to indicate the apparent changes that have taken place during the Tertiary.

The best-known fauna of the Eocene and Oligocene comes from the estuarine-deltaic sequence in the Fayum area in Egypt. The upper Eocene marine Birket-el-Qurun beds contain primitive cetaceans and sirenians, some of which occur also in the overlying Quasr-el-Sagha beds together with the oldest known Proboscidea, Moeritherium and Barytherium. The former occurs also at sites in Libya, Mali (Soudan) and Senegal, thus demonstrating their wide distribution. The lower Oligocene Jebel-el-Quatrani beds include eight additional proboscideans, the unique embrithopod Arsinotherium, and more than 20 species of hyracoids which represent a highly diversified group. It may be inferred that all these orders were differentiated in Africa; the Sirenia, which are abundant in the middle and upper Eocene of Egypt, must also have developed in this region and at least a Paleocene age is indicated for the uniquely African basic stock. It is very probable that the diversification of the hyracoids resulted from the existence of many unoccupied ecological niches for a precocious group and that their rapid decline resulted from the development of more successful competitors. The Fayum rodents are unique, but the hyaenodont carnivores, anthracotheres, bats and solitary insectivore are not notably divergent from the Holarctic or "world fauna". Great interest attaches to the five genera and six species of Primates, two of them recently described by Simons (1962); the four families include Cercopithecidae and Pongidae, suggesting that Africa was the centre for the initial radiation of the anthropoids, whether their prosimian ancestors were also originally indigenous or not.

The early Miocene fauna is most completely known from the region in and around the Kavirondo Gulf of Lake Victoria in Kenya, but also from nearby Napak in Uganda and from Losodok on the west side of Lake Rudolf. Some 50 genera with 60

TABLE I - MAIN TERTIARY FOSSIL MAMMAL SITES

		Northwest Africa	Libya	Egypt	East Africa	Southern Africa
PLIOCENE	U	Mascara	Gasr-es-Sahabi			
	L	Wadi-el-Hammam		Wadi Natrun	Fort Ternan	Klein Zee
MIOCENE	U	Beni Mellal				Malembe
	L		Jebel Zelten	Wadi Faregh Moghara	Kavirondo Gulf region, Losodok, Napak	Namib
OLIGOCENE	U	(no upper Oligocene sites known)				
	L	Jebel Bon Gobrine	Zella	FAYUM SERIES: Jebel-el-Quatrani beds		
EOCENE	U	In Tafidet, M'Bodione Dadere	Jebel Coquin	FAYUM SERIES: Qasr-el-Sagha beds; Birket-el-Qurun beds		

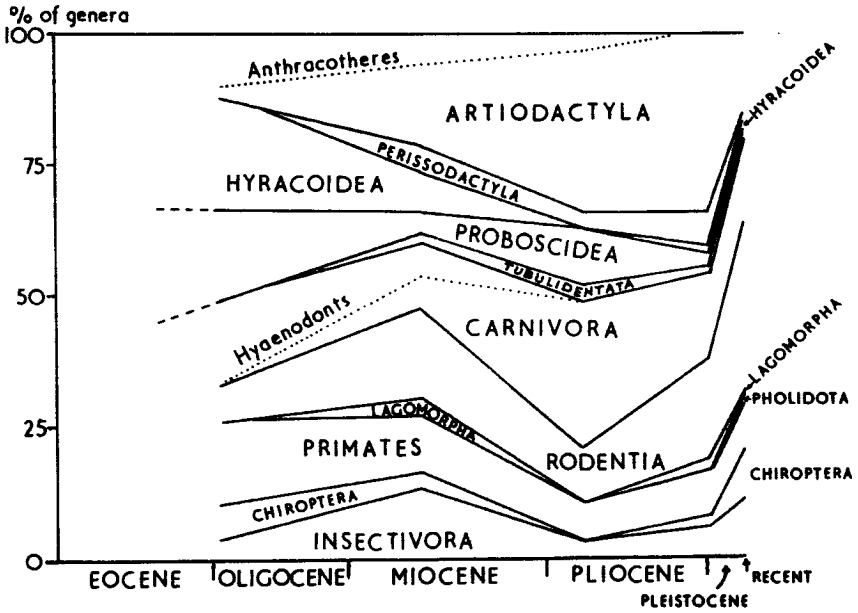

Figure 1 - Variation in percentage composition of mammalian faunas during the Tertiary and Quaternary in Africa

species have been described but there are many new finds yet unreported. Other sites ranging from South-West Africa to Egypt and Morocco show the existence of an essentially similar assemblage over the whole continent and serious ecological barriers were probably lacking. The proboscideans, hyracoids, hyaenodonts and anthracotheres follow naturally from the Oligocene. The varied insectivores include the earliest known chrysochlorid, and there are three genera of Tenrecidae (Butler and Hopwood 1957), not formerly known outside Madagascar. The earliest African lorisids accompany a varied assortment of important pongids. Asiatic-type ochotonid lagomorphs make their African debut, as do the characteristically African Pedetidae and Bathyergidae, as well as the primarily African Thryonomyidae. New elements which seem to have originated outside Africa include some perissodactyls (rhinoceroses and chalicotheres) and a few fissiped carnivores closely allied to European canids and felids; undescribed herpestine viverrids occur and it is likely that a small unspecialized viverrid stock arrived in the later Oligocene in time to give rise to the varied living African types and to reach Madagascar. A typical tubulidentate, Myorycteropus (MacInnes 1956), supports Colbert's (1941) derivation of this peculiar and already very specialized creature from a condylarth ancestor. In addition to the anthracotheres, the Artiodactyla include the chevrotain which, though earlier than the Asiatic fossils, is probably an immigrant. No cervoids are known, although an early giraffid was understandably mistaken for one; there is a very strong probability that the Giraffidae originated in Africa although they later differentiated greatly in Asia (Arambourg 1961). Primitive Bovidae occur and there are four genera of early Suidae; some of these are allied to near-contemporary European forms and it is uncertain whether the two groups arose in Africa-Arabia or came in from Eurasia in the late Oligocene.

The Pliocene fauna is scantily known so that particular interest and importance attaches to a new site at Fort Ternan, east of the Kavirondo Gulf in Kenya, where Miocene beds are overlain by Pliocene deposits that have yielded a new primate, a mastodont, a giraffe and many other new fossils (Leakey 1963). In Egypt the Wadi Natrun furnished a cercopithecid primate, the oldest African mustelid carnivores, a machairodont felid, and a suid ascribed to the Indian genus Sivachoerus. In the Pliocene hyenas and hippopotamus appear for the first time; so also does Hipparion which became widespread in North Africa but is not known south of the Sahara, where a distinctly different hipparionid, Stylohipparion, occurs in the Pleistocene. The hyenas and hippopotamus are probably indigenous but Hipparion is an import that may have arrived at the end of the Miocene after which the Sahara became a significant ecological barrier. The Pliocene was also the period of development of the Kalahari desertic region.

THE PLEISTOCENE MAMMALS

The glacial and interglacial alternations in Europe caused wide latitudinal changes in ecological conditions, which are reflected in the "more arctic" or "more tropical" nature of the successive fossil faunas. With the geographical limitations placed on movement, failure to adapt meant failure to survive. Although temperature changes and periods of higher or of diminished rainfall affected Africa, neither the exact chronology nor the ecological effects have been evaluated adequately. Forest areas expanded and contracted, and the desert and steppe encroached upon the normal savannas, but these Pleistocene fluctuations apparently served merely to shift the boundaries of the vegetational and ecological zones allowing the mammals to move with them. There is no evidence of wholesale extinction or gross population transfer, nor does climatic change seem to have been responsible for inducing abnormal speciation, although it may have resulted in some cases of physical and genetic isolation.

The Villafranchian fauna of the early Pleistocene consists almost entirely of extinct species and may contain 30-40% of extinct genera; yet the general assemblages are typically Ethiopian in character. During the middle Pleistocene, more and more living species replace archaic forms until, in the upper Pleistocene the fauna is essentially that of the present. The late upper Pleistocene extinctions have left a somewhat impoverished modern fauna. The Suidae and Bovidae, in particular, have been greatly diminished in variety. Compared with four living species from three genera of African pigs, there are at least a dozen valid additional fossil species and half as many additional genera. The alcelaphine Bovidae to-day comprise three genera with twelve species, but the fossil record provides fourteen more and also eight further genera, some of them decidedly aberrant and reflecting a long evolutionary history in Africa. Gazelles are abundant in the Pleistocene and many are found as fossils in South Africa, where they no longer occur although the Springbuck and Impala occupy similar ecological niches. These two genera, and also the Gerenuk of East Africa, demand a long lineage which had branched off from the gazelle stock well back in the Pliocene. The evolution of the African elephant within the continent is now clear, as also is that of other fossil proboscideans which have been confused with Asiatic forms. Hyaena and its degenerate offshoot Proteles are also autochthonous and there is reason to believe that much differentiation of carnivores has taken place in Africa itself so that there is little reason to require more than fleeting contacts with Eurasia during either Pliocene or Pleistocene time. The role of Africa in human evolution is well known and man may be regarded as one of the many "exports" from the continent.

GEOLOGICAL HISTORY

Through most of geological time Arabia has been basically part of the African continent; the Red Sea rift developed as a terrestrial trough in the Oligocene, was invaded from the Mediterranean in the Miocene and connected through to the Indian Ocean in the Pliocene. The pre-Mediterranean Tethys sea cut Arabo-Africa off from Eurasia during much of the Mesozoic and Tertiary. The principal periods of temporary connection occurred during the Paleocene elevation of the Arabo-African block, during the late Oligocene orogenesis in the Atlas and the Alps, and again at the end of the Miocene. The character of the Eocene-Oligocene fauna accords well with expectations from the entry in the Paleocene of a ferrungulate stock, already possessing early anthracotheres and hyaenodonts but primitive enough to allow the proboscideans, hyracoids and sirenians to develop as new elements, as did astrapotheres, notungulates and litopterns in South America. Insectivores and early primates must also have been part of this early fauna. In late Oligocene times there was an export of the African stocks to Eurasia in exchange for importation of perissodactyls, fissiped carnivores, and perhaps basic suid and bovid elements. A lesser interchange took place at the end of the Miocene, contributing African elements to Eurasia and admitting hipparionids to Africa. During the Pliocene great diversification took place among the African Bovidae and Suidae in particular, but the Ethiopian region was effectively isolated from Eurasia and similarities in developments seem to be convergent. True horses entered the Ethiopian region at the end of the Pliocene, possibly before the southern end of the Red Sea broke through the Straits of Bab el Mendeb. The Villafranchian faunas of northern and central Africa share many common elements which indicate that the Sahara was not an impenetrable barrier at this time. Thereafter, although Ethiopian forms trickled northwards, the few Eurasiatic elements which filtered along the north African coast did not penetrate southwards and the African fauna died out in Europe.

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THE LIVING MAMMALS OF AFRICA. R.C. Bigalke, Alexander McGregor Museum, Kimberley, South Africa.

The living mammals of Africa South of the Sahara, thus of the Ethiopian Region minus Southern Arabia, form the subject of this paper. Darlington (1) has pointed out that the Sahara has a very limited, specialised fauna with mixed relationships and that Southern Arabia can be considered as a complex transition area. By omitting these regions the peculiarities of the mammals of Africa proper will be highlighted more clearly. It must be pointed out that the taxonomy of many groups is chaotic. The numbers of species and especially of superspecies listed are only as accurate as our present state of knowledge allows; this is probably not very accurate.

The mammals of Africa thus defined are as follows:-

	Species	Super-species
INSECTIVORA		
Potamogalidae	3	2
Chrysochloridae	13	9
Erinaceidae	9	3
Macroscelididae	16	9
Soricidae	ca 102	ca 35
CHIROPTERA		
Pteropidae	30	20
Rhinopomatidae	2	2
Emballonuridae	10	7
Nycteridae	17	ca 12
Megadermatidae	2	2
Rhinolophidae	23	11
Hipposideridae	14	10
Vespertilionidae	86	44
Molossidae	ca 45	ca 24
PRIMATES		
xLorisidae	2	2
xGalagidae	6	4
xCeropithecidae	36	17
xColobidae	5	4
xPongidae	2	2
PHOLIDOTA		
xManidae	4	3
LAGOMORPHA		
Leporidae	?ca 9	?ca 5
RODENTIA		
Sciuridae	ca 35	ca 21
Anomaluridae	6	5
Pedetidae	1	1
Muridae		
Murinae	ca 89	ca 56
Dendromurinae	ca 20	ca 11
Otomyinae	21	8
Cricetinae	2	2
Gerbillinae	ca 74	ca 31
xRhizomyidae	ca 14	ca 10
Gliridae	ca 6	ca 6
Dipodidae	1	1
xHystricidae	ca 7	ca 2
Thrynomyidae	2	2
Petromyidae	1	1
Bathyergidae	ca 14	ca 8
Ctenodactylidae	3	? 1
CARNIVORA		
Canidae	10	9
Mustelidae	8	7
xViverridae	33	25
Hyaenidae	3	2
Protelidae	1	1
Felidae	9	? 9
TUBULIDENTATA		
Orycteropodidae	1	1
PROBOSCIDEA		
xElephantidae	1	1
HYRACOIDEA		
Procaviidae	10	4
SIRENIA		
Trichechidae	1	1
PERISSODACTYLA		
Equidae	5	5
xRhinocerotidae	2	2
ARTIODACTYLA		
Suidae	3	3
Hippopotamidae	2	2
xTragulidae	1	1
Giraffidae	2	2
Bovidae	77	52

(For an explanation of the underlining and symbols used in this list see Zoogeography below)

MORPHOLOGICAL AND ECOLOGICAL TYPES

The Chrysochloridae are subterranean mole-like eaters of earthworms and insects. They show convergence with two families of sub-

terranean rodents, the Rhizomyidae and the Bathyergidae. The close resemblances between these latter two groups are apparently entirely convergent since they have quite different affinities within their order (2). Both feed on roots and tubers.

The Macroscelididae are on the whole larger than the Soricidae and are adapted to drier surroundings. They show a tendency to morphological and ecological convergence with two groups of mainly like-sized rodents which are typical of the arid areas, the jerboas (Dipodidae) and the gerbils (Muridae, Gerbillinae). All three have long hind legs and can hop bipedally, this mode of progression being most highly perfected in the jerboas. All three also shelter in burrows (but some elephant shrews in rock crevices). However the elephant shrews are diurnal and insectivorous and at least one species can climb trees (3) while the two groups of rodents are nocturnal, vegetarian and purely terrestrial.

The Soricidae are the smallest predators while the Muridae are the small granivorous, herbivorous or in some cases omnivorous "key-industry" animals. (Very little is known about murid diet & ecology). It is of interest to note however that rats and mice have not radiated greatly in the forest zone. Possibly the smallest members of the Galagidae, Anomaluridae and Sciuridae occupy the "mouse niche" there. Interesting too is the fact that the one species of the genus Mystromys is the only true member of the Cricetinae in Africa. Lophiomys, the Maned Rat, is rather doubtfully included in this subfamily but is porcupine-like both in appearance - it has an erectile dorsal crest of coarse hairs - and in habits and diet (4), although not in its affinities within the order. Africa has only a few giant rodents. Cricetomys (Murinae H & B.15in.T.17in) which eats mainly seeds and the Hystricidae (up to ca 30 lbs), which take coarse vegetable matter, are nocturnal, burrowing forms. The cane rats(Thrynomyidae) weigh up to 14 lbs, live in dense cover near water, swim well and eat grass, shrubs and bark.

Arboreal forest- and, to a lesser degree, savanna-dwellers have evolved in several groups. Amongst the Primates the Lorisidae are creatures whose slow movements and strong grasping reflexes are reminiscent of the sloths, which group they also resemble in some adaptive skeletal features (5). The pottos are however omnivorous, probably with a preference for animal food, while the sloths are of course vegetarian. The Galagidae are mouse- to rabbit-sized, quick and agile tree dwellers. When on the ground the partly terrestrial savanna species use their long tails as balancing aids when they leap bipedally. The galagos are omnivorous, occur singly or in pairs and most but not all are nocturnal. Monkeys of the families Cercopithecidae and Colobidae are diurnal and social, they feed on leaves, fruits and flowers, and some to a limited extent on eggs and other animal foods. Most live in the forest trees and only one arboreal species (C.aethiops) is distributed in the savanna zone. There are two large, mainly terrestrial forest forms (Mandrillus) and several terrestrial savanna species (Chaeropithecus i.e. the baboons, and Erythrocebus) which are omnivorous. The African forest monkeys have speciated to a remarkable degree. Of the two large apes (Pongidae), both forest dwellers, the chimpanzee is probably mainly arboreal but partly terrestrial and the gorilla mainly terrestrial but partly arboreal. Both are vegetarian and the gorilla seems to meet most of its requirements from a few plant foods (6).

-3-

Three rodent families are essentially arboreal. The Sciuridae are co-dominant with the Cercopithecid monkeys in the trees of the forests and have speciated at least as much. But not all forest squirrels are arboreal and one genus (Xerus) contains burrowing, herbivorous ground-squirrels of the arid areas. Forest squirrels are omnivorous. The Anomaluridae show convergence with the flying squirrels (Pteromys, etc.) of the Holarctic and Nearctic but probably have no close affinities with the Sciuridae (2). All except Zenkerella have a membrane stretched between the fore- and hindlimbs and between hindlimbs and tail which enables them to glide from tree to tree. Sharp claws and a row of sharp, horny scales on the underside of the tail aid them in running quickly up treetrunks. Some anomalurids are nocturnal and some diurnal and they feed on fruit and flowers (7). The dormice (Gliridae) are like small, slow-moving squirrels and are also omnivorous (? mainly carnivorous). Some species and subspecies are restricted to the forest zone but others live in trees in the savanna and even amongst rocks. Three species of Procaviidae are arboreal, one of them, Dendrohyrax dorsalis, occuring only in forests. They probably live mainly on leaves. Two viverrid genera Poiana and Nandinia appear to be purely arboreal forest carnivores (? omnivores).

Aquatic carnivores of two different orders which display remarkable convergence are the otter shrews (Potamogalidae) which live in forest streams and feed mainly on crustacea (7) and the familiar otters of the family Mustelidae, which are mainly fish-eaters. The marsh mongoose Atilax (Viverridae) is also partly aquatic and carnivorous and the little known aquatic civet Osbornictis seems to feed at least partly in the water. The manatee (Trichechidae)is an obligatory aquatic herbivore and the Hippopotamidae spend much of their time in the water but feed mainly on land grasses. Other swamp dwellers, especially some highly adapted Bovidae, will be dealt with.

Two groups are specialised eaters of ants and termites. The pangolins (Manidae) are covered with horny scales, roll up when alarmed and have a long, sticky tongue. Both they and the aardvark (Orycteropodidae) are nocturnal. Orycteropus is a large and rather piglike termitophile which digs burrows and also has a long tongue. The aberrant canid Otocyon (see (8( ) and Proteles (Protelidae) feed largely but not exclusively on termites.

Several families have produced small herbivores confined to rocky habitats. Two genera of Leporidae, Petromus (Petromyidae), the Ctenodactylidae and most of the Procaviidae occupy this niche. Some of the Leporidae fill the niche of small, cursorial open-country herbivores but from this size-class upwards almost all the terrestrial plant eaters have evolved by a truly remarkable radiation within the order Artiodactyla.

The pigs (Suidae) are partly grazing but mainly rooting herbivores whose very large canines are an adaptive feature. They take carrion, grubs etc. in addition to plant food; two are forest forms and one occurs in savanna country. The Giraffidae have a very long-necked form which browses on the tops of trees and a short-necked leaf-eating forest species. The Bovidae dominate the African scene with antelopes from the size of hares to the eland and buffalo. There are grazing and browsing forms in all the biotic zones and the radiation in this family will be analysed in some detail. The Equidae have not speciated a great deal but they are successful and abundant large grazers outside the forests.

The other perissodactyl family Rhinocerotidae is represented by two very large savanna species, one mainly a grazer and the other a browser. The largest herbivore of all is the elephant. (Elephantidae) which uses its proboscis to pluck a variety of plant material.

We come finally to terrestrial Carnivora. The Hyaenidae have exceptionally strong jaws and large teeth with which they can chew bones. They eat carrion and also kill living prey on occasion. Most of the Canidae are about the size of a fox-terrier dog and feed on carrion, a variety of insects and small animals and fruits. Lycaon hunts in packs and kills antelope of various kinds, filling the "wolf" niche. The Felidae range from a species smaller than a house cat to the lion; they hunt and kill a very wide range of prey. In addition to the members of this group already dealt with, the Mustelidae contain only a few species of weasel-sized nocturnal predators with warning colours. The rather odd omnivorous ratel, Mellivora, also belongs here. On the other hand the Viverridae have radiated extensively. They range from insect-eating dwarf mongooses to the dog-sized civet Viverra which preys on a variety of smaller birds & mammals.

ZOOGEOGRAPHY

Africa has a bigger variety of mammals (50 families) than any other zoogeographical region. It ranks second only to South America in the number of exclusive families (underlined with a solid line in the list above; nearly exclusive families are underlined with a broken line). The strongest faunal relationships are with the Oriental Region (shared families marked x in list) and the affinities with South America and Australia are very slight. In the words of Darlington (1) the African mammal fauna "is a diverse mixture of more or less widely distributed families, families shared with the Oriental Region, and exclusive families, and a few families with other relationships".

The biotic subdivisions of Africa have been discussed by Davis (9) and I follow him in recognising 7 zones. The fauna of these zones is discussed briefly and marked faunal gaps are mentioned.

SPECIATION AND RADIATION - Recent work on Southern Africa shrews (10) and murids (9) is discussed. Speciation and radiation in the Cercopithecid monkeys (review of Tappen 11), Sciuridae and Viverridae is discussed briefly and a fairly detailed analysis of these phenomena in the Bovidae is presented, some attention being paid to relevant behavioural characteristics.

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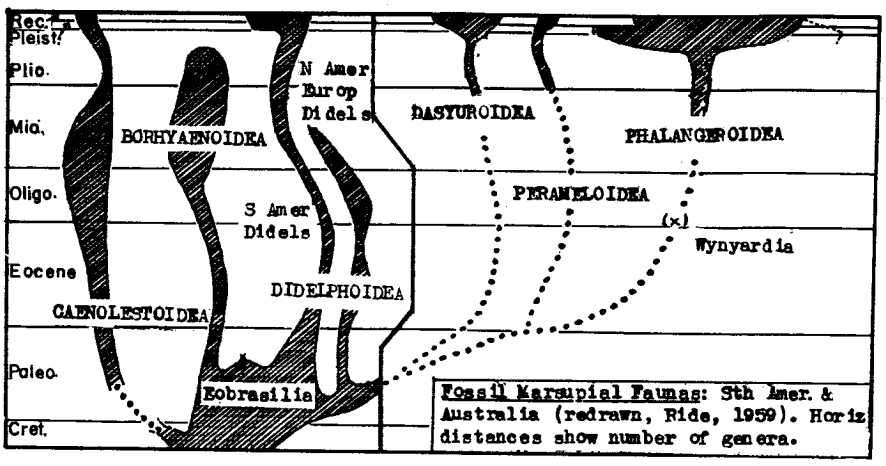
THE MAMMAL FAUNA OF AUSTRALIA. Allen Keast, Department of Biology, Queen's University, Kingston, Ontario, Canada.

Mammals presumably reached Australia in the late Cretaceous. The Tertiary fossil record is, however, a blank with the exception of the Eocene/Oligocene Wynyardia (phalanger), about 8 forms that can now be allocated to the Miocene, a few Pliocene types, and a fair Pleistocene fauna. This contrasts with a reasonable knowledge of the fossil faunas of South America.



It is a matter for speculation as to why Australia apparently received only marsupials (and monotremes?), but not placentals, from the late Mesozoic "World Continent" fauna when South America was colonized by both. One suggestion is that Australia was already an island and that the ancestral marsupials only were capable of "island hopping" (Simpson, 1961).

The Fossil Faunas

Such as they are known these may briefly be reviewed:

Late Eocene/Oligocene: The oldest Australian fossil, Wynyardia, is now regarded as a more or less generalized phalangerid.

Miocene: Gill (1957) has recently demonstrated that beds near Beaumaris, Vic., containing remains of a Nototherium-type diprotodont and a Sthenurus-like macropodid are Upper Miocene, whilst Glaessner et al. (1960) describes a Miocene kangaroo from Hamilton. Extensive excavations in the Lake Eyre basin in recent years by Prof. R.A. Stirton have revealed what apparently is a primitive Oligocene - early Miocene fauna (Etadunna Formation). In it are a koala (Perikoala), a dasyure, a bettongid wallaby, a little palorchestid kangaroo, plus an interesting genus intermediate between the phalangers and macropodids. It has a primitive phalangeroid molar dentition with sectorial premolars, a potoroine-like skull and jaws, and hind limbs little modified for saltation (Stirton, 1961; Tedford, 1961). Of the Tertiary fossils so far described it best approaches a stem stock.

Pliocene: Stirton and Gill also describe several Pliocene forms, these again being mostly primitive members of modern groups.

Pleistocene: The chief interest of this fauna lies in its spectacular types (reviews: Anderson, 1933; Howchin, 1930; David, 1950). Foremost amongst these are a kangaroo that must have stood 10-12 ft. high (Palorchestes), and a group of robust kangaroos (Sthenurus) with extremely short faces, massive jaws, and browsing dentition (Raven and Gregory, 1936). Thylacoleo, the so-called "cave-lion" was a leopard-sized phalangeroid predator with a remarkable shearing dentition. The Diprotodontidae, a group of heavy-bodied quadrupedal herbivores include Diprotodon (size of rhinoceros) and Nototherium (up to bullock-size), plus slightly smaller forms like the striking Euryzygoma whose 2 ft. long skull is characterized by a remarkable extension of the cheek bones (apparently to support cheek pouches). The trend to gigantism is also seen in Pleistocene wombats, one of which (Phascolonus gigas) is described as being as large as a tapir. From the quantitative viewpoint Pleistocene fossils include a couple of species of platypuses, a few dasyures and peramelids, several phalangers, nearly a dozen wombats, and perhaps 15 macropodids. Most of these have been placed in modern genera. Many are based on imperfect material. The extinct Diprotodontidae have been divided into about 5 genera and 13 species.

Summary: In the absence of a fossil record it is not possible to say whether or not Australia has had a succession of distinct faunal assemblages, like South America, or whether the theme has been the more simple one of a progressive modification and radiation. Nevertheless, certain known historic events must have initiated striking changes, especially the sudden Pliocene-Pleistocene "switch to aridity" (Crocker and Wood, 1947) - note marked northward contractions of range of certain tropical animals since the Tertiary, vide the fossils of cuscus from Victoria (Gill, 1957), crocodiles from Victoria and South Australia (Gill, 1961), the ratite Casuarius from central N.S.W. (Miller, 1962), and Zaglossus from southwest Australia. Nevertheless, Diprotodon apparently survived until perhaps 7,000 B.P. and extinction of Thylacinus and Sarcophilus from the mainland is recent. A highly significant historic event must have been the Miocene-Pliocene arrival of murid rodents and, latterly, the appearance of the dingo and primitive man.

The Contemporary Fauna of Australia (excl. New Guinea)

This amounts to some 3 species (2 superspecies)* of egg-laying monotremes, 110 (87) marsupials, and 120 (75) placentals. The monotremes fall into two well-defined family groups (based on Platypus and Echidna). In modern classifications the marsupials are grouped into 3 superfamilies (Dasyuroidea, Perameloidea, Phalangeroidea), and 6 (or 7) families. The placental fauna is restricted to murid rodents, bats, and the dingo, a canid believed

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*Figures to be regarded as provisional.

Superspecies: A monophyletic group of entirely or essentially allopatric species that are morphologically too different to be included in a single species (Mayr, 1963). This category, since it eliminates allopatric forms of uncertain status, provides the best basis for comparing faunas.

to have been introduced by aboriginal man perhaps 6,000 years ago.

The small monotremes, unknown as fossils pre-Pleistocene, occupy two specialized ecological niches. The Platypus, with its ducklike bill, webbed feet, and beaverlike tail, is aquatic and feeds on bottom-dwelling crustacea, worms, etc. The spiny termite eating Echidna is terrestrial and has powerful digging limbs and strong claws, a conical beak and protrusible tongue.

The rodents, of which there are about 83 species (44 superspecies) fall into two groups, typical Murinae, and the endemic Hydromyinae (water-rats). The group is believed to have arrived by "island hopping" during the Miocene or early Pliocene, and has been supplemented from time to time. Simpson (1961) draws attention to the high number of endemic genera. As a group they occupy the typical herbivorous "rodent niche". Hydromys is largely carnivorous. The bats have had a comparable history of colonization and differentiation in Australia but the degree of endemism is relatively less. Major ecological levels exploited include the typical aerial insectivorous niche (most species), fruit and nectar-feeding (the large Pteropodidae). Macroderma is predatory.

Marsupials occupy almost all the major life niches in Australia. They have developed an amazing diversity of body forms, the unique nature of which has long attracted attention. Thus, genera have attained the superficial body forms of dogs, cats, moles, weasels, shrews, rats, marmots, and anteaters, and there are a range of arboreal groups, including gliders. The large-herbivore niche is occupied by the saltatory kangaroos and quadrupedal wombats.

The numbers of species comprising the superfamilies and families of the Australian marsupials are as follows: Dasyuroidea, Dasyuridae, mostly small mouse- and rat-sized insect eaters and predators, the cat-sized "marsupial cats", Tasmanian Devil, and marsupial wolf, plus Myrmecobius, the ant-eater, 33 species (26 superspecies); Notoryctidae, the marsupial moles, 2 species (1). Perameloidea, Peramelidae, the bandicoots, long-snouted rat- and rabbit-sized forms, most of which fossick or dig for invertebrate food, 12 species (9). Phalangeroidea, Phalangeridae, the arboreal marsupials, are mostly foliage, fruit, or insect-eaters, but they include the specialized leaf-eating Koala, the long-snouted Honey Mouse (Tarsipes), and 4 species with gliding membranes, 21 species (21). Phascolomidae, the wombats, are heavy-bodied, short-legged, fossorial herbivores, 2 species (2). Macropodidae, the herbivorous wallabies and kangaroos, range in size from that of a small rabbit to the large Grey and Red Kangaroos, old males of which may stand 6 ft. high, specialized for a wide range of habitats including rock and tree dwelling, 38 species (30).

Active speciation is occurring in the Australian marsupials, chiefly in peripheral refuge areas (Keast, 1959).

Isolation, Continental Size, and Faunistic Richness

Two characteristics of Australia are that it is the most isolated of the continents, as well as the smallest of them. It is desirable that the significance of this on the fauna should be tested, both from the viewpoint of number of basic ecological types that have been able to reach it and the number of species in the different categories that it maintains. This can best be achieved by comparing the fauna with that of Africa, a continent that has many similarities as an environment yet has been in

intermittent contact with the continents of the Northern Hemisphere.

Category	AFRICA 11,700,000 sq.m. No.species	and sup.sp.	AUSTRALIA 2,974,581 sq.m. No.species	and sup.sp.
Total Mammal Fauna	1314	(824)	235	(168)
No.species/one million sq.m.	109	(69)	78	(55)
Small insect-eaters and predators (shrews, etc.)	±140	(±60)	24	(15)
Subterranean moles	28	(18)	1	(1)
Long-snouted fossicking and digging invertebrate feeders	37	(18)	14	(10)
Weasel-like predators	62	(60)	3	(3)
Large predators and scavengers	26	(21)	2	(2)
Arboreal herbivores, mixed feeders	82	(62)	22	(21)
Rodents (less arboreal squirrels)	485	(328)	40	(32)
Rabbit-sized herbivores	42	(31)	9	(7)
Large terrest. herbivores	117	(88)	29	(23)
Bats, fruit and blossom feeders	29	(19)	9	(7)
Bats, insectivorous	249	(125)	27	(23)

NOTE:-Figures are provisional, meant as general guide only.

It will be seen that Africa, with 4 times the area, has 5 times as many superspecies as Australia, 824 compared to 168 (69 instead of 55/million sq.m.).

Australia has approximately the proportion that might be expected of small insect-eaters and predators (the smaller dasyures) arboreal herbivores (phalangers and 2 tree-kangaroos, African counterparts primates and sciurids chiefly), rabbit-sized herbivores (the small macropodids), large terrestrial herbivores, and bats. There is a marked deficiency in burrowing mole-like invertebrate eaters but this is made up by a higher number of diggers. There is a marked (relative) inbalance in rodents and in their associated predators (medium-sized dasyures in Australia and mustelids and viverrids in Africa). Only two large predators and scavengers occur in Australia (dingo and Thylacinus), with one other being known as a fossil (Thylacoleo). Africa has a wide range of felids, hyenids and canids (note: all canids are included here though foxes and jackals may be largely insect-eaters and general feeders).

Indications from the above are that insular Australia has only a slightly smaller total number of superspecies than would be expected from its smaller size but that several ecological categories are markedly out of balance compared to Africa. The former suggests that there may be some general correlation between number of superspecies and surface area available. The latter indicates that despite isolation preventing various ecological types from colonizing Australia, the native fauna has failed to develop

a complete range of types and certain niches apparently remain partly unoccupied.

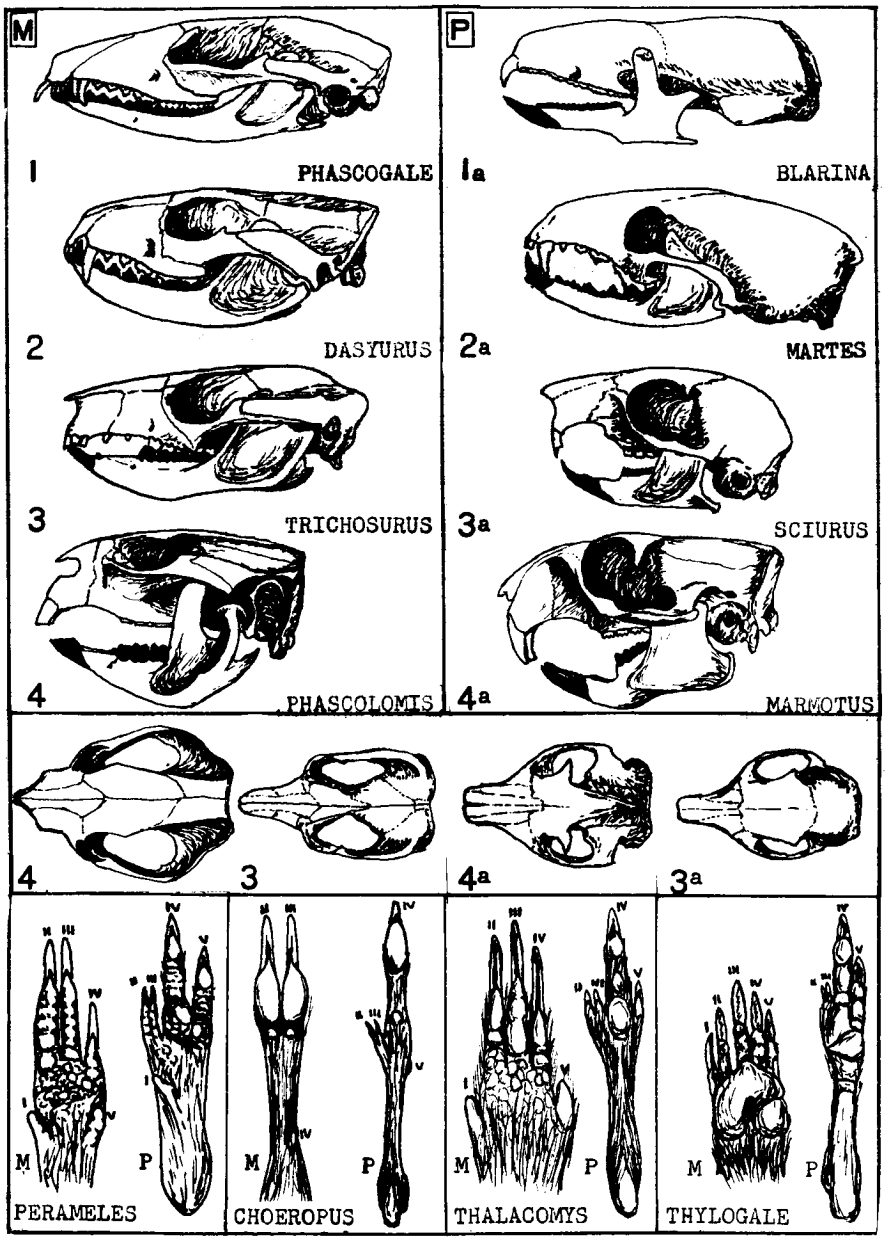
Convergence in Body Form, Australian Marsupials and Some Holarctic Placentals

Convergence, the tendency of evolutionary lines to produce similar forms and structures in response to comparable environmental demands, is an important facet of evolution. The Australian marsupials, in particular, have attracted attention in this regard and the tendency has developed to think of them as closely duplicating comparable placentals. This, however, is misleading for each animal must be looked upon as a mosaic of (partly) independent characters being acted upon by selection at different rates and in different ways. Marsupials and placentals have, moreover, rather different gene pools. The significance of these points will be brought out by a comparison of the skulls of 4 pairs of marsupials and placentals that have been regarded as instances of convergence.

The Phascogalinae are small, generalized marsupials, which are believed to approximate to the stem stock. Phascogale, however, without reduction in the canines, has the anteriormost pair of incisors exaggerated in the direction of the "pincers" of the specialized soricid shrews, many members of which are direct ecological equivalents (top figure not to scale). Dasyurus maculatus is a cat-sized predator, arboreal and with enlarged canines. Martes pennanti (Mustelidae) approximates to it ecologically and in general size, body form and dentition. It, however, has carnassials whereas Dasyurus has developed an effective counterpart, a shearing action being obtained between adjacent premolars and grinding by the compression of the upper teeth on an inner platform of the lower ones. Trichosurus vulpecula (Phalangeridae), a generalized, arboreal herbivore with 6 incisors and small, persistent canines is sluggish and slow-moving compared to the lithe, agile Sciurus carolinensis (Sciuridae). The rodent incisors of the squirrel permit it to eat nuts in addition to softer vegetable matter. Only limited convergence is involved here. Phascolomis mitchelli (Phascolomidae), the wombat, and Marmotus, a sciurid, are heavy-bodied, short-limbed, fossorial herbivores, both having continuously-growing "rodent" incisors. In this case, however, the marsupial is the better adapted, having a markedly stronger and more powerful frame (apart from being larger), and heavy lower jaws (it consumes coarser foods than Marmotus). The smaller, nocturnal, eyes are sunk deep in the solid, bony skull, whereas Marmotus has secondarily had to develop supraorbital processes (compare with Sciurus) to protect its eyes. In the wombat the neck muscles attach to a flattened posterior skull surface but in the marmot a prominant lambdoidal crest has appeared posterior to the expanded bain cavity. Thus alternate pathways to a common end are suggested.

Also illustrated is an example of convergent radiation in the syndactylous feet (not to scale) of 3 Australian peramelids (bandicoots). Perameles is the generalized type, whilst in the running Choeropus (Pig-footed Bandicoot) the pes is a single "hoof". Thalacomys (Rabbit Bandicoot) is partly saltatorial and its foot inclines towards that of the macropodids (vide Thylogale). The manus may retain a general form or be modified independently to the pes, as in Choeropus.

These examples illustrate that these "ecologically equivalent",



U: Convergent & non-convergent trends, skulls of 4 "ecologically equivalent" marsupials & placentals. L: Convergent radiation in syndactylous foot of Peramelidae, compared to macropodid (latter redrawn from Jones, 192[illegible]).

counterparts, far from being truly convergent, are a blend of convergent and non-convergent, specialized and primitive, characters. As suggested by the skulls, in some ways the placental may be the better adapted, in others the marsupial.

The degree to which the overall body form and individual structures show convergence probably varies with the severity of the demands associated with the particular niche, this being great for fossorial types (note, especially, the moles), fairly marked for large predators (e.g. Thylacinus and canids), but least for arboreal forms (especially where there is no competition). The "non-convergent" body form of macropodids can probably be explained by the group having an innate tendency to saltatory locomotion and there being no selective forces against it. The dentition of the kangaroos is, however, of the herbivore type and the group has evolved a rumen (Moir et al. 1954).

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Please refer to addendum in the Appendix of the Program.

Mammalian Evolution on the Southern Continents

Synthesis and Summing-up

George Gaylord Simpson

The principal zoogeographic feature shared by the southern continents is that all three have been from one point of view dead ends and from another separate arenas of evolution during the Cenozoic. All have existed as units throughout that time with approximately their present sizes, shapes, and geographic relationships. Whatever may have been true at earlier times or of other organisms, it is now beyond reasonable argument that there has been no interchange of terrestrial mammals directly between any two of these continents during the Cenozoic. Discussion of continental drift, of possible southern intercontinental land bridges, or of a supposed southern transoceanic continent therefore would have no pertinence for the present symposium.

Such interchange as has occurred has been between each southern continent and (apart from islands) the continent to which it is now geographically closest: Australia with southeastern Asia; Africa with Europe plus southwestern Asia (especially the latter); South America with North America. Longitudinal faunal spread was continually possible within a fairly unified (warm to cool) temperate zone through Europe, Asia, and North America during the Cenozoic. Movement to and from Africa and South America was mainly latitudinal and went across climatic zones. The southeast Asia-Australia route is, however, primarily longitudinal and in a single, little-changing climatic zone. The southern continents are dead ends in that there has been no mammalian migration through any of them to a third region.

All three of the southern continents have been more or less isolated with consequent evolution of highly autochthonous and endemic faunas in the Tertiary. The degree and constancy of isolation have, however, been strikingly different in the three. Australia has been an island continent throughout the Cenozoic, and probably even longer. South America may or may not have been connected with North America in the late Cretaceous, early Paleocene, or both, but it was certainly separate and highly isolated from sometime in the Paleocene until the latest Pliocene or earliest Pleistocene. Africa had intermittent, narrow sea barriers against Eurasia especially in the early Cenozoic and an increasingly rigorous ecological barrier in the later Cenozoic, but nevertheless had rather continual and sometimes extensive faunal interchange.

All three continents had marked climatic and ecological changes, but with only a few broad features in common. All were probably affected by the phenomenon, better established for the northern continents, of increasing sharpness of climatic zoning through most of the Cenozoic and movement of zonal boundaries toward the equator. None had continental ice caps. In all the southern continents deserts developed in the later Cenozoic, but these played quite different roles in the three. In Australia deserts and semi-deserts now occupy most of the continent and are central, older ecological zones becoming marginal to north, east, and south. Indeed Australia as a whole may have undergone an ecological revolution in the late Cenozoic. In South America desert and semi-desert, although extensive, are marginal and are occupied by an attenuated, locally

adapted representation of the general continental fauna. Africa has similar marginal deserts in the south, but its vast northern desert has become one of the major faunal barriers of the world and makes of north Africa a faunal region quite distinct from that of sub-Saharan Africa.

Africa and South America, but not Australia, have very extensive equatorial lowlands with rain forests. These have probably been present since the early Cenozoic or before and they include the most continuously available ecologies for Cenozoic mammals on those continents. It is therefore likely that they have been source areas from which a part, but evidently not all, of the mammals of the surrounding woodlands and savannahs were derived. (Geological evidence conclusively disproves earlier suggestions that the Orinoco-Amazon-Paraná basins of South America were occupied by seas during much of the Cenozoic and that their present forests are young habitats, lately stocked from surrounding uplands.)

All three continents have mountains, but those of Australia are so comparatively low and local as not to have very distinctive mountain faunas. Some African mountains reach great heights but as isolated and geologically young volcanic peaks, so that here, too, one can hardly speak of a mountain faunal facies, above quite low taxonomic levels, at least. In South America the situation is different, for here the Andes form a high, extensive, and continuous cordillera. Some Andine uplift occurred around the mid-Tertiary, but it was not fully developed until almost the end of the Tertiary. The high Andes and the lowland deserts that are largely a secondary consequence of the Andine uplift are in fact the most recent South American habitats and must be viewed as marginal rather than as source areas. The Andes are a formidable barrier to faunal movement, but their north-south orientation and their flanking by temperate faunas on both sides during the Pleistocene have prevented their becoming a major faunal boundary.

Africa is the largest of the three continents and has much ecological variety. Although smaller, South America is still more diversified, with far more extensive mountain and temperate lowland areas in addition to equally diverse tropical habitats. The numbers of recent mammalian taxa on the two continents have the same orders of magnitude. (Completely comparable counts are not available, and precise analysis of taxa: area ratios would be unreliable.) Those two apparently represent balanced or equilibrated faunas. Australia is much the smallest of the three southern continents and is also the least varied topographically, climatically, and ecologically. It is not surprising that it has the fewest mammalian taxa at all levels and also somewhat fewer species, at least, per unit area. Whether or in what sense its mammalian fauna is impoverished or unbalanced is still moot. All of the major mammalian ecological categories recognized by Keast are represented. The suggestion that some are underrepresented and some lesser niches unoccupied can be related to a probably rather recent ecological upheaval and the suggestion (especially by Gill) that consequent heightened speciation is still under way.

The extensive taxonomic differences among the three faunas are related not so much to ecological factors as to two basic historical differences: the continents started out with different assortments of mammals in the early Cenozoic, and their degrees and durations of isolation have been quite different. Ancestors of the monotremes may have been in Australia since the middle or early Mesozoic. The ultimately far more important marsupials may have reached there in late Cretaceous or early Tertiary,

probably by island-hopping from Asia although the evidence is poor and all indirect. Bats have been intermittently island-hopping into the region probably since the Eocene or even the Paleocene. Murid rodents have been coming in, also intermittently and by island-hopping, probably since the Miocene. Other mammals have been introduced by man. Isolation has been high and continuous, so that the few basic stocks of nonvolant mammals have diversified markedly and with great endemicity within the region. The bats, however, have surprisingly low endemicity, decidedly less than in South America, which may be imputed in part to ease of dispersal but in greater part to the fact that southern Asia is in about the same climatic zone as New Guinea, the principal center of bat evolution and dispersal in the Australian Region.

The basic stocks in South America included only marsupials, edentates, and primitive ungulates. Surely some and probably all came from North America in the late Cretaceous or early Paleocene - whether by island-hopping or a filter bridge is dubious. Thereafter South America was isolated until around the end of the Tertiary and an extremely diversified, wholly endemic, eventually balanced fauna evolved there. The diversity was increased by two more island-hopping groups from Central America (then part of North America) around the mid-Tertiary: rodents and primates. Union (or re-union) with North America at the end of the Tertiary produced a tremendous faunal interchange and unbalanced the fauna by multiplication of taxa. Consequent extinction produced the present again approximately balanced fauna, about half of which is of late Cenozoic North American origin.

The history of the African fauna is much more complicated than those of Australia and South America and is less well understood than either even though much better documented than that of Australia. Its basic stocks seem to have been about the same as the early placentals of Eurasia and North America. Semi-isolation fostered the origin of numerous taxa, even up to the ordinal level, in Africa. There was, however, rather frequent selective interchange with Eurasia, the details of which are still very poorly known. The modern fauna is an extremely complex mixture of autochthons and groups of Eurasian, even ultimately of North American, origin.

Among the special problems for further synthesis are: radiation and faunal stratification within the continents; convergence of southern to northern faunal elements; spread of southern autochthons to northern continents; differential Pleistocene extinctions; the roles of Indonesia and New Guinea in relation to Australia, of Central to South America, and of North to sub-Saharan Africa.

EVOLUTION OF MAN

A symposium organized for the
XVI International Congress of Zoology

by

John A. Moore
Department of Zoology
Barnard College and Columbia University
New York

THE EARLY TERTIARY PRIMATES AND THEIR ANCESTORS. Malcolm C. McKenna, Dept. of Vertebrate Paleontology, The American Museum of Natural History, New York 24, New York.

Ancient fossil primates and primate-like insectivores are becoming known at a slow but accelerating rate. From a paleontological standpoint, study is greatly limited by the scanty distribution of known fossiliferous deposits laid down at the very time when the order Primates was emerging in the Paleocene, the earliest epoch of the Tertiary. The non-marine Paleocene fauna is the most poorly known fauna of the whole Cenozoic Era. Only in North America is there a reasonably complete sampling of mammalian evolution throughout the whole Paleocene, and only that continent has yielded a glimpse of the middle Paleocene evolution of the primates, though they were almost certainly distributed over all northern continents and perhaps also Africa during a large part of the Paleocene and possibly even in earlier times. It remains to be seen how representative of these earliest primates the North American sample is, but at present we have no alternative but to interpret the North American sample with suitable caution.

The earliest known primates occur in the middle Paleocene of the United States, where six different genera have been recognized. Only jaws and teeth of these animals have been described. The dentition indicates that they were all clearly primates, but evidence from the ear region and feet of certain archaic late Paleocene and Eocene primates shows that in non-dental features the earliest primates did not possess some of the commonly accepted hallmarks of the order.

MAMMALIAN FAMILIES NEAR THE PRIMATE-NONPRIMATE BOUNDARY

Several mammalian families are either primates or are closely related to primates though more meaningfully placed in the Insectivora. These are the Tupaiidae, Plesiadapidae, Leptictidae, Microsyopidae, Mixodectidae, and Apatemyidae.

TUPAIIDAE: The most primitive living primates are the tupaiids. No Tertiary fossils can be referred to the Tupaioidea with any certainty at present, e.g., the Anagalidae. Anagalids have a primarily tympanic bulla and other features which cannot be reconciled with tupaiid anatomy.

Living tupaiids are more similar to living prosimians than to living insectivores, but it should be remembered that, prior to the Miocene, fossil insectivores of an archaic Cretaceous and early Tertiary radiation are far more abundant as fossils than the hedgehogs and their allies, which represent a later radiation. It is to the archaic insectivores, not to such archetypal

standbys as Echinosorex, that tupaiids should be compared.

The tupaiid auditory bulla, like that of other primates, is usually regarded as an outgrowth of the petrosal, but is ultimately of entotympanic origin. Early in tupaioid history the entotympanic fused to the petrosal, and only traces of this early fusion are to be seen in modern tupaiids. The tympanic ring plays no part in the formation of the bulla, but lies free within it as in the Leptictidae.

Tupaiid internal carotid arterial circulation has become simplified by the loss of the archaic medial carotid branch, which previously would have passed along between the petrosal and the basioccipital-basisphenoid. A large stapedial branch of the internal carotid artery remains, but the ramus inferior of the stapedial artery has been lost except in the most primitive living tupaiid, Ptilocercus. On the other hand, the promontory artery of Tupaia is reduced, as in lemurs.

Tupaiids have enclosed the surviving branches of the internal carotid artery in bony tubes, a step beyond the condition exhibited by plesiadapids, leptictids, and early Tertiary insectivores in general. Some macroscelidids, erinaceids (Brachyerix), and pantolestids also possess either partly or wholly encased internal carotid systems. The primitive condition is for the internal carotid system to be free, its branches lying in grooves, and complex, a medial branch and a large ramus inferior of the stapedial branch having originally been present in the ultimate ancestors of primates.

PLESIADAPIDAE: The plesiadapids are the most primitive fossil primates in which more than the dentition is well known. Their remains are common Paleocene fossils in both Europe and North America and skeletal material, including the skull, is available from both continents. Simons has remarked that the Plesiadapidae have specialized markedly in reduction of the dentition and are in effect an isolated group of early primates with little bearing on problems of higher primate origins. That may be, but the plesiadapids are of great interest if one's attention is focused upon broader matters, such as the origin of the order and its relationships to other mammalian orders such as the Rodentia and Dermoptera.

D. E. Russell has discovered much new information about Plesiadapis demonstrating that the Plesiadapidae were dentally primates at a time (late Paleocene) when their feet still had fissured claws, their skulls lacked postorbital bars, and their ear regions still possessed a large promontory artery crossing the promontorium in an open groove rather than in a bony tube.

Apparently the stapedial artery as well was not encased in a bony tube. Nevertheless, adult Plesiadapis possessed an auditory bulla, apparently composed of an entotympanic fused completely to the petrosal--a characteristic primate feature seen even in the Tupaiidae.

No cranial material is known of Pronothodectes, the earliest known and most primitive plesiadapid, but on the basis of the ear region of the Microsyopidae (Cynodontomys), whose dentition suggests derivation from the early Plesiadapidae, we may expect to discover that the earliest plesiadapids lacked an ossified bulla and that the entotympanic was not originally fused to the petrosal. Both the plesiadapids and the microsyopids appear to have originated from ancestors that would be broadly defined as unspecialized members of the insectivore family Leptictidae.

LEPTICTIDAE: The leptictids are insectivores known from the Cretaceous to the Oligocene in North America, and from the beginning of the Tertiary mammal record (late Paleocene) to the middle Eocene in Europe. No leptictids are known from Asia, though the Cretaceous family Zalambdalestidae of Mongolia is closely related. Although the leptictids are long-ranging, most of our knowledge of their anatomy is based upon Oligocene specimens thirty million years removed from the time when their anatomy would have been of crucial importance to the student of insectivore and primate affinities. There are features of Oligocene leptictid anatomy that are clearly specialized, such as the reduction of the first and fifth toes and fusion of the tibia and fibula high on the shaft of the former (in contrast to Prodiacodon, in which fusion is distal), but these modifications are regarded as post-Paleocene, just as the fusion of tibia and fibula in Tarsius is surely of late date.

Oligocene leptictids are the only mid-Tertiary insectivores to possess an ear region of the basic type from which the primate ear region must have arisen. A bulla is present and is composed of an entotympanic bone (contrary to statements by various authors) rather than an expanded tympanic ring or a descending wing of the petrosal, basisphenoid, or alisphenoid. In life the entotympanic of Oligocene leptictids lay closely appressed to the alisphenoid, basisphenoid, and petrosal, but did not fuse to them. For this reason only the most perfectly preserved fossil specimens retain the entotympanic and fewer still possess the tympanic ring, which lies free, wholly within the entotympanic. Although we have no knowledge of the ear region of Cretaceous or Paleocene leptictids, the evidence of the microsyopid ear region indicates that the leptictid entotympanic was formerly cartilaginous rather than ossified. Prior to its ossification, fusion of the entotympanic to the petrosal would lead directly to the morphology that came to characterize the primate bulla.

In the Oligocene the leptictids still retained a rather primitive system of internal carotid arterial circulation in the ear region, although the system was more specialized than that of some other early Tertiary mammals. Upon nearing the region of the posterior lacerate foramen the internal carotid divided into a medial and a lateral branch. The medial branch, though the homologue of the internal carotid of therapsids, pantolestids, canids, etc., was small relative to the lateral branch and passed along between the petrosal and basioccipital to a foramen at the basioccipital-basisphenoid suture, where it entered the braincase. The tract for this artery is almost impossible to observe in any but perfectly preserved skulls, and these must be dissected by petrosal excision. Plesiadapis and other primates have lost this branch of the internal carotid altogether, though it occurs in some rodents. The lateral branch of the leptictid internal carotid continues to the promontorium, where it again divides, producing the stapedial and promontory arteries. Grooves on the petrosal for both of these large arteries are easily seen in leptictids. The stapedial artery passes dorsad toward the stapes and the promontory artery runs forward in its groove to a foramen between the petrosal and the alisphenoid bones. Near the foramen the promontory groove becomes a deep trench, but the sides of the trench do not close over to form a tube, nor are any of the internal carotid branches encased in bony tubes except in one known specimen, in which the right internal carotid is briefly bridged by bone just prior to its division into promontory and stapedial branches. The major features of the internal carotid arterial pattern of leptictids characterized members of the plesiadapid-like family Microsyopidae as well (e.g., Cynodontomys), and the pattern appears to have persisted in the most primitive primates (e.g., Plesiadapis), but later primates, including tupaiids, have modified the pattern even more by encasing the promontory artery and stapedial artery in bony tubes.

MICROSYOPIDAE: The Microsyopidae and Mixodectidae were believed to be separate families by early workers and both families have been repeatedly transferred back and forth between the insectivores and primates. Later workers merged the two families as one, but they appear to be validly separable groups of mammals even though they may eventually prove to be derived from approximately the same source. For the present, the mixodectids are placed on the insectivore side of the insectivore-primate boundary and the microsyopids are placed on the primate side. The Microsyopids occur in the Eocene of North America (Cynodontomys, Microsyops, Craseops) and Europe (Alsaticopithecus) and show enough dental similarities to the Plesiadapidae to suggest derivation from early plesiadapids, possibly in Europe. The microsyopid ear region, however, is similar to that of leptictids, with one outstanding exception: In Cynodontomys the lack of an

ossified entotympanic is suggested by the lack of rugosity or vascularization of the surfaces of the petrosal, alisphenoid, and basisphenoid bones in the area that would normally contact the entotympanic. Such clues concerning the entotympanic are nearly always present in well-preserved Oligocene leptictid skulls from which matrix has been carefully cleared. Thus the ear region of at least one microsyopid is possibly even less like that of advanced prosimians than that of Plesiadapis. But like Plesiadapis, Cynodontomys possesses a large groove along the petrosal for the promontory artery, and both Microsyops and Cynodontomys lack a postorbital bar.

Thus, microsyopid teeth indicate close community with the plesiadapids, but the ear region seems to indicate a primitive leptictid insectivore level of organization. The known cranial material of Plesiadapis is late Paleocene in age, however, so that if we may revert for the moment to an argument that might have been used by a late Paleocene comparative anatomist, perhaps earlier plesiadapids such as Pronothodectes would have lacked not only a fused petrosal-entotympanic bulla but even an ossified entotympanic altogether. Such primitive animals may also have possessed a medial branch of the internal carotid artery.

The general shape of the skull of Microsyops is clearly somewhat adapid- and plesiadapid-like, but that of Cynodontomys shows similarity to the leptictids as well, so that it is almost certain that there will be continuing debate about microsyopid affinities and the proper borders of the order Primates. Detailed analysis of the question has not been presented yet.

MIXODECTIDAE: The Mixodectidae constitute an insectivore family near the base of the primates. They appear to be restricted to the middle and late Paleocene. The group possesses various specialized dental features which prevent them from being ancestral to primates, but enough similarities to plesiadapids are present to suggest possible relationship. Almost nothing is known of the skull or skeleton, and what is known has been inadequately described and figured, so that most speculations about the affinities of the family are almost groundless. The group needs intensive study.

APATEMYIDAE: The insectivore family Apatemyidae is still regarded as a primitive offshoot of the primates by a number of authors, but the structure of the auditory region indicates that they are best placed in the Insectivora, as Hürzeler has suggested. Hürzeler removed the family from the primates because they lack a postorbital bar, lack an ossified bulla, and possess a strong promontory artery crossing the promontory of the petrosal in a groove, but in 1959 D.E. Russell demonstrated that in the

Paleocene the genus Plesiadapis still possessed a promontory artery lying in a groove and had not yet developed a postorbital bar. Of the three characters chosen by Hürzeler, only the lack of a primate-like bulla remains diagnostic if one considers Plesiadapis an unquestioned primate. However, the archaic insectivore-like dentition of the earliest apatemyid, Jepsenella, and the insectivore-like ear region of known apatemyid skulls are enough to remove the apatemyids from the primates, though they are certainly related to primate ancestors and probably originated from some early leptictid-like animal. The place of this origin is unknown, but North America may be somewhat more probable than Europe because apatemyids are unknown in the European Paleocene. Apatemyid remains are not common fossils, however, and it is always possible that they will be found in Europe as exploration becomes more complete.

CONCLUSIONS

Fossil primates occur in sediments at least as old as the middle Paleocene, approximately sixty million years ago. Several distinctive primate lineages can be traced back to that ancient period, suggesting an even more ancient origin in the early Paleocene, but lack of sufficient suitable Paleocene deposits hampers the search for evidence of the earliest primate beginnings. In the late Paleocene, at least one lineage of primates (Plesiadapidae) still retained certain characters not characteristic of later primates but which would be expected to occur in primate ancestors: fissured claws, the lack of a postorbital bar, a large promontory artery lying in a groove, and the lack of a bone-encased stapedial branch of the internal carotid arterial system. A still more primitive ear region is possessed by the Eocene microsyopids, which lack an ossified bulla formed by a petrosal-entotympanic fusion. Both the Plesiadapidae and Microsyopidae agree in a number of primitive features, however, and together represent closely related offshoots from the very beginnings of the order. Dentally, both families diverge from the primate main stream and both possess features which suggest relationship to the Dermoptera and Rodentia. But both families were dentally members of the order prior to the time when primates acquired certain generally accepted hallmarks. The foot structure, dentition, and ear region are examples of mosaic evolution and complicate the problem of assignment of boundaries to the Primates. These different aspects of primate anatomy appear to have crossed taxonomically convenient thresholds at different times.

The order Primates appears to have arisen from Cretaceous or early Paleocene leptictid-like insectivores lacking certain minor specializations seen in Oligocene Leptictidae.

EVOLUTION OF THE PRIMATES TO MAN. Elwyn L. Simons, Dept. of Geology and Museum of Natural History, Yale University, New Haven, Connecticut.

The scope of the subject covered by this title requires that only an outline be given of the highlights reflected from the stream of primate evolution through time with primary emphasis on the main stages leading up to the origin of Man.

As is well known this subject has been historically more controversial than might be wished for in the true spirit of scientific inquiry. During the past ten years the speaker has had the opportunity of studying many hundreds of original fossil primate specimens. Materials belonging to nearly all species of the more than 110 fossil genera of the Order Primates described to date were analysed. This survey, now nearly completed, has been motivated principally by the premise that more direct observation and less second-hand citation of opinions of earlier authors is a crucially important practice if we are to learn as much as possible from presently known fossil materials of this order. Drawing from extensive research observations, hopefully in an impartial manner, primate evolutionary history, throughout its sixty-million-year duration is summarized.

Three general aspects of the evolutionary study of the higher Primates are treated: 1) major unsolved problems and poor application of knowledge in the historical study of Primates are considered together with suggestions for improving standards of future work; 2) the main stages of prosimian evolution are discussed; and 3) the significance of discoveries of the past two years in changing and enlarging knowledge of the rise of Anthropoidea from Prosimii and the later emergence of Hominidae is set forth.

SOME PROBLEMS OF PRIMATE PALEONTOLOGY

Scanty evidence relating to the geographic region of differentation of this order is considered, as well as the possible place of initial appearance of Old World Anthropoidea, of Hominoidea and of Hominidae. The need for much additional field collecting in the specific areas where further finds could most likely help bring about solution of some of these fundamental questions is dealt with. Questionable methods and past weaknesses of approach to the study of fossil Primates are criticized. The fairly widespread, recent practice of rejecting as Primates little-known and poorly understood taxa previously assigned to this order by competent students is analysed. Examples are given of the manner in which this tendency detracts from accurate understanding of primate evolution, and the proper procedure for such transferals (when actually necessary) suggested. A case is made that continued widespread transmission of certain fallacies about human and other primate origins is fundamentally due to the fact that there are a great many more persons interested in, and willing to deal with, anthropogenesis, than there are scientists trained to take into account properly all of the diverse aspects of such studies. Past departmentalization of scientific training probably lies at the source of this difficulty. The subject is

truly interdisciplinary, drawing as it does from evolutionary biology, taxonomy, osteology, anatomy, geochronology, stratigraphy and field geology. Fortunately modern changes in scientific education in this country and in Europe particularly are making it easier for students to acquire the diversified background necessary for contributing to primate paleontology and to the question of human genesis.

THE MAIN STAGES OF EVOLUTIONARY PROGRESS AMONG PROSIMII

Although broad outlines of the major steps in evolutionary development leading up to the appearance of Man are now fairly well known, detailed phylogenies are not available for most primate groups. For instance, it is not yet possible to trace any primate species successions from early to late Cenozoic times.

As has been discussed in the preceding paper the best-known early Primates of the Paleocene appear to represent aberrant side branches which did not long survive the beginning of the Eocene epoch. These early species, nevertheless, are of great interest in indicating the gradual transition from non-Primates to Primates. It may be that some of the less completely known Paleocene Primates, such as Palaechthon and Plesiolestes, will eventually prove to be near the ancestry of one or more of the primate families of initial early Eocene appearance, but this possibility cannot be clarified without further research on primate materials in the Paleocene collections at Princeton University.

At the beginning of the Eocene prosimians of lemur-like and tarsier-like aspect appear, nearly simultaneously, in Europe and North America. Due to non-recovery of warm-climate faunas of this time period elsewhere, particularly in equatorial regions, the possible distribution of early prosimians outside these two continents remains unknown. Three major sorts of Primates which have variously been divided into from three to six different families, appear at this time. These are the loosely defined Adapidae, including such well known forms as Pelycodus and later Notharctus, and Smilodectes in North America and Adapis in Europe. Species of these genera together with allied forms, insofar as we know, during the Eocene did not advance beyond the "lemuroid" condition represented today by the Malagasy lemurs, which they closely resemble both postcranially and in the auditory region. Dental specializations in notharctines and adapines do not trend toward those of modern lemurs; this appears to indicate that known Eocene species of Adapidae were not directly ancestral to the living varieties of lemurs. On the other hand, the Adapidae if broadly defined could well represent the family from which modern lemurs and possibly the lorises differentiated (Simons, 1962a). A second main type of primate of the early Eocene is represented by the more or less tarsier-like Anaptomorphidae of North America and the Microchoerinae (or Necrolemurinae) of Europe. The anatomy and taxonomic interrelationships of these small creatures is outlined. Relative to estimated body size, anatomorphs and the Necrolemur group, like the Recent tarsier, were large-brained.

They are generally characterized by many "progressive" or advanced features such as short faces, anteriorly shifted foramen magnum (implying a hopping mode of progression) and by varying degrees of anterior dental reduction and specialization. A considerable number of Eocene species are assigned, at least tentatively to these two families (or subfamilies). The best known of these species, such as Tetonius homunculus (Anaptomorphidae) from the Wasatch, early Eocene of North America, and Necrolemur antiquus from the later Eocene of Europe seem definitely to have reached the "tarsioid" grade of organization (Simons, 1961). In fact, the latter species together with some closely allied forms from France, Germany and England can, in my opinion, be assigned to the existing family Tarsiidae. For those not preferring acceptance of a vertically composed family of such great temporal duration, the name Microchoeridae may be preferable.

Omomyidae, another major group of Primates of initial Eocene appearance occurs in Europe, Asia and North America and is, with one exception, restricted in temporal range to this epoch. Collectively members of this family show greater resemblances to Anthropoidea, than are to be found among other early prosimians and could well represent the taxon from which all true Anthropoidea differentiated, a probability which is also sound zoogeographically in view of their known holarctic distribution. Certainty on this point must wait, however, until better knowledge of cranial and post-cranial anatomy is available for members of this family. Partial evidence derived mainly from skull and limb remains of a species of the North American middle Eocene omomyid genus Hemiacodon shows that this member of Omomyidae cannot correctly be regarded as having reached a "tarsioid" grade of advance (Simpson, 1940). This observation in turn implies that Anthropoidea in its earliest differentiation may not have passed through a tarsioid grade of organization. Nevertheless, anatomy of both recent Tarsius and Eocene Necrolemur clearly indicates that so-called tarsioids probably originated in the same segment of early Prosimii which produced the radiation (or radiations) of Anthropoidea, but with the divergence happening at the prosimian grade. In spite of the foregoing knowledge, serious gaps in the geologic record of Primates prevent any very definite attachment of early Tertiary species successions to those of the later Cenozoic. Nevertheless, it is likely that some known Paleocene types, at least at the generic level, do pertain to ancestry of living forms. As with most attempts to trace phyletic lineages in given groups of fossils the need to discover a great many more connecting links remains.

NEW EVIDENCE ON THE RISE OF OLD WORLD MONKEYS, APES AND MAN

On the question of differentation of cercopithecoid monkeys and hominoids (apes and men) discoveries made in Oligocene deposits in Egypt during 1961 and 1962 by Yale expeditions under the writer's direction change many previous ideas and add new data on the nature of the initial appearance of these two superfamilies. A new small primate, Oligopithecus savagei (Simons, 1962b) exhibits the typical dental formula of Miocene-Recent

Old World Anthropoidea, 2.1.2.3., together with the most primitively constituted lower molars known in this suborder. Premolar heteromorphy and slightly bilophodont molars, together with other characters of resemblance suggest relationships on the one hand to Eocene Omomyidae and on the other to Miocene-Recent cercopithecoids. Further finds of this small mammal could strengthen the possibility now based entirely on the evidence of this single fragmentary jaw, that cercopithecoids arose from Old World omomyids.

To date, no significant information whatever on upper tooth structure of earliest (Eocene-Oligocene) African or Eurasian Anthropoidea has been published. This has been a serious gap in our knowledge, because, being somewhat more complex than lower cheek teeth, upper premolars and molars allow for more accurate appraisal of taxonomic affinity. New analysis of the previously poorly known upper molars of Pondaungia cotteri from the latest Eocene of Burma (reported here) together with last winter's discovery by the Yale expedition of several partial upper dentitions of Apidium and of isolated upper teeth of Propliopithecus greatly add to the data available for consideration of the prosimian sources of the higher Primates of the eastern hemisphere. The dental evidence of these three early species on the crucial point of emergence of Hominoidea from Prosimii is here announced for the first time.

About two dozen mandibles of the Egyptian Oligocene primate Apidium moustafai discovered in 1961 and 1962 also reveal much new knowledge of the nature of earliest Old World Anthropoidea. The close relationship of this species to the celebrated Parapithecus fraasi is analysed. Primarily on the basis of an aberrant dental formula it is concluded that neither of these species could be directly ancestral to later Primates. However, it can be stated with some assurance that Parapithecidae had passed beyond the prosimian grade of advance and, taken together, materials of these two species do indicate much that is of fundamental significance regarding dental patterns and construction of teeth among earliest hominoids.

Several new finds of hominoids of the later Miocene and earliest Pliocene are mentioned. The possible pertinence of Oreopithecus from Tuscany to the line of direct human ancestry is analysed and rejected. Restudy of the Old World dryopithecines, taken together with the evidence of a series of important recent discoveries of these Primates in Kenya, Uganda, and the Siwalik Hills of India, greatly clarifies the problem of the taxonomic place of origin of the family of Man (Hominidae). Nevertheless, limited knowledge of cranial and postcranial anatomy still leaves many pertinent questions, such as the time of appearance of bipedalism among our ancestors, entirely unanswered.

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EARLY PHASES IN THE EMERGENCE OF MAN. F. Clark Howell, Department of Anthropology, University of Chicago, Chicago 37, Illinois.

The basic unity of the hominoid stock, long recognized on comparative anatomical grounds, has been further confirmed by serological, immunological, karyological, and even behavioral investigations. The origin of the hominoid stock remains unknown, but it dates from a time within the Paleogene; a tarsioid source, perhaps to be found among the omomyids, is favored by some experienced workers. Fragmentary remains of several (at least three or four) different hominoids are known from the Fayum Paleogene (lower Oligocene) in Egypt. And substantial hominoid diversity is documented in the fossil record of the lower Neogene in eastern Africa. Both hylobatine (Limnopithecus) and pongine (Proconsul, Sivapithecus, and another, as yet unnamed) taxa, mostly with several species, existed in eastern Africa. In younger Neogene deposits of Eurasia other, distinct, (non-hominid) taxa, both pongine (Dryopithecus, Sivapithecus, Sugrivapithecus, Bramapithecus, some of the latter being only specifically distinct probably) and hylobatine (Pliopithecus), are distinguishable. This diversity is further enhanced by the now well-known higher primate Oreopithecus, which is clearly hominoid, but is so distinctive as to represent a distinct (oreopithecine) lineage. And Ramapithecus (including Kenyapithecus), Afro-Asian in distribution, and non-pongine on present (limited) evidence, represents another distinct taxon, perhaps even a separate lineage. These forms, plus the existence of an extinct (non-hominid) hominoid, Gigantopithecus, in the Cromerian stage of the Pleistocene of southern China, attest to the extraordinary past diversity of the hominoid superfamily by comparison with the paucity of surviving higher taxa. Existing (cercopithecoid) simians, with a diversity of taxa exhibiting significant divergences in their ecological adaptations, may in fact afford a more representative group for analogy with late Cenozoic hominoids.

Hominoids disappeared from the fossil record of Europe in the upper Neogene. They persisted, probably with increasingly reduced diversity as a consequence of habitat restriction, in the subtropics and tropics of Asia and Africa. Many (though not yet all!) students of primate evolution accept hominid origins to constitute a distinct radiation within the Hominoidea, dating from some time within the Neogene. There is still no consensus as to the ancestral taxon nor is there evidence to fix more precisely the time and place of origin. In the writer's opinion the most appropriate ancestral taxon is Ramapithecus. Its dento-facial structure appears to be strikingly (proto-) hominid. If confirmed this would delineate a source for hominid origins around the time of the (poorly known) Miocene/Pliocene boundary. Admittedly much more extensive fossil material is necessary and further fieldwork, especially in upper Neogene deposits, is sorely needed. For the moment then the initial phases of hominid emergence are still essentially unknown.

The past decade has witnessed, however, substantial advances in knowledge of some earlier phases of man's emergence. Such progress has been largely the result of intensive (though not yet extensive) field investigations in Africa. Comparable efforts still must be made in the Asian subtropics. Overall, however, the

data does afford some inferences into the ecology of very early hominids.

AUSTRALOPITHECUS

RELATIVE AND ABSOLUTE AGE. Hominids date back (at least) to the very early Pleistocene. The relative geological age of early hominid taxa from southern African cave fillings has been much discussed, often on the basis of quite inadequate data. The problem has become much clarified in recent years as their contexts and associations were properly studied and evaluated. Moreover general advances have been made toward a better understanding of the late Cenozoic succession, including (especially) the mammalian faunas, of the Ethiopian region. All the more recent studies point toward a post-Tertiary, Basal Pleistocene age; persistence into the Lower Pleistocene (=early Middle Pleistocene of some other authors) is claimed, but wholly unproved. This conclusion is suggested by varied geological evidence, but especially by associated mammals. These are practically all, where reasonably well known, extinct species; and a very substantial number of extinct genera are also represented. Such assemblages are comparable with, and the presumed equivalents of the Villafranchian faunal stage of Europe. In Europe and Asia this stage was complex and the time encompassed of relatively long duration by comparison with the (subsequent) 'glacial' Pleistocene; perhaps twice (or more) the duration is a minimum estimate. However it is still difficult to effect reliable intercontinental comparisons with Africa due to the (generally) dissimilar composition of the mammal faunas.

'Absolute' age determinations will eventually obviate this difficulty. Some results from potassium-argon (K-Ar) radiometric assays are most promising. Such assays would indicate an antiquity in excess of 1.5×10^6 years for early hominid occurrences in eastern Africa, at least one taxon of which is the same as previously recorded from cave fillings in southern Africa. A guess-estimate for the known time span of the (inclusive) taxon Australopithecus is ca. one million years, that is between 2×10^6 and 1×10^6 years. Since this taxon comprises lesser taxa the origin of the lineage, and the lesser taxa, is obviously very considerably older.

TAXA. At least two taxa of very early hominids existed in the Basal Pleistocene of Africa. These have been variously distinguished systematically as of generic, subgeneric, or specific rank. These taxa are best regarded as congeneric, and referred to the genus Australopithecus. The customary subfamilial distinction (Australopithecinae) is of doubtful utility (or validity). The apparently oldest taxon, (Australopithecus) or A. africanus, is well known from material from three localities (cave sites) in southern Africa. It may also be represented (tentatively regarded here as A. cf. africanus) at one locality (at least two sites, MK and FLK NN1, Bed I, Olduvai Gorge) in eastern Africa. The other taxon, (Paranthropus) or A. robustus, is well known from one locality (two cave sites) in southern Africa, one locality in eastern Africa (FLK Main, Bed I, Olduvai Gorge), and probably one locality in Sudanese (Chad basin) Africa. Isolated teeth, possibly referrable to cf. Australopithecus

(resembling most A. robustus) are also present at the BK site, lower Bed II, Olduvai Gorge. The distribution of this taxon was possibly also extra-African, since one locality (Sangiran) in central Java has yeilded incomplete mandibles (and teeth) which fall within the range of variation of the African population sample.

SUCCESSION AND/OR COEXISTENCE OF TAXA. In southern Africa A. africanus appears earlier and is succeeded by A. robustus. And one site (Swartkrans) yielded abundant remains of A. robustus as well as dento-facial fragments of another hominid; the latter, originally described as Telanthropus capensis, has been referred to Homo erectus more recently, although it is demonstrably similar (in skeletal parts preserved) to A. africanus and is even possibly conspecific with the latter. In eastern Africa, where A. robustus is represented at Olduvai Gorge (Bed I), it appears that another hominid (possibly A.? africanus) coexisted with it. However, it is still uncertain whether A. robustus replaced the other taxon, as an effect of ecological displacement under competitive conditions, or whether there was ecological coexistence and hence differential utilization of available niches in the same habitat.

LOCOMOTION. The morphology of the (incompletely known) lower limb skeleton of Australopithecus indicates adaptation for erect terrestrial bipedalism, hallmark of the hominid radiation. However, some structural peculiarities of the ilium (weak iliac pillar and cristal tubercle) and the ischium (elongation), as well as the proximal femur, suggest that capabilities for walking were limited by comparison with those for running. The essentially hominid foot skeleton, in the form of the ankle joint and the (inferred) pattern of weight transmission, also broadly substantiates this view.

HABITAT AND RANGE. The habitat of Australopithecus was terrestrial and, so far as may be inferred, principally non-forested situations. There is no positive evidence to indicate the exploitation of lush lowland or montane evergreen forest. The habitat evidently varied from grass- or shrub-savanna to deciduous woodland savanna. Sources of permanent water, whether in hilly limestone plateaus where caverns, fissures and sinkholes were common, or amidst small and shallow lakes and flooded marshy areas, fed by highland streams in active volcanic landscapes, were perhaps important factors in determining (limiting) distribution. More wooded habitats, either fringing water courses or mantling slopes of adjacent volcanoes, were probably close by. Such transitional zones or ecotones would afford maximum abundance and diversity of animal and plant life. Annual rainfall, estimated from sediment studies of southern African cave fillings in which Australopithecus occurs, apparently varied from present-day ranges (in the same region) to approximately forty percent drier and wetter. The damper episodes reflect 'pluvial' climates which were very likely colder as well as wetter than present-day regional climates. In southern Africa A. africanus existed (principally) under drier conditions, whereas A. robustus existed under conditions comparable to, as well as substantially damper than those of the present.

DIET AND CULTURALLY-PATTERNED BEHAVIOR. The nature of the dietary adjustments of *Australopithecus* has aroused considerable controversy. The earlier taxon (*A. africanus*) has been regarded as primarily omnivorous, with moderate to substantial meat-eating proclivities; and the later taxon (*A. robustus*) has been thought to have been essentially vegetarian. The available paleoenvironmental evidence surely provides no support for the view that either taxon exploited habitats in which there were numerous, abundant, readily available, and edible plant species comparable, for example, to that of the wholly vegetarian gorilla. Cave fillings in southern Africa, in which these taxa are associated with other vertebrates (principally mammals), do not afford incontrovertible evidence of their diet. Carnivores, whether hyaenids and/or felids, may have occupied these localities (and hyaenid coprolites are recorded from several localities) and might have been responsible, at least in part, for the bone accumulations. And yet there is some material from these accumulations which is quite convincing as representative of hominid food debris, consequent upon meat-eating habits.

Thus far these cave fillings have provided some 127 species of 80 genera of mammals. About 35 percent of the genera and perhaps 80-90 percent of the species are extinct. The total inventory includes: insectivores (7 genera, 9 species); bats (1 genus); cercopithecoid primates (6 genera, 13 species); lagomorphs (2 genera); rodents (22 genera, 32 species); canids (2 genera, 7 species); herpestines (3 genera, 4 species); hyaenids (4 genera, 12 species); felids (5 genera, 9 species); hyracoids (1 genus, 3 species); equids (2 genera, 3 species); chalicotheres (1 genus); rhinoceros (2 genera); hippopotamus (1 genus); giraffids (2 genera); suines (4 genera, 5 species); and bovids (15 genera, 21 species). No forest or desert species are represented; all are broadly characteristic of savanna grassland or shrub grassland habitats. There are noteworthy differences between sites in the occurrence and/or frequency of certain taxa, in the occurrence of certain size classes among bovid species, in the preponderance of certain age classes of some species, and in the differential occurrence of skeletal parts of different species. Each such problem merits further study and quantitative analysis.

Open-air sites at Olduvai Gorge in eastern Africa, with their remarkable preservation, now afford new insight into the matter. The locality represents a basin, once part of the Neogene drainage to the Indian Ocean, filled with volcanic, fluviatile and lacustrine sediments in the course of the Pleistocene. The lower series of conformable deposits (Bed I), up to forty meters thick, comprise subaerial tuffs and lacustrine clays (western sector) or predominantly fluvial tuffs and conglomerates (eastern sector), the latter with intercalated lava flows: a widespread marker bed (indurated flaggy tuff) delimits the top of the series. Radiometric assays (K-Ar) indicate an age for the series in excess of a million years. The climate was relatively dry throughout; although minor more arid fluctuations may have supervened there is still no proof of substantially damper ('pluvial') conditions. The (mammal) fauna is comparable with that of the (upper) Villafranchian ; nearly all species and many genera are extinct.

Five excavated sites in the Bed I series record evidences of hominids. Two sites (MK, DK) overlie slightly the basalt flows; two sites (FLK Main, FLK NN 1) are slightly higher in the series; another site (FLK N 1), in clays, just underlies the marker bed at the top of the series. The sites all lie largely about the eastern and southern margins of the basin, in relation to shallow water (clay) and subaerial (volcanic ash) deposits. Some sites presumably occur on what were once dessicated mud-flats.

All these sites preserve more or less abundant concentrations of vertebrate skeletal remains (including many mammals). Although large mammals occur of course in Bed I sediments these sites generally preserve only small species [rodents, insectivores; also some fish, amphibians, reptiles (tortoises, lizards), and birds] or the young of medium-sized forms (pigs, antelopes, equids, etc.). At all sites stone artifacts (in lava, quartz, quartzite), referrable to an Oldowan stone industry, are found in association with the dense concentrations of split, fragmented, and splintered bone. A high proportion of the stone assemblage comprises natural pieces, merely utilized pieces, or only rudimentarily fractured pieces, all of which have been carried into the site. Finished tools, including chopping-tools and various flake implements, comprise only a quite small proportion of the assemblage. Other types of tools (or weapons), including fashioned spheroidal stones and pointed chopping-tools, which are presumably indicative of particular activities, make their appearance in low frequency in the younger sites (like FLK N1). A not dissimilar industry continues into the lowest deposits preserved elsewhere (HWK locality) in Bed II, where several occupation sites are under investigation (no hominid skeletal remains are yet known). At one site (DK) in Bed I a puzzling arrangement of blocks of basalt, sometimes heaped up into small piles, forms a circumscribed feature on the occupation surface.

Students of hominid evolution are not yet in agreement that these occurrences prove either culturally-patterned behavior (eg., regular tool-making) or a meat-eating diet for Australopithecus. Either taxon might (conceivably) have been the prey of the other, or of another 'more advanced' hominid (taken, by some workers, to have been Homo). No stone tools are known in association with A. africanus at the three principal southern African localities (Taungs, Sterkfontein Main, and Makapan Limeworks). However, a substantial assemblage (nearly 300 artifacts) occurs at a younger level at Sterkfontein (Extension Site) in association with dento-facial fragments of A. africanus. The second of the two explanations offered above has been proposed to account for this occurrence. The problem is clearly in need of further investigation, and may only be solved by new discoveries.

The hand skeleton (of A. cf. africanus), with its spatulate though rather short thumb, and some primitive features of the digits and also the carpus, was surely capable of a strong power grip though precision grip (pulp-to-pulp opposability) was presumably limited. Manipulatory behavior, even coupled on occasion with some aspects of the food quest, is marked in free-ranging chimpanzees, doubtless as it was to an even greater extent in proto-hominids. Largely terrestrial life, in non-

forested habitats, selected for efficient cursorial bipedalism. The addition of substantial protein to the diet, through exploitation of small vertebrates (especially mammals), similarly selected for the motor habits and correlative structure to permit efficient tool-use and, eventually, the modification and fashioning of natural stones into sharp-edged tools for special types of activities. Tool-making and meat-eating were doubtless correlated habits. The fundamentally hominid type of dentition (small anterior teeth, spatulate canines, non-sectorial premolars, no diastemata) of Australopithecus already suggested the liklihood of substantial manipulatory behavior and tool-use, if not proper tool-making, once carnivorous behavior was habitual.

HOMO ERECTUS

RELATIVE AND ABSOLUTE AGE. This taxon is represented in the fossil record by Lower Pleistocene (=early Middle Pleistocene of other authors) times, defined on mammalian faunal grounds. Radiometric assays (K-Ar) permit age estimates of ca. 500 (±100) thousand years for beds of (later) Lower Pleistocene age, which contain Homo remains in southeast Asia and eastern Africa. However, this is a minimum estimate since occupation sites (eastern Africa) or actual skeletal remains (Java) of Homo of still earlier Lower Pleistocene age antedate (stratigraphically) these occurrences (and postdate African occurrences of Australopithecus). Hence the genus may have been differentiated as much as a million (or more?) years ago.

TAXA. A number of hominid taxa, distinct at the generic or specific level, have been proposed for this time range. However, the known skeletal remains are justifiably subsumed in the genus Homo, probably represented by a single, variable species, Homo erectus.

HABITAT AND RANGE. This species was widespread judging from occurrences (skeletal remains) in southern Asia (three localities in Java), in northern Asia (Choukoutien Locality 1), in northern Africa (Ternifine, Algeria) and in eastern Africa (site LLK, Olduvai Gorge, Bed II); in Europe only an isolated mandible (Mauer) may be referred to this species (Homo cf. erectus). Fragments of a hominid, perhaps Homo cf. erectus, are also represented in western Asia, at the site of Ubeidije, central Jordan valley. All of these occurrences, except Mauer and those in Java, occur in archeological contexts. More widespread evidence of range is afforded by stone artifact occurrences in geological contexts, dateable on stratigraphic grounds, principally in riverine and littoral situations. Although notable gaps in distribution are manifest (eg., central-western Asia, east-central Europe) early populations of Homo were surely dispersed over most of the Asian subtropics, with penetrations northward into (temperate) middle latitudes of Eurasia. The time and place of the differentiation of the taxon is still unknown; an African source is likely, in which case neither the Saharan zone nor the transgressive seas of the earlier Pleistocene constituted barriers to hinder dispersal.

LOCOMOTION. The fully human pattern of locomotion was doubtless

perfected (though postcranial skeletal material is very incomplete, and remarkably rare). Such final transformations in hip, thigh and foot permitted fully relaxed standing posture, full lower limb extension, and sustained walking, presumably with a characteristic stride, over long distances.

CULTURALLY-PATTERNED BEHAVIOR. Tool-making capabilities were enhanced, to judge from persistent habits of manufacture, manifestation of new techniques for initial preparation of raw materials, and modes of fashioning diverse, selected sorts of stone into tools (and weapons). There was standardization of tool forms and these tend to show a broad pattern of geographic distribution. New tool types made their appearance in substantial variety. Offensive stone missiles are probable, as is the development of the thrusting spear. Fire, traces of which are infrequently preserved in geological contexts, was demonstrably utilized, whether for heat, cookery (or other purposes) is uncertain. Only a few occupation places of very early *Homo* populations are known and have been properly excavated. These include localities (not all of the same age) in eastern Asia (Choukoutien Locality 1), eastern Africa (several sites in Bed II, Olduvai Gorge), northern Africa (Aïn Hanech in part), and western Europe (Torralba and Ambrona in Spain.

DIET. Hunting was important as a basis for subsistence. Meat-eating was not only increased, but doubtless formed a stable portion of the normal diet. Most of the mammalian faunal spectrum was exploited; the prey included many or most of the largest of herbivorous species, both solitary forms as well as gregarious 'herd' species, and a variety of small mammals. Sites like those mentioned above preserve substantial, even prodigious quantities of skeletal remains of slaughtered and butchered mammals. Some indication of the level of cultural capability, as well as requisite plasiticity for local ecological adjustment, is afforded by the diversity of game species exploited, and the corresponding distinctions in occurrence, habitat preference, size of aggregation, and their species-specific patterns of behavior.

Hunting, as a new way of life, surely set very different requirements on early hominid populations. Selection pressures were markedly altered and profound changes in human biology and culture ensued. This adaptation, according to many students of the problem, was a critical factor in the emergence of fundamentally human institutions.

CONCLUSIONS

The past quarter century has seen remarkable progress in the accumulation of new data bearing on long term hominid evolution. Aside from paleoanthropological fieldwork much of this progress is a consequence of advances in related fields of the natural sciences (stratigraphic geology, mammalian paleontology, palynology, radiometric geochronology). The influence of modern evolutionary biology is also, at last, beginning to be felt.

In the evolution of the Hominidae notable transformations were effected in the morphology and functional capability of locomotor

system, dentition, and skull, in physiological processes, in diverse constituents of behavior, in populations and their structure, and in ecological adjustment. The new evidence brought forward on extinct taxa, *Australopithecus* and *Homo erectus* in particular, has provided an extraordinary impetus to human evolutionary studies. And yet it is clear that students of the problem, acquainted with the formulations of modern evolutionary biology, must continue to be aware of the paucity of current knowledge and the inadequate understanding which we have thus far gained.

SOME FEATURES OF THE LATER EVOLUTION OF MAN. J. S. Weiner, Environmental Physiology Research Unit, London School of Hygiene and Tropical Medicine.

The concluding stages of the evolution of Man, from the supersession of Pithecanthropus (H.erectus) to the establishment of the modern polytypic biospecies, have taken a complex course. This is not surprising in view of the fairly long time span and the extensive geographical distribution involved. The time-span is of the order of 300,000 years and for this we have two good absolute dating indicators. 'Chellean' man of Bed II at Olduvai, East Africa, dating back some 250,000 years by the potassium-argon isotope method, marks (as we should expect at this stage) a morphological transition from Pithecanthropus to Homo, with sapiens-features in evidence perhaps to a slight extent (we still await a definitive comparative study). The earliest specimens displaying clear sapiens features and referable to Homo are Steinheim and Swanscombe man and these date from the second (Mindel-Riss) interglacial, a period, again according to the K-A method, some 400,000 years ago. A period of this duration is about sufficient for the full emergence of a distinct species; and certainly for a high degree of sub-speciation (Rensch).

The spatial dispersal of Homo, like the temporal, is of course a highly important evolutionary factor. The distribution of Homo at its beginnings some 360,000 years ago, cannot be stated with any certainty in view of the poverty of the fossil evidence, though we can be sure that the Hominidae as a whole had attained a very wide distribution as judged by both fossils and implements. What seems significant is that the hominidae generally including the harbingers of Homo (Steinheim-Swanscombe in Europe, Chellean in East Africa) were still confined to warm climatic conditions. Only with the discovery of fire-using by the advanced variety of Pithecanthropus at Peking in the middle Pleistocene was life made possible in the cooler conditions of the interstadials of the 3rd and 4th glacial periods. Consequently, from about 100,000 years ago, that is from the last interglacial onwards, the genus Homo began to spread widely reaching a world-wide scale some 50,000 years ago with representatives in Southern Africa (Rhodesian Man), East Africa (Kanjera Man), the Middle and Near East (Mt Carmel, Galilee, Shanidar, Crimea), the Mediterranean Littoral (Combe Capelle, Monte Circeo), Central Europe (Neandertal Man), Western Europe (Fontechevade, Neandertal), China (Upper Choukoutien) and Java (Ngandong). Australia and America were occupied much more recently.

This great phase of radiation, like all rapid evolutionary radiations, meant that the genus Homo had come to occupy a great diversity of ecological niches. The complex phylogenesis attending this adaptive radiation, poses many problems. Two of these merit special attention. The first is a question of systematics. What status should be accorded those Upper Pleistocene populations, some of which in their "type" characters seemed to have attained such a high degree of distinctiveness than many anthropologists have claimed for them full

species rank? This applies in particular to the 'extinct' varieties in three continental areas - Neandertal man, Rhodesian man and Ngandong man.

The second problem is biologically more important and also more refractory - to attempt an explanation particularly in selectionist and adaptational terms - of the variation associated with the world-wide radiation of Homo in his latest phases. The bearing of this topic on the question of race formation is obvious.

On these questions I will have occasion to allude to observations and experiments carried out in the anthropology laboratory at Oxford over the past ten years.

Speciation

One of the persistent elements of taxonomic reasoning which enters into the assessment of the status both of the extinct varieties of Homo of the Upper Pleistocene and of the surviving present-day varieties, is nothing more than the old "type" concept. Once the "type" had been specified it was almost automatically accorded a high taxonomic rank. As more and more individuals were deemed to correspond to the "type", the taxonomic rank was thereby reinforced. In practice the opposite tended to happen; when the material was impartially and statistically re-examined the observed variation was often seen to be too great to justify the original distinction. This has happened to all the important specific categories into which Homo was believed, at one time or other, to have differentiated - H.steinheimensis, H.neandertalensis, H.krapinensis, H.rhodesiensis, H.soloensis, etc. (for full discussion see Campbell).

The belief in a radiative or kladogenetic species formation was favoured by the separate continental provenance of Neandertal, Solo and Rhodesian man, and also by the available time element. Rensch (p.91) finds "that the average (sic) age of the species is somewhere between 100,000 and a few million years." A time-span of 300,000 years would appear to have sufficed for the emergence of several Pleistocene species. But for the several Upper Pleistocene species to have evolved in this way, separate ancestries at the Pithecanthropus level as well as an independent existence would have to be demonstrated.

Does the evidence really support this? It seems to me that both temporally and spatially the gaps between the "non-sapiens" and the "sapiens" forms have been very largely filled in and we now recognize a great deal of morphological overlapping between them. The existence of "intermediates" between Neandertal man and modern man and the occurrence of "sapiens" features in Rhodesian and Solo man have been set out in many papers and need no detailed documentation here. The continuity of resemblance between these forms is strikingly and

accurately expressed by the use of multivariate methods to specify degrees of affinity. A recent study of this kind, by B. Campbell and the author, (of the Swanscombe remains) has led us to a concept of a "spectrum" theory to describe the spatial and temporal continuity we now know to exist from Pithecanthropus onwards. On this concept the most reasonable view is that all forms of Homo, present or extinct, are referable to one species only (H.sapiens) and within this species we can recognize geographical and local subspeciation of varying degrees. What we recognize as classical neanderthal (and also as Rhodesian and Solo man) would represent geographical race formation of a distinctive but not absolute kind. A strong case for a particularly high degree of subspeciation for classical Neandertal under conditions of climatic and geographical isolation has been made out (Howell). Even so the existence of populations contemporary with Neandertal merging morphologically into more sapiens-like varieties (e.g.Krapina man) means that neandertal is more properly regarded as a constellation of certain characters occurring in high frequency in a particular region.

There has always been an extraordinary reluctance to accept that intrapopulation variability (and hence interpopulation overlap) in a polytypic species like Homo sapiens is necessarily (on genetic grounds) very great at all stages of its evolution. Le Gros Clark pointed out that within present day H. sapiens the range of size of the mandible is as great as that between so-called Meganthropus and Pithecanthropus. This point is strikingly borne out by two mandibles from the single Krapina site, which many would have regarded a priori as representing at least two different species! (see fig.)

The magnitude of the variability within modern sapiens was clearly demonstrated by Miss Tildesley many years ago. From her table it is clear that of 25 characters only one has a mean intrapopulation variance less than the variance of the interpopulation means. In other words the range within 'races' or populations is greater by a factor up to 200% than the range of the interpopulation means.

That the conventional specification of races or subspecies is based in reality on a more or less arbitrary selection of criteria can be illustrated by one character on which so much reliance is placed - skin colour. The Europenoid or Caucasoid race is usually contrasted with the Negriform on the basis amongst other things of an absolute difference in skin colour. The fact is that a high proportion of Caucasoids in South India and South Arabia are as dark skinned as many Africans, and conversely there are Negroid peoples who are as light coloured as some North African or Indian groups (fig.). I refer of course to the Bushmen and Hottentots who have for long been placed by the taxonomists into a subspecies or major race of their own - the Khoisaniform or Capoid (Coon). The genetic evidence shows clearly that they are undoubtedly variants of the Negro-African stock.

Adaptation

The widespread ecological distribution of H.sapiens even in the early hunting, subsistence stages poses many questions of adaptation and selection which are biologically more important and interesting than the exercises in race taxonomy with which physical anthropologists have occupied themselves so obsessionally for so long and with little benefit to the subject. The serious study of these questions is only at its beginning but already a number of far-reaching discoveries have been achieved, notably the demonstration of balanced polymorphism in the haemoglobin and blood group systems.

The analysis of adaptation and adaptability is a difficult pursuit not only in the quest for suitable field and laboratory data but also because it raises complex theoretical issues (cf Waddington, 'The Strategy of the Genes' 1957). Some of these are not perhaps as well appreciated as they deserve. In this connection two prime characters deserve special consideration - skin colour and bodily physique. Here it is possible only to discuss briefly the implications raised by variation in human skin colour.

Skin Colour

A sound understanding of the biological significance of differences in skin colour demands that many more detailed distribution studies using spectrophotometric methods (Weiner, 1951) should be carried out. No anthropological character has figured so much in race taxonomy yet the amount of reliable information is meagre in the extreme. Reflectance values have only recently been obtained for West Africans (Barnicot), South African Bantu, Hottentots, Bushmen, Central African Bantu (Weiner, Singer, Tobias, Harrison), for Europeans (Barnicot, Lebegue) and small samples of Asian Indians and South American Indians (Weiner et al). But even this is not sufficient for a detailed geographical comparison of the relation between exposure in contrasting habitats and the colour of untanned skin and of the tanning capacity. For example we do not know the exact shades and range of skin colour of the so-called Oceanic Negroes; it seems possible (Gajdusek, personal communication) that varieties of brown colour occur in New Guinea different from any in Africa. Similarly it is likely that many Congo pygmies are lighter in hue than is generally supposed (from coloured photographs supplied by Turnbull). We particularly need reflectance data on Amazonian Indians and, for comparison, on Eskimo and other Mongoloid groups.

Our knowledge of the adaptive and selective value of dark skin colour in regions of high ultra-violet intensity has been well reviewed by Harrison (1959). Special attention should be given to the investigations of Thomson (1951) and of Schirere (1958) as these provide important evidence of the protective value of deep melanin pigmentation against the

deleterious effects of ultra-violet radiation.

There is, however, a rather more profound problem implicit in the adaptive value of dark skin colour, in so far as this character falls into the category of pseudo-exogenous adaptations (Waddington). These are adaptations "in which the animal exhibits characteristics similar to effects which can be called forth as direct, exogenous (i.e. by the external environment) adaptations, but which on investigation are shown to be hereditary and independent of any particular environmental influence" (Waddington). Dark skin colour is of course a genetically determined character though we are by no means sure of the number of genes involved. Tanning of light skin by ultra-violet radiation mimicks this character and, as far as we know, confers very much the same benefit. What is the connection between the two categories of dark skin colour? Is this an example of Waddington's phenomenon of the genetic assimilation of adaptive characters? Direct proof is of course in this instance not possible but it is important to see whether a plausible prima facie case can be made in favour of this possibility. If genetic assimilation seems a likely explanation for the co-existence of 'genetic' and 'physiological' dark pigmentation (it is of course not the only possible explanation), it would also throw light on the widespread occurrence of dark skin colour quite possibly based on rather different genotypes.

'Tanning' is clearly the outcome of a capacity to respond to ultra-violet light. This capacity of adaptability is obviously developed to a variable degree in light-skinned groups. There is no difficulty in supposing that natural selection will operate to improve the capacity to respond to the environment. This improvement in capacity is an essential precondition for successful genetic assimilation. The optimum adaptive response involves the establishment of a canalized system or pathway of development. Following Waddington's argument this is a situation in which natural selection can act on many factors concerned in the epigenesis of the adaptive response, quite distinct from the specific genes concerned in the direct environmental effect. The general 'genetic background' will be altered. "There will then be two ways in which genetic fixation of the originally acquired adaptive character may take place. Either a gene-mutation occurs which suffices to direct development into the channel which has been prepared for it; or the remodelling of the epigenetic landscape goes so far that what was initially the side-valley, reached over a threshold, becomes the most easy path of change, so that one cannot point to any particular genes as being responsible for switching development into it."

In the case of dark skin colour, support for its genetic assimilation through an epigenetic process of the kind envisaged by Waddington may perhaps be found in a comparison of the darkening process of the new-born African (or Melanesian or Tamil Indian) with that accompanying ultra-violet irradiation of the light-skinned. A knowledge of the genetics of skin colour in many different populations is also necessary.

This example (which is of course far from conclusively argued) has been given only to illustrate that in the study of adaptation amongst living populations physical anthropologists have a rich and exciting field open to them.

NEW TECHNIQUES FOR SYSTEMATICS

A symposium organized for the
XVI International Congress of Zoology

by

Charles G. Sibley
Department of Zoology
Cornell University
Ithaca, New York

APPLICATION OF MODERN TECHNIQUES TO PROBLEMS IN THE SYSTEMATICS OF THE PROTOZOA.* John O. Corliss, Department of Zoology, University of Illinois, Urbana, Illinois, U.S.A.

No matter how we define or delimit "the Protozoa" we shall have difficulty recognizing exact boundaries of subgroups within the complex, at all taxonomic levels. Such problems do not seem to be so vexatious - at least at the crude stages of alpha and beta taxonomy - in many other animal groups. The reasons are clear: the majority of the 50-60,000 described species of protozoa (extant + extinct) are very small, requiring high-powered light microscopy for adequate examination; their bodily organization is subcellular in nature, thus presenting for comparative study no tissues or organs, only minute cytoplasmic organelles or, occasionally, secreted materials; few groups have left fossil records (tests or skeletal elements), and specimens so preserved are subject to the two preceding limitations. The generally cosmopolitan distribution of protozoa and the widespread lack of recognized sexuality in many groups constitute further impediments to progress in elucidation of problems in the systematics of these microorganisms (Corliss, 1960, Syst. Zool., 8: 169-190).

Because of obvious dependence on prior developments in the parallel field of microscopy, much of the taxonomic work in protozoology is still in the alpha or descriptive phase. Thus protozoan systematics, in this important respect, lags nearly a century behind the taxonomy of certain other animal groups (e.g., the vertebrates). I must emphasize this, for there are some biologists who seem to think that every protozoologist should be concerned solely with gamma taxonomy, carrying out elegant and refined studies on speciation and subspeciation oblivious of the far from solved, important, basic questions at the other levels of systematics which demand attention first.

The problems facing the systematist interested in the Protozoa are not especially novel. They require that he attempt recognition of presumed "degrees of affinity" among the organisms he is studying by use of whatever reliable data he can collect. There are essentially two main levels of interest in classification of protozoa today: one is concerned primarily with the lower taxa; the other, with only the ordinal or still higher groups. Protozoologists involved in work of the first kind ideally analyze extensive series of species, many of which probably they themselves have collected (possibly with attempts to culture) and preserved in the form of stained preparations. Workers interested in possible interrelationships at the top levels of the classificational hierarchy, on the other hand, may limit their studies - often of a speculative nature - to synthetic approaches, depending heavily on data available in publications of others. Fundamentally, however, in either type of work the same principles are ultimately involved and the requisite factual material is derived from the same basic analyses of anatomical and other characteristics of the organisms, or populations of organisms, being investigated.

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In order to focus our attention on techniques which may be utilized profitably in comparative taxonomic study of the Protozoa, I must first review - very briefly - the nature of the major protozoan features of value (or potential value) in such work. Handicapped by the lack of multicellular tissues or organ-systems in their organisms, protozoologists generally must fall back on more subtle anatomical variations, located either intra- or extracellularly, or on physiological differences, either broadly nutritional in nature or related to other functional aspects in the life cycle. Ecological features may be of considerable importance, especially in the case of symbiotic forms for which "host-parasite interrelationships" take on definite taxonomic significance. Understandably, however, comparative morphology has been and still is the mainstay of protozoan systematics; what is more, it is likely to remain so, in one guise or another, for many decades to come. Some ten major classes or categories of anatomical features of great usefulness in protozoology have recently been discussed elsewhere (Corliss, 1962, Symp. Soc. gen. Microbiol., 12: 37-67) and thus need not be repeated here: they range from non-living products of the organism to nuclear characteristics, from organelles of locomotion to components of the feeding apparatus. Utilizable non-morphological characteristics of protozoan species are less numerous. They include ecological considerations of all kinds, a broad spectrum of refined physical and biochemical properties, genetic, morphogenetic, and phylogenetic factors.

Problems in the systematics of the Protozoa demand attention from all three of the following approaches: detection or recognition of features of taxonomic value, which may involve development of new or improved techniques; proper analysis of such data, once they have been collected; and sensible incorporation of the results of such analyses into new concepts of group-interrelationships at the taxonomic level under consideration. The remainder of the present paper must be limited to the first of these three aspects, using lantern slides in illustration of points made; but, for the sake of completeness, allow me now to dispose very briefly of the second and third approaches mentioned, since all three are - in practice - inextricably involved.

Proper analysis of data rightly implies a statistical approach. I regret to say that protozoologists often have been guilty of neglecting such treatment of their material, even with regard to such simple procedures as determining means of body measurements. The use of populations of organisms rather than single - or only a few - isolated specimens is also to be encouraged. I must mention here the field of "numerical taxonomy" (Sneath & Sokal, 1962, Nature, 193: 855-860), the controversial concepts of which have so recently stirred the world of systematics. Application of the numerical or "neo-Adansonian" approach to determining affinity or similarity among taxonomic units is a subject of great interest: it will be receiving detailed attention in contributions of other speakers in the present Symposium. To my knowledge no one has yet specifically attempted to use the method

in protozoological taxonomic work, although very recently a noted protozoologist has ardently advocated adoption of some sort of "computer system of taxonomy" for all animal and plant groups (Jahn, 1962, J. Parasit., 48: 656-663).

The matter of conceptual treatment of taxonomic problems is one of perhaps greater importance in revisory work than is commonly supposed. Reinterpretation of "old" data has been responsible for a number of sound changes in protozoan schemes of classification. True, such theoretical work has frequently combined new treatment of "old" facts with careful consideration of new information derived from fresh analytical approaches. But my point is this: new techniques and new data are not solely responsible for advances in systematics; in fact, new ideas may be of far greater significance, especially at levels above the categories of species and genera.

When one mentions "new techniques for systematics" one thinks primarily of new methods for obtaining data, data which are either different from or similar to those obtainable by procedures conventionally employed in the past. In protozoology such new techniques fall roughly into two groups: those already successfully in use (although still relatively "new"), on a greater or lesser scale, and those of theoretical interest and potential importance but of very limited application to date. I must restrict my remarks primarily to examples of the first group; and even here I must be highly selective.

Many modern cytological techniques can be employed to advantage in study of protozoa. Those primarily of a refined cytochemical or biophysical nature, however, are seldom of value from a taxonomic point of view. Certain of the precise methods for studying nuclei (including the indispensable Feulgen nucleal reaction, too seldom employed by taxonomists) are of potential usefulness in the gamma stage of systematics and occasionally at the highest levels of classification - e.g., in consideration of the origin of nuclear dualism in the ciliate Protozoa (Raikov, 1963, Proc. 1st int. Conf. Protozool., Prague, 1961, in press) - but rarely have they been so applied to date. The relatively recent application of cytological methods of silver impregnation, on the other hand, has had far-reaching consequences in major areas of taxonomic protozoology; thus these techniques warrant our further attention.

Although the exact chemistry of the reaction is not perfectly known, a number of protozoan organelles in species belonging to diverse taxonomic groups consistently appear to be impregnated with silver after exposure to a solution of silver nitrate or silver proteinate which subsequently has been reduced by ultraviolet light or by such a chemical as hydroquinone. Few of these so-called argentophilic structures represent new anatomical discoveries, but their revelation by methods of silver impregnation is so complete, so striking, and so convenient that they have rapidly been accepted as characteristics of nearly indispensable value in the systematics of the groups concerned. Also, by

virtue of the constancy of appearance of these sometimes very minute organelles throughout populations of organisms belonging to many species, they have come to be considered as "fundamental" features within the groups which possess them. Thus such features are now valued in phylogenetic considerations as well as in less speculative areas of taxonomy such as construction of practical schemes of classification. Finally, the availability of such techniques has permitted tracing the fate of the argentophilic organelles throughout entire life cycles: therefore such neatly revealed structures have become of significance in the field of morphogenesis, as well as in classification and phylogeny.

Most popular for the parasitic or symbiotic flagellate Protozoa is the Bodian Protargol silver technique (Honigberg & Davenport, 1954, Stain Tech., 29: 241-246). It reveals with particular clarity the several elements of the so-called mastigont system: flagella, basal bodies (singly or as components of the blepharoplast-complex), undulating membrane, axostyle, costa or cresta, nuclei, parabasal body, the kinetoplast of trypanosomes, and other even more inconspicuous structures, some of which are discoverable only by use of this technique. Differences in sizes, shapes, numbers, and position (as well as presence or absence) of these organelles can now be determined with the greatest precision. Thus the characteristics so revealed are collectively of wide utility at both lower and higher taxonomic levels within the hierarchy of the Zoomastigophora.

In the case of ciliate Protozoa the stable complex known as the infraciliature has, in recent years, been recognized for its fundamental worth in systematics, primarily because of the ease in revelation of its principal constituent, the ciliary basal bodies, through use of methods of silver impregnation. Leading variations of ciliate silver techniques include the Klein "dry" (no preliminary fixative used) method (Klein, 1958, J. Protozool., 5: 99-103), the Chatton-Lwoff refinement (fixatives used and organisms embedded in gelatin: Corliss 1953, Stain Tech., 28: 97-100), and - again - the Bodian Protargol method (Dragesco, 1962, Bull. Micr. appl., 11: 49-58). The wealth of anatomical details available from both the buccal and somatic infraciliary systems and from elements of the entire "Silberliniensystem" (only part of which is shared in common with the infraciliature sensu stricto) can be analyzed most profitably in comparative investigations at all taxonomic levels. The important morphogenetic phenomenon of stomatogenesis can be investigated with similar precision. With the Protargol technique the intact external ciliature is also revealed, as well as certain subpellicular fibrillar systems and the nuclei. The Chatton-Lwoff method, more popularly used at the present time (although even it is far too often neglected by ciliate protozoologists whose work would benefit by their adoption of it), may be successfully applied to parasitic as well as free-living forms, to encysted as well as trophic stages in life cycles, to marine or brackish species as well as fresh-water fauna.

Incidentally, for protozoologists interested in designating type-specimens - an action seldom taken to date for Protozoa (but see Corliss, 1963, J. Protozool., 10, in press; and Weiser, 1963, Proc. 1st int. Conf. Protozool., Prague, 1961, in press) - the permanent preparations resulting from the use of the silver methods cited above are ideal.

I have mentioned that the development of much of the broad field of protozoology has been dependent on that of the science of microscopy. Of several major improvements or additions to the latter field in recent years, two are contributing significantly to the betterment of taxonomic protozoology. Phase optics has now become standard equipment in the protozoologist's laboratory: the ease with which cilia, flagella, fine pseudopodia, stalks, and fibrils can be seen will greatly increase the inclusion of such organelles in future systematic studies of a comparative nature. Other structures ordinarily invisible, or practically so, because of only slight density differences are also revealed to advantage. Phase microscopy allows a profitable revival of the study of living material, an old practice of much merit too often abandoned by modern workers.

Even more recently the advent of electron microscopy has had a tremendous impact on many areas of protozoology, with taxonomy obviously being one of these. The higher resolution of the electron microscope, once the difficult problems of fixation, embedding, sectioning, etc., have been successfully surmounted for the material under investigation, makes possible much more detailed and refined comparative studies of the subcellular organization of the protozoan body. The data so made available are often directly applicable to taxonomic problems at various levels in the classificational hierarchy. Furthermore, entirely new organelles may be discovered, structures potentially of considerable value in both "practical" systematic work and in problems of phylogeny. Examples of this have recently come to light in ultrastructural investigations of a number of parasitic species belonging to groups whose taxonomic affinities have always been difficult to determine or even suppose. In such genera as Plasmodium, Haemamoeba, Laverania, Lankesterella, Sarcocystis, and Toxoplasma the following newly discovered organelles have been found in common: a conoid or apical ring at the anterior end of the body; a system of convoluted tubules, extending posteriad through the cytoplasm from the anterior end; and a micropyle, a minute lateral pore located along the body wall some distance from the anterior end. Thus these forms are all certainly "good" Sporozoa (Garnham et al., 1963, Trans. Roy. Soc. Trop. Med. Hyg., 57: 27-31).

I have singled out only a few modern techniques for direct inclusion in this paper: I wish to stress that various "old" techniques, whether or not modified in more recent times, are still very serviceable in protozoan taxonomy, especially if the data so made obtainable are treated properly. Also I should mention again that a number of new methods for studying protozoa which

are excellent are not particularly applicable to problems in systematics. Refined investigations of biochemical attributes (nutritional requirements, metabolic pathways, products of biosynthesis: Holz et al., 1963, in Leone, ed., Taxonomic Biochemistry and Serology, Ronald Press, N. Y., in press) of many species fit into this last category, although valiant attempts are being made by some workers to apply analyses of such data meaningfully to taxonomic questions. Theoretically it is not difficult to envision advantages of a biochemical approach, but practical results of usefulness available to date are sparse. The use of differences in DNA base composition, now determinable with facility by CsCl density gradient centrifugation or from comparative thermal denaturation temperatures, offers an attractive approach (Schildkraut et al., 1962, Nature, 196: 795-796). How promising it will really be, however, depends on the success of extensive investigations involving a large number of protozoa. Both closely related and taxonomically widely separated species must be included to establish a fair test of the technique's utility in systematics.

Finally, I should like to mention the genetic approach to problems in the taxonomy of Protozoa. The greatest barrier to widespread profitable application of such an approach is the extremely common absence of observable phenomena of sexuality among these microorganisms, pointing up the painful taxonomic problem of the nature of a species among predominantly asexually reproducing forms (Sonneborn, 1957, in Mayr, ed., The Species Problem, AAAS Pub., Wash., D.C., pp. 155-324). A second frustrating difficulty is our inability to handle most of the protozoa which do show sex (e.g., the Sporozoa) according to the rigid laboratory procedures requisite to proper genetic studies. The diploid (micronucleus) Ciliophora have served as the most favorable material to date, although even here only a few species have lent themselves to truly refined work which is of any significance at the lowest taxonomic levels. Essentially all problems of gamma taxonomy (population dynamics, subspeciation, etc.) among the Protozoa must await further improvement in culturing the organisms under controlled conditions and further knowledge than we now possess concerning their modes of sexuality, their physiological life cycles, and their cytogenetics in general.

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The Use of Hybridization in the Determination of Phylogenetic Relationships

by
Clark Hubbs
Department of Zoology, The University of Texas

Many methods have been used to ascertain phylogenetic relationships and new scientific techniques are often quickly applied to systematic zoology. The additional information is often extremely valuable in working out problems of relationships: however, many of the modern studies can be affected by convergence and parallel evolution, problems that have long disturbed morphologic taxonomists. For instance similar food sources might lead to similar body chemistry, etc. These complications may occur, but are less likely, in studies using hybridization success as an index of phylogenetic relationship.

Interspecific hybridization has long been known in animals (Hertwig, 1936). Most studies have shown that closely related species can be crossed much easier than either can be hybridized with more distantly related species (Patterson and Stone, 1952; Hubbs, 1955; Nikoljukin, 1952, etc.). Some of the hybrids are known to be totally or partly fertile (Kosswig, 1961; Duyvené de Wit, 1960, etc.) and are generally considered to have parents that are closely related. Many other kinds of hybrids can attain the size of their parental forms, but are sterile; likewise, most workers consider the parental species to belong to the same or closely related genera. In more distantly related crosses, the resulting offspring succumb at earlier and earlier ontogenetic stages as the parental phylogenetic relationship becomes more distant. Suzuki (1957 a, b, and c) exposed fish eggs to sperm from males with various degrees of relationship and obtained extremely close correlation of degree of development from interclass (none) to interspecific (mature size) hybrids.

There are several dangers involved in the utilization of hybridization success as an index to phylogenetic relationship. Perhaps the most universal is the possibility of gynogenetic development (Moore, 1955). Obviously this can be avoided by the discovery of the effects of paternal chromatin in the hybrid. In crosses between distantly related animals the hybrids often die as blastulas and the functional presence of paternal chromatin is difficult or impossible to demonstrate before gastrulation. Likewise, the morphological and developmental differences tend to become progressively less with increasingly closer relationship so that some of the problems are retained in crosses between rather closely related animals.

Another possible complication involves reinforcement of isolating mechanisms (Dobzhansky, 1940). The difficulty in rearing hybrids between two species of treefrogs (Johnson, 1961) does not necessarily indicate distant relationship, because there is little question that hybrid inviability is an effective isolation mechanism between these sibling species. Other members of the genus Hyla will cross with both members of the genetically incompatible species pair.

Differences between the survival of reciprocal matings can also cause difficulties. Interspecies group hybrids involving Bufo americanus group females generally hatch and the reciprocals seldom gastrulate (Thornton, 1955). Hybrids involving members of the Fundulus majalis species group have a similar difference between reciprocal matings except that it is the males that can be hybridized (Newman, 1914). Obviously the one sex is no more distantly related than the other to a second species.

It is obvious that the general level of hybridization success is associated with the degree of phylogenetic relationship. Moreover, the ontogenetic levels of potential survival are common to many multicellular animals. Therefore, it is possible that a comparative phylogeny could be based on hybridization studies. It is unfortunate, however, that the available evidence

indicates that certain animal groups are more readily hybridized than are others. For instance mammalian hybrids appear to be rarer than avian hybrids. Similarily Patterson and Stone (1952) have reported that most intrageneric Drosophila crosses do not survive to maturity. At the other extreme many hybrids between closely related fish genera are known to occur occasionally in nature (Hubbs, 1955), and interfamilial hybrids have been produced repeatedly by artificial hybridization. It is inconcievable that the hybridization capacities are the same and that all of the differences in taxonomic ranking of the parents of successful hybrids involves different degrees in lumping and splitting of higher taxa. In addition, the differences may well involve the complexities of courtship and the internal environment for sperm in females of internal fertilizing species (Patterson, 1946). Although these factors may often by paramount, they can not account for other differential hybridization capacities such as the low success of hybrids in the genus Rana (Moore, 1949, and others) compared with the relatively high survival of other anuran hybrids in the genera Bufo (Blair, 1961) and Pseudacris (Littlejohn, 1961). A similar difference in hybridization capacity occurs between the relatively easily hybridized genus Fundulus (Hubbs and Drewry, 1960) and the much more difficult to hybridize genus Crenichthys (Hubbs and Drewry, 1962) which belong to the same fish family. Minamori's (1957, etc.) studies on cobitids indicate that similar problems occur among these Japanese fishes.

Despite the above complexities, there is no question that hybridization potential can be used to indicate phylogenetic relationships especially when the data conform to conclusions obtained from studies using other systematic techniques. It is possible that other modern techniques will permit the application of hybridization data to closely related species. Wasserman (1960) has shown that the degree of chromosomal synapse in Drosophila hybrids correlates with phylogeny. Similar studies on other organisms maybe exceedingly useful, especially in forms with chromosome pairing during mitosis so that the hybrids do not have to be reared to maturity before the technique can be applied.

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IMPORTANCE OF BREEDING CALL IN AMPHIBIAN SYSTEMATICS

William E. Duellman
Museum of Natural History
University of Kansas

Within the last fifteen years the development of acoustical recording and analyzing devices has provided biologists with the means to make quantitative studies of animal sounds as they pertain to behavior, ecology, and systematics. Insofar as systematics is concerned, the groups that have received the most attention are Homoptera, Orthoptera, Aves, and anuran amphibians.

Techniques

The techniques used in the study of animal sounds consist of the recording of the sounds on magnetic tape by means of a tape recorder in the field or in the laboratory. In the laboratory tapes can be played back on a recorder equiped with an amplifier, and the signals can be transferred to a spectrographic analyzer, such as a Vibralyzer or Sonagraph. By the use of these machines, sounds can be analyzed with respect to time, pitch, and intensity. The resulting spectrograph is a visual impression of the sound.

The sounds produced by most amphibians and insects consist of rather simple notes or groups of notes, as compared with the vocal abilities of birds to produce sounds of variable pitch, resulting in an array of melodies. Some frogs have a call consisting of a group of notes; such a series of notes is known as a call-group. Other species produce only a single short note, whereas others have a long continuous note or trill, such as that produced by most toads of the genus Bufo. Various components of the calls can be analyzed, as follows: 1.) Note repetition rate — the rate at which notes of a call-group containing two or more notes are produced; this is measured in notes per minute. 2.) Call rate — the rate at which call-groups are produced; this also is measured in calls per minute. 3.) Trill rate or pulse rate — the frequency in time at which pulses of a note are produced; this is measured in pulses per second. 4.) Duration — the actual length of the note or call, measured in seconds. 5.) Frequency range — the lowest and highest frequencies of the call; this is measured in cycles per second. 6.) Fundamental frequency — that frequency of sound resulting from air passing over the vocal cords and causing them to vibrate at a frequency primarily dependent on the tension of the vocal cords; this is measured in cycles per second. 7.) Dominant frequency — that frequency resulting from the resonating of the fundamental frequency or one of its harmonics with greater emphasis than any other frequency; this is measured in cycles per second. 8.) Intensity — loudness of the call; this can be measured in decibels on a sound level meter.

Function of the Breeding Call

Some of the sounds made by animals are not associated with breeding. Except for echo-location in bats, the sounds produced by mammals apparently are chiefly territorial in function. Little is known of the function of sounds produced by fishes. Many frogs and toads have warning calls and distress calls that are not associated with courtship. Field observations and experimental evidence show that in the Orthoptera and in anuran amphibians females usually are attracted by the calls of the males of their own species. The calling male stimulates other males of the same species, and in many frogs and toads the calls serve to attract other males to the breeding site. The situation is more complex in birds, for the function of the call in attracting females also is of territorial significance in repelling other males. Of primary concern to the systematist are those sounds produced by animals at the time of courtship and breeding. In this respect, the breeding calls of anuran amphibians have been shown to be important ethological isolating mechanisms.

Use of Breeding Calls in Species Recognition

From our still rather limited knowledge of the breeding calls of amphibians it seems apparent that each species has a distinctive breeding call that differs from the breeding calls of, at least, sympatric species. Consequently, with a little practise the biologist can learn to recognize the species by means of their breeding calls. Of greater importance is the biological significance of the breeding calls.

Although some frogs are solitary callers, most frogs and toads characteristically congregate at ponds and streams to breed. The males arrive at the breeding sites first; their calls attract other males and the females. Auditory orientation has been studied in several species, and the results of these studies show that the breeding call is important in attracting sexually aroused males and receptive females to the breeding site. Experimental studies have shown that females of a given species have a positive response to the calls of males of their own species and an indifferent response to the calls of other species. These responses have been observed in frogs in which the calls are nearly indistinguishable to the human ear. Thus, the discriminatory ability of some frogs is well developed.

Sympatric and synchronic breeding in frogs and toads is a common phenomenon. A dozen or more species sometimes breed in the same pond at the same time. In such situations various courtship isolating mechanisms are in operation. Of these, mechanical isolation or structural differences prevent interbreeding in some; size apparently is the most important structural difference in frogs. The calling or breeding location at the breeding site serves to isolate some species, but the most

significant factor which serves to isolate most species of frogs is the breeding call. Sympatric and synchronic breeders have calls that differ in various ways. Significant differences have been found in the range of frequency (pitch), including variation in the dominant and fundamental frequencies, trill or pulse rate, note repetition rate, and duration.

Differences in call sometimes can be attributed to obvious structural differences in the species. Frogs having internal vocal sacs, which usually are relatively small and lack the distensibility of external vocal sacs, resonate relatively low frequencies. In many toads of the genus Bufo there is a correlation between the size of the toad and the frequency and trill rate of the call. In large toads the trill rate is slow, and the frequency is low; by comparison, smaller toads have a faster trill rate at a higher frequency.

In sympatric and synchronic breeders isolating mechanisms sometimes do break down, resulting in hybridization. In hybrids of Scaphiopus, Bufo, and Hyla that have been studied, the calls are intermediate between those of the parental species. In some characteristics the calls may more closely resemble those of one parent, or they may differ from the calls of both parents. The evidence gained from the calls of hybrids and from observations on isolated frogs raised in the laboratory indicate that the breeding calls are inherited and not learned.

Use of Breeding Calls in Showing Relationships

In studies of interspecific relationships, characteristics of the breeding calls can be used in conjunction with morphological characters and information pertaining to ecology and behavior. At the present time insufficient data is available to determine what, if any, geographic variation occurs in breeding calls. In some species, Hyla versicolor for example, two kinds of breeding calls are known. Experimental hybridization of the two call-types from Texas resulted in abnormally low development, indicating genetic incompatibility between the call-types. Further investigations are necessary to determine whether these call-types are indicative of individual populational differences in breeding call without respect to geographic distribution, or whether Hyla versicolor, as now recognized, consists of two sibling species differing in breeding call and possibly other characters yet to be discovered, as indicated by the results of experimental hybridization of the two call-types in Texas.

Related species of frogs usually have similar breeding calls, and differences seem to be most distinct in sympatric species. In the Hyla eximia group in the Mexican and northern Central American highlands, the calls of the various species differ mainly in duration and repetition rate. All of the calls consist of series of notes; in some species there are a few long notes per call-

group; in others, many short notes. Apparently Hyla eximia has two call-types; possibly two sibling species have been confused under this name. The Middle American hylid genus Ptychohyla is composed of two groups of species. The breeding calls of the three allopatric species in the Ptychohyla euthysanota group consist of a single long note. Interspecific differences are found in the frequency ranges and pulse rates. The breeding calls of the two allopatric species in the Ptychohyla schmidtorum group consist of a series of short notes; specific differences occur in call rate and frequency. Data obtained from the breeding calls of Ptychohyla substantiate the systematic conclusions resulting from morphological and ecological evidence. Throughout most of the range of the genus one species in the euthysanota group occurs sympatrically with a species in the schmidtorum group. The sympatric species breed synchronously, and there is no evidence of hybridization.

In the Hyla microcephala group composed of four species having a combined range from southern Mexico to Colombia, all species have a breeding call consisting of a series of short squeeks. The primary differences in the calls of the various species are in the positions of the dominant and fundamental frequencies. Of the four species in this group, only two are partially sympatric — Hyla phlebodes and Hyla microcephala in Panamá. The breeding calls of these two species are more different than the calls of the other allopatric species.

In frogs of the hylid genus Smilisca pulse rate and note repetition rate apparently are the primary differences in the breeding calls. The greatest differences occur in sympatric species.

From the evidence available from the few groups of frogs and toads that have been studied, it is apparent that various qualities and quantities of the breeding calls are of systematic importance in different groups. This suggests that positive responses by females are initiated by different qualities in the calls in different species.

In some cases a knowledge of breeding calls was the first clue to the relationships of a species. For example, until recently Hyla arenicolor was thought to range from southern Utah and Colorado southward on the Mexican Plateau with a disjunct population west of the Colorado Desert in California. The breeding call of the population in California provided evidence that this population is different from the eastern population. Further investigations revealed morphological differences to substantiate the differences in breeding calls. As a result, the population in California is now recognized as a separate species. Similar results have been obtained from studies on the Australian leptodactylid frogs of the genus Crinia.

Problems in Using Breeding Calls

At the present time an adequate number of recordings of the breeding calls is available for few species of frogs. Consequently, investigators wishing to use characters of breeding calls in systematic studies of a group must devote a considerable amount of time in the field to obtain the recordings. Obtaining a good series of recordings often is difficult, for after the calling frogs are located, it is necessary to record isolated individuals. Recordings containing several individuals of the same or different species in the background often are not suitable for certain kinds of analyses. Recording equipment, as now available, is fallible, especially under field conditions, where humidity may affect the speed of the tape.

Frogs are ectothermal animals whose metabolism and therefore breeding calls vary with temperature. Limited investigations have shown that call rate, pulse rate, and pitch vary with temperature. Consequently, differences in temperature have to be taken into consideration when comparing calls of different individuals or different species.

At the present time little is known about phonoreception in frogs. In order to determine what components of the breeding call are significant in attracting females and thereby acting as isolating mechanisms, extensive studies on call discrimination are needed.

Although in sympatric species selective pressures may operate more intensively on courtship isolating mechanisms, such as the breeding calls, than on certain morphological characters not directly associated with reproduction, similarities in breeding calls can develop in allopatric species through parallelism and convergence. Therefore, in the absence of other criteria, similarities in breeding calls should be used with caution in attempting to determine relationships. In using breeding calls as systematic characters it should be kept in mind that the breeding calls, like other characters, are subject to individual and probably geographic variation. Used in conjunction with ecological and geographical information and behavioral, biochemical, and conventional morphological characters, breeding calls are extremely useful in determining phylogenetic relationships.

THE APPLICATION OF CHROMOSOMAL ANALYSIS TO TAXONOMY OF SOME NORTH AMERICAN SCIURIDAE

CHARLES F. NADLER
Northwestern University Medical School, Chicago, Illinois

The study of mammalian chromosomes in the past was based chiefly on serial sections of testis. Evaluation of chromosome number and morphology by this technique was time consuming and often difficult due to inadequate dispersal of chromosomes within the cytoplasm. Advances in cytologic technique, since 1952, have provided improved methods for the study of somatic cells in metaphase. These cells contain well dispersed chromosomes suitable for determining the diploid number. Current methods also have the advantage of enhancing the structural detail of the chromosomes, thus facilitating morphologic analysis.

Application of cytologic data to animal taxonomy has been most successful among insects, especially Drosophila. Comparison of chromosomal patterns has clarified relationships between some species and aided in detection of sibling species of Drosophila and other insects. In contrast, chromosomal data have not been utilized as extensively in mammalian taxonomy. Some investigators, especially Matthey and Makino, have done excellent work in describing and compiling chromosome numbers of many mammalian genera but these data have not been utilized extensively by taxonomists. Recently chromosomal patterns or karyotypes have been reported in detail from humans, mice, hamsters and primates but, except for the latter mammals most of this information has not been applied to taxonomy.

The purpose of this paper is to review recent techniques for mammalian chromosome analysis with special emphasis on their application to taxonomy. Findings obtained during chromosomal investigations of some North American Sciuridae (Eutamias, Tamias, and Spermophilus) will be discussed to demonstrate the applicability of chromosomal data to mammalian taxonomy.

Methodology. Various tissues may be used for chromosome preparations. Bone marrow is the tissue of choice for studying most small mammals because of its high mitotic index. A disadvantage is that the animal must be killed to obtain the marrow. When marrow can not be used, tissue from the kidney or skin may be grown in tissue culture to provide cells for chromosome analysis. Human chromosomes are now commonly analyzed from cultures of peripheral blood.

Three technical advances have provided the basis for current methods of chromosomal analysis. First, hypotonic solutions were used to swell cells and disperse the chromosomes in the cytoplasm (Hsu 1952). Second, when cells were treated with colchicine, or its derivatives, and hypotonic solutions even better chromosome preparations were obtained (Tjio and Levan, 1956 and Ford and Hamerton, 1956). Colchicine inhibits spindle formation, arrests cell division in metaphase and aids in the

dispersal of chromosomes. Furthermore, colchicine causes contraction of the chromosomes and divergence of the sister chromatids from their point of common attachment at the centromere. The latter effect is important because it permits accurate determination of chromosome morphology based on centromere position and arm lengths. Lastly, various workers introduced techniques that provided preparations with chromosomes lying in a single plane. These procedures consisted of either squashing the cells between a slide and cover glass or air drying a cell suspension on a slide. These three advances not only aided the task of counting chromosomes but made detailed morphologic study possible. All current methods also require cellular fixation, usually with 1:3 acetic-alcohol, and staining with either acetic-orcein stain or the Feulgen reaction.

A simple procedure for analyzing chromosomes from the bone marrow of rodents will be described in detail to illustrate the use of the newer cytologic techniques. The entire procedure takes 5 hours and does not require tissue culture to obtain dividing cells. Animals are injected intraperitoneally with 0.05% colchicine or colcemide at a dose of 0.01 ml/gm body wt. One and a half hours after injection the animals are killed, the femurs removed and the femoral epiphyses amputated. A needle with trochar that nearly fills the marrow space is inserted through the marrow cavity. The extruded marrow is grasped with forceps and transferred to a small petri dish containing 1% sodium citrate. Then the marrow is cut into 1-2mm pieces and left in the hypotonic citrate for 20 min. at room temperature. Using forceps, the pieces of marrow are next transferred to a watch glass containing a stain-fixative made of one part 1NHCL and 9 parts acetic-orcein. (The stain is made by dissolving 2 grams of synthetic orcein in 45cc of hot glacial acetic acid, cooling to room temperature and then adding 55cc of distilled water. The stain is filtered before each use.) The watch glass, containing marrow and stain-fixative, is heated gently, but not allowed to boil, and then allowed to cool to room temperature for 30-60 minutes. Finally, a piece of marrow is placed on a slide, a drop or two of acetic orcein stain is added and a cover glass is gently lowered over the tissue. Using the point of a blunt lead pencil, the cover glass is tapped over the tissue to disperse the cells. Excess stain is removed by blotting gently with filter paper. The cells are then squashed by exerting firm thumb pressure on filter paper overlying the cover glass. The preparations are made permanent with the dry-ice method (Conger and Fairchild, 1953). Ten or 15 slides per animal will provide about 40 cells for chromosome counts and 5-10 suitable for morphologic study. Cells with well spread chromosomes are counted at 970x. Some variation about the modal chromosome number will be noted when counts are obtained from squash preparations. Usually 85 to 95% of the cells contain the diploid number while the remainder have lower counts due to cell rupture

and chromosome loss during squashing. In contrast, the air drying procedure for cell flattening results in less variation in chromosome counts because cell rupture is infrequent. Photomicrographs of the best cells are used to study chromosome morphology and determine the animal's karyotype. Chromosomes are cut from enlarged photographs and arranged in pairs according to size and centromere position. Chromosomes are called metacentric when the centromere is located in a median position, submetacentric when the centromere is between the middle and end of a chromosome and acrocentric when the centromere is terminally located.

Chromosomes in chipmunk (Eutamias and Tamias) taxonomy.

The author has analyzed chromosomes from 7 of the 16 species belonging to genus Eutamias. Fifty-two animals were studied and all had a diploid chromosome number of 38. A total of 1600 bone marrow cells were counted. Analysis of 205 karyotypes from the 52 chipmunks demonstrated two types of chromosomal patterns. The first pattern found only among the karyotypes of E. minimus (11 specimens), was characterized by 4 pairs of metacentric chromosomes, 6 pairs of submetacentrics, 8 pairs of acrocentrics, a large submetacentric X and a small dot-like Y chromosome. The second chromosomal pattern differed from that of E. minimus and was found in all 41 specimens of E. quadrivittatus, E. umbrinus, E. speciosus, E. amoenus, E. townsendii and E. dorsalis. Karyotypes of these species contained 5 pairs of metacentric chromosomes, 6 pairs of submetacentrics, 7 pairs of acrocentrics, a large submetacentric X and a small dot-like Y chromosome. The fact that the diploid number was always 38 and the karyotype did not vary within an individual or species suggests that chromosomal characters of chipmunks are probably stable and may be of taxonomic value. Studies of pelage, and external and cranial morphology enabled Howell (1928) to identify individual species and he described 5 species groups of chipmunks. Later, White (1953) studied the baculum (os penis) of Eutamias and made the significant discovery that each chipmunk species had a baculum of characteristic morphology. Three major types of bacula were present and White therefore concluded there were only 3 species groups in Eutamias. Chromosomes were analyzed from species belonging to all 3 of White's species groups and 4 of the 5 groups proposed by Howell. Comparison of chromosomal characters with those used by Howell and White indicates that chromosomal characters will differentiate only E. minimus from the other species studied. Therefore the baculum, external and cranial characters are more useful to the taxonomist for identifying species of Eutamias. However, Howell and White, using these characters, arrived at differing conclusions regarding the number of species groups and the species comprising them. It is of interest that among the 7 species of Eutamias studied by the author, only 2 chromosomal patterns could be identified. These two "chromosomal species groups" differ from the groups of either Howell or White.

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To further explore chromosomal characters at the supraspecific level, 5 specimens of Tamius striatus from the second, monotypic chipmunk genus Tamias were studied. All had a diploid number of 38 and a karyotype containing 5 pairs of metacentrics, 6 pairs of submetacentrics, 7 pairs of acrocentrics, a large submetacentric X and a small dot-like Y chromosome. Although the karyotype of T. striatus closely resembled that seen in E. quadrivittatus, umbrinus, speciosus, dorsalis, townsendii and amoenus there were definite differences in the size of certain chromosome pairs. Comparison of the two chromosome patterns seen in Eutamias with that seen in Tamias suggests that the 3 patterns are about equally related. Chromosomal characters are therefore in agreement with the conclusions by Howell, White and Moore (1959) that there is a close relationship between Tamias and Eutamias. In brief, chromosomal characters do not replace other characters used in chipmunk taxonomy. Nevertheless, chromosomes provide a new approach to the study of supraspecific relationships which have not been completely clarified by existing characters.

Chromosomes and taxonomy of ground squirrels (Spermophilis)

Chromosomes have been analyzed from 5 of 8 species of ground squirrel belonging to the subgenus Spermophilus (Citellus). These animals, in contrast to chipmunks, exhibited variation in both chromosome number and karyotype among species. The diploid number was 30 in S. beldingi beldingi, 32 in S. columbianus columbianus and 34 in S. armatus and S. richardsonii elegans. Chromosome counts from a single specimen of S. townsendii indicated a probable diploid number of 46. Comparison of karyotypes revealed an interesting difference in the Y chromosome structure. S. beldingi and S. columbianus each had a minute metacentric Y chromosome while S. armatus had a small acrocentric Y, and S. richardsonii had a larger acrocentric Y chromosome. The Y chromosome was not definitely identified in S. townsendii. Another striking feature of the karyotypes was the absence of acrocentric autosomes in S. beldingi, columbianus, armatus, and richardsonii. In contrast, the karyotype of S. townsendii contained 10 or 11 pairs of acrocentric autosomes. Among the above species of Spermophilus both chromosome number and morphology appeared to be stable characters suitable for species identification. A positive correlation was noted between higher diploid numbers and greater numbers of acrocentric chromosomes. This observation may possibly be explained by a mechanism of karyotype alteration called centric fusion. Centric fusion, a well known process in karyotype evolution of insects, produces a metacentric chromosome by fusion of two acrocentrics. A small fragment containing a centromere is also a product of centric fusion and is usually lost during subsequent cell divisions. If this mechanism was operating, the karyotype of S. townsendii with a high diploid number and many acrocentrics, would be more ancestral than the karyotype of the other species examined. Support in favor of the centric fusion mechanism is suggested by

the fact that S. beldingi and S. columbianus have low diploid numbers, no acrocentrics and have minute metacentric Y chromosomes. The Y chromosomes may be the retained fragments usually lost after fusion.

Cranial and pelage characters currently used in ground squirrel taxonomy accurately differentiate the species of Spermophilus. However, chromosomes also provide apparently reliable characters for species identification. Chromosomal characters may, in addition, provide a basis for determining phylogenetic relationships in the subgenus Spermophilus.

Conclusions. Current methods of chromosome analysis have the advantage of speed, reliability and simplicity. They provide two taxonomic characters, diploid number and chromosome morphology, that appear to be consistent in individual chipmunks and ground squirrels. No chromosomal variability was found within a species, although the samples were small.

Certain problems pertinent to mammalian cytotaxonomy can not be resolved by present methods. Mammalian mitotic chromosomes, unlike those of Drosophila can not be analyzed for intrachromosomal structure or chromomere pattern. Inversions, duplications or translocations are not easily detected, although they may be occasionally suspected by gross change in chromosome morphology. This disadvantage in methodology is apparent especially in the case of different species with identical karyotypes. Intrachromosomal analysis or visualization of chromomeres might permit identification of such species as well as clarify karyotype evolution.

Taxonomic characters may be used for identification of a specimen. However, to achieve this purpose, characters should be consistent for all members of a category. The chromosomal characters described for Eutamias, Tamias and Spermophilus appear to fulfill the criterion of stability. Characters may also indicate relationships between categories and the use of chromosomal characters to determine such relationships among some Sciuridae has been discussed. Application of chromosomal characters to the taxonomy of other mammalian genera seems warranted.

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THE USE OF CARTESIAN COORDINATES FOR COMPARING RELATED FORMS.

Ronald Strahan, Dept. of Zoology, University of New South Wales, Kensington, N.S.W., Australia.

Although numerous other criteria may be used, most taxonomic decisions are based upon morphology. However, due to the difficulty of dealing with complex shapes and relations in ordinary language, the taxonomist is often unable to describe those morphological differences which, in his opinion, are most significant. For the limited purpose of identification or construction of keys this is often unnecessary, since other simpler correlates such as ratios of lengths of parts of the body may be sufficiently unique. Nevertheless, reliance upon such characters may obscure the evolutionary relationships which it is the function of taxonomy to display.

Faced with the difficulty of communication by words, the taxonomist may seek other means. D'Arcy Thompson, in his volume, "On Growth and Form", drew attention to the usefulness of Cartesian coordinate systems for describing a two-dimensional shape but for most purposes, of course, a photograph or drawing provides an equally useful record. Cartesian coordinates are superior to simple pictures, however, when the shapes of two or more forms are to be compared. If the aim is to describe the shapes in mathematical terms, each may be inscribed within a standard rectangular coordinate system, and the differences between them represented numerically by different values of the equivalent coordinates. If, on the other hand, one is interested in the relations between the shapes, the rectangular grid fitting one of them may be deformed so as to fit the others, and the degree of deformation then gives a measure and a description of the differences in shape.

Such coordinate grids may also be used, as Thompson pointed out, to interpolate any number of stages intermediate between two related forms, thereby setting out the geometrically simplest path whereby one shape may be transformed into the other. Thompson used this technique to set out several hypothetical evolutionary series, but subsequent workers have suggested that his technique of transformations is better applied to problems of development than of evolution. In fact, it is rarely used in either field.

The author has used a combination of Thompson's methods in an attempt to elucidate the relations between some of the major groups of the Agnatha, starting from Watson's hypothesis that the single nostril of the cephalaspids, anaspids, lampreys, and hagfishes indicates that each of these must have been derived from (or possessed in its life-history) a form with a head resembling that of the ammocoete larva of the modern lamprey. In the course of this, a modification of Thompson's technique has been devised which may be applicable to other problems of phylogeny.

Thompson's method of transformations was used to compare the

adult heads of representative agnathans with that of the ammocoete larva, but this led to confusion in which it was impossible to perceive any direction of evolutionary change. This difficulty of determining direction (or worse, a spurious implication of direction) is a serious fault of Thompson's method as he himself has used it. The adult form upon which the rectangular grid is erected is taken to be the 'starting point', and the deformed grids of the other forms suggest how these may have been derived from it.

Morphogenesis is the vehicle of evolutionary change, so the most appropriate starting point from which to explore change of form in the course of evolution is the embryo of the ancestor.

In the case in question - the morphological relations between the major Agnathan groups - embryology offers little help, since the lampreys are the only group of which the development is known, while the cephalaspids and anaspids are known only as adult fossils. The drastic step was therefore taken of 'predicting' their development. A basic agnathan embryo or Bauplan was deduced from considerations of the essential relationships between myomeres of the head, the gills, the buccal cavity, and the nasal and hypophysial anlagen, and a rectangular grid was erected upon this diagram. Intermediate forms were interpolated between this and the known adult conditions to provide a predicted development for each type.

No degree of accuracy is claimed for these predictions (although there is a close resemblance between the 'predicted' and actual development of the lamprey), but each suggests the probable major morphogenetic movements leading to the respective adult conditions,

This leads to a denial of a primitive role to the ammocoete. It seems far more likely that the monorhinal condition arose first in the cephalaspids (or their ancestors) as a result of a great rostrad shift of the branchial region with respect to the brain, and that the anaspids and lampreys retained this condition in spite of a secondary caudad shift of the branchial region.

These deductions depend upon a number of very slender hypotheses but, even if this scaffolding be taken away, the results can be defended by orthodox morphological arguments. By drawing attention to possible patterns of morphogenesis (and, by corollary, to those which are highly improbable), this modification of Thompson's method offers a tool for exploring relationships in areas where the more reliable and orthodox bases of evidence are scanty.

TIME, SPACE AND COMPUTERS. Sergius G. Kiriakoff, Zoological Institute, State University of Ghent, Belgium.

As everybody knows, modern biological systematics is basically different in its aims and methods from Pre-Darwinian systematics. Before Darwin, any causal basis for classification was lacking, because the only possible conception regarding the origin of species was that of special creation. Structural similarity was, then, the only possible criterion for grouping organisms. Since the concept of organic evolution became universally accepted, common descent has become the basis of the various biological systems. This has resulted in the broadening of the character concept, which now includes besides structure, such features as physiology, ethology, biochemistry and genetics. Features based on time and space have also come to be considered.

The modern tendency ("The New Systematics") is sometimes opposed to the "Old" or "Typological Systematics". Strictly speaking the opposition is not one of principle, because characters can only be interpreted as similarities and dissimilarities. The degree of phyletic relationship has to be measured by the degree of similarity. This is no longer limited to morphological structures and thus has become an "overall similarity". It therefore has a quantitative rather than a qualitative significance.

Similarities and dissimilarities are essentially typological concepts but phylogenetically minded students must try to think in purely phylogenetic terms. Phyletic relations must be looked upon as completely independent from the "Gestalt" or holomorph. Of course, similarity is very generally characteristic of phyletically related organisms or groups. That, however, is not a principle or an axiom, but merely an empirical observation.

The factors of time and space are not 'characters' in the usual sense and therefore cannot be used in typological systems. They are, however, of great significance in systematics, via two sciences closely related to the latter, viz., biogeography and paleontology.

Modern biogeography should be based on the following principles (cf. Kiriakoff, 1961):

1. Continuity of most of the continental masses and oceans.
2. Vavilov's rule of parallel variations.
3. Simpson's theory of the probability of passive distribution.

Item 2 has a particular bearing on our subject. Indeed, Vavilov's rule of 'homologous series in the inheritance of variability', as defined in 1920 (cf. English translation, 1950) includes the following statements (Vavilov, 1950: 75):

1. Species and genera that are genetically closely related are characterized by similar series of heritable variations with such regularity that knowing the series of forms within the limits of one species, we can predict the occurrence of parallel forms in other species and genera. The more closely related the species and Linneons in the general system, the more resemblance will there be in the series of variations.

2. Whole families of plants in general are characterized by definite cycles of variability occurring through all genera and species making up the family.

As I have pointed out (Kiriakoff, 1956) Vavilov's rule can be of paramount significance in biogeography and hence in systematics, and more especially in the so-called 'difficult' cases of geographical distribution. The latter mostly refer to organisms inhabiting parts of the Gondwanian complex (South America, South Africa, Indo-Australia) and are being variously explained on the basis of theories such as that of Wegener and that of transoceanic bridges. The inadequacy of all these hypotheses (at least in some cases) is well known. It would seem that Vavilov's rule (quite unnecessarily called by Verheyen paramorphogenesis) offers in such cases a more probable explanation. I shall discuss two cases.

A. Red-breasted Nuthatch (Sitta canadensis auct. plur.).

The passerine genus Sitta has a Holarctic and partly oriental distribution. Among its fifty-odd members, one group of forms generally considered as a polytypic species is Sitta canadensis L., with subspecies distributed as follows:

Sitta c. canadensis L.: North America, S. c. villosa Verreaux: West and North China to Southern Manchuria and Korea; S.c. whiteheadi Sharpe: Corsica.

All these have black crowns in the male. The following are also often considered as conspecific:

S. krüperi Pelzeln: Asia Minor, Caucasus. Forepart of crown black; S. yunnanensis Ogilvie Grant: Yunnan. Crown gray.

Some authors have different views regarding the taxonomy of these nuthatches: Vaurie (1957) considers the two last named forms as not conspecific. Löhrl considers S. canadensis and S. whiteheadi as good species, and so does Stresemann who, however, considers S. villosa to be another good species (cited in Vaurie, 1957: 22, footnote).

B. Blues of the subfamily Brephidiinae (Lycaenidae, Lepidoptera).

Four species of very small Blues have been placed by Stempffer (cf. Stempffer and Kiriakoff, 1952) in a separate subfamily Brephidiinae, because of the aberrant male genitalia, in conjunction with a rather similar facies. These are:

Brephidium exile Boisduval: California, New Mexico, Texas, Mexico, Central America, Venezuela; Br. isophthalmum Herrick-Schaffer: Florida, Louisiana, The Antilles; Oraidium barberae Trimen: Cape District, Natal, Transvaal.

In both cases, the groups discussed are of relatively recent descent (later than Cretaceous). Now, there is strong evidence that the Atlantic was already in existence in Middle-Secondary (cf. 'Sympoisum' 1952), so that neither continental drift of Wegener nor the theory of transoceanic bridges can be used. There remains the classical explanation by "migrations" from the so-called "cradle-region" towards the periphery, with subsequent extinction of all the intermediate populations. Cradle-regions are to be sought for somewhere in Eastern Eurasia, for the nuthatches as well as for the blues. Both groups must, then, have had at one time a very extensive area of distribution, covering for the former, the whole of the Holarctic region, and for the latter, besides that, parts of North and South America and of Africa. The widely scattered

present areas of distribution presuppose a wholesale extinction of populations over huge areas. This is hardly acceptable.

Now, the explanation by means of parallel variations is very much simpler. Distinctive characters of S. canadensis and of Brephidiinae are by no means exceptional in their respective groups. There are several other species of Sitta displaying a black cap or black parts of the crown. Also, some species of Lycaenidae (e.g. of the genus Lycaena, the Coppers) show, in the male genitalia, peculiar features not unlike those found in Brephidiinae. Genes that control the black cap and the peculiar shape of tegumen and of penis, are accordingly in many species of both groups, without any geographical discrimination, and there is nothing unusual in the fact that they could have originated in various regions, as a result of parallel variations in members of rather homogenous groups.

So much for space. Regarding time, it is obvious that it also has played a role in the cases discussed. Long and complicated migrations around the globe and subsequent extinctions undoubtedly require a long time - much longer than the defenders of such hypotheses realize.

Time is also of great significance in the question of the relative age of phyletic series in connection with their taxonomic value. Hennig's theory (1950) of taxonomic equality of groups of equal geological age has been questioned,and even considered (Simpson, 1961) as "undesirable" for taxonomic purposes. Still, absolute age as a taxonomic criterion has the advantage of being wholly objective albeit mostly only moderately precise. It should at least be used in phylogeny, although it seems to me that refraining from its use in classification results from the still operating typological complex, and maybe still worse, from sentimentality. At least the higher groups of animals could be arranged on Hennig's principle.

In the first part of this paper I have tried to show the importance for systematics of the factors of time and space. Both do not properly belong to the holomorph, and accordingly cannot be handled by the method of overall similarity. As I have recently pointed out (Kiriakoff, 1962) that is a very serious drawback, the more so because there is no way to avoid it. That alone should be enough to show the shortcomings of the numerical method. But there is more.

From some reactions to my paper, I have gathered that the "real issue" - to use the celebrated phrase of Sir Julian Huxley - has not been properly understood. Hope has been expressed that the opposite points of view could be brought into harmony with each other. I believe the hope is fallacious and I shall try to show why.

a. Phylogenetically-minded taxonomists are trying to arrange the organisms according to their phyletic affinities. These are based on organic evolution which can be defined as the grand total of the phyletic series of organisms, each series showing the history, and thus representing the evolution, of an organsim or of a group of organisms. Each series is a succession in time of individual organisms, related to each other by the objective, physical fact of descendence. No one, I feel sure, not even the most obdurate typologist or Neo-Adansonian, will ever venture to affirm that mother-to-daughter relationships is not the

truest, the most real bond between organisms.

Consequently, having chosen the criterion of actual descendence, the phylogeneticists have chosen not only the 'best' but the only strictly 'natural' criterion. There is not much doubt that nowadays no scientist would ever dare to deny that.

b. Neo-Adansonians are trying to arrange the organisms according to their phenetic affinities. Their choice is based on the following considerations:

1. Phylogeny is not a criterion that can be used for a classification because we lack most of the information about descendence, and most therefore replace facts by speculatinns.

2. Overall similarity is objective reality, and its degree can be established in a very precise way by means of numerical methods. All the characters that form the overall similarity are claimed to possess the same value.

Consequently, numerical methods are to be preferred to classical or phylogenetic methods.

I agree with the Neo-Adansonians that the pure phylogenetic system cannot be built up because of lack of information. The 'best' possible system contains some proportion of speculation, and in a sense it must be considered as an ersatz-system.

Secondly, it probably may be assumed that numerical taxonomy is being considered by its proponents as a (very good) substitute for the pure phylogenetic systematics, for reasons given below.

We are accordingly confronted by two types of ersatz-systems, viz.

a. The one called, somewhat euphemistically, the modern phylogenetic system.

b. The other based on overall similarity, without any consideration of the origin or the phyletic relations between the organisms.

Neither is strictly typological but both have an admixture of typological thought, the former with an addition of phylogenetic principles and methods, the latter differing from the pure typological system in the absence of idealistic types, and thus being built on strictly empirical lines.

Now, we have to make a choice between those two types. Recently (Kiriakoff, 1962) I have tried to explain why I prefer the first ersatz-type. Some additional arguments may, however, prove useful.

What the Neo-Adansonians are asking us is,in fact, to ignore the information won during the whole century as a result of the Darwinian revolution. They will probably indignantly deny the accusation, and yet that is what they are doing. For, although they are using the same facts and the same data (except those where the holomorph is not involved, e.g. time and space) as the evolutionists, they eliminate from their conclusions any evolutionist consideration. To say, for example, that species A should be placed nearer to species B than to species C because it has 91.029% common characters with B as against only 91.016% with C, is not a conclusion based on evolutionist considerations, notwithstanding any assertions to the contrary.Now, it could be, if the Neo-Adansonians renounced the principle of equality of characters, a thing they will never do. They may call it objective, but they should

know better, and I am sure they do. The whole study of evolution shows that there is no such thing as equality of characters, and the evidence is so overwhelming and, moreover, so well known to everybody, that to make an abstraction of it in so-called scientific work, constitutes a crime against science.

Every Neo-Adansonian knows or should know that equality of characters is a fiction, a mere convention, and in deliberately accepting the principle of equality, the Neo-Adansonians have lost the right to claim any objectivity in their work.

No evolutionist would ever accept the principle of equality, nor, as I have pointed out previously, would he accept having phylogenetic principles superseded by that of overall phenetic similarity, a concept that has no roots in the scientific thought of this century. We would not accept it, because, I repeat, we know better.

So do, I am sure, the Neo-Adansonians, and yet they deliberatly disregard organic evolution to return to creationist thought and creationist methods.

I now ask you: Is a reconciliation between the two schools possible? My answer is emphatically NO.

The Neo-Adansonians claim that their results mostly confirm those reached by what they call "classical" methods. That means, I should think that the latter are not so bad, in fact not bad at all. Why, then, abandon them?

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BLOOD GROUPS AND THE SYSTEMATICS OF MARINE ANIMALS. John Cushing, Dept. of Biological Sciences, University of California, Santa Barbara, Goleta, California.

A number of investigations conducted during the past decade have established the value of blood group studies on populations of marine animals (Cf. references and names of principle investigators in the American Naturalist, XCVI, no. 889, 1962, pgs. 193-246). This value derives its significance from immunogenetic concepts established by research on human and domestic animal blood groups. Such research has shown that the specificity of blood group antigens is genetically controlled in a simple manner unaffected by environmental influences. This knowledge makes it possible to treat blood group antigens as "genetic tags", subject to the laws of population genetics and useful in comparisons of populations at the subspecific level of systematics. Such comparisons are concerned with establishing the extent to which a species is subdivided into genetically isolated subpopulations (races, stocks) and the identification of these subpopulations as they are distributed in the sea. These comparisons are based on the axiom that population samples which differ significantly with respect to the frequency of occurrence of the same blood group antigens are probably from reproductively isolated stocks.

Although genetic experiments with marine animals are generally out-of-the-question, information based on criteria derived from serology and population genetics shows that the mechanism of inheritance of blood groups in these forms must be essentially the same as that of experimental organisms. Additional support for this conclusion is derived from limited observations on the blood groups of parents and offspring of some species, and from the consistency with which relative phenotype frequencies of blood groups are maintained in specific populations from year to year.

Blood group antigens are usually detected by capillary, slide or tube agglutination tests using reagents obtained from isoimmune, heteroimmune and normal serums; as well as extracts of plant materials. Isoimmune serums are most apt to provide the best reagents, but may not be readily prepared. Erythrocytes are usually obtained from living or recently killed animals, but can also be obtained from animals that have been dead for a few days if kept under favorable conditions. Glycerol-freezing has been used to advantage in the collection and preservation of samples.

Blood group polymorphism occurs in all of the species that have been seriously investigated. Several studies are far advanced, subpopulation units having been recognized in species with extended and even world-wide oceanic distributions. These include representatives among clupeoids, elasmobranchs, salmonids, tunas and whales. Some of these studies have reached the point

where it is feasible to start considering the ecological and evolutionary factors that are responsible for the polymorphisms and genetic isolations that are observed.

In addition to their value in subspecific systematics, erythrocyte antigens provide characters for comparisons among species as these generally have some antigens of such high frequency of occurrence as to be "species-specific". Furthermore, related (and even unrelated) species may also have antigens that are very similar in their specificities and polymorphic variation as, for example, the Ju antigens of whales, the Tg antigens of tunas, and antigens with specificities akin to those in the human A, B, O, system.

Soluble antigens occur in serum and body fluids that have specificities similar to those found on erythrocytes. These antigens offer considerable promise for studies in subpopulation research, particularly with respect to the plankton and other marine invertebrates. (Some of the researches referred to were supported in part by a contract with the Office of Naval Research, Biology Branch).

Enzymes and Nucleic Acids in Systematics

Allan C. Wilson and Nathan O. Kaplan
Graduate Department of Biochemistry,
Brandeis University, Waltham 54, Mass., U.S.A.

Degree of genetic homology between animals can now be estimated from studies on informational macromolecules (nucleic acids and proteins). Techniques for studying the composition and structure of such macromolecules are advancing rapidly. Measurement of degree of genetic homology is therefore likely to become a relatively simple matter in the near future. Systematics will undoubtedly come to depend heavily on these techniques.

Proteins can provide indirect estimates of degree of genetic homology because the amino acid sequence of a protein is a direct translation of the base sequence in a segment of genetic material. However, a technical breakthrough will be required to make amino acid sequence determinations feasible for systematic studies. In the meantime, alternative methods of comparing protein structure in different animals can be used. The fingerprint method, in conjunction with data on amino acid composition of peptides, is probably one of the best alternatives to sequence determinations. Much faster, and probably just as reliable, are quantitative immunological methods using antibodies against pure proteins. The quantitative micro-method of measuring complement fixation, the micro-C'F method, is probably the method of choice because it is a hundred to a thousand times more sensitive than the quantitative precipitin and complement fixation methods previously used in systematic studies. Electrophoretic, chromatographic, and physical methods are likely to be less valuable because they measure fewer aspects of a protein's structure than the above methods.

Enzymes are better suited than other proteins, from the viewpoint of methods, for taxonomic investigations. The catalytic property helps greatly in the identification, purification and isolation of enzymes. In immunochemical studies with antibody against a pure enzyme, cross reactions can be studied in crude extracts of tissues since the concentration of enzyme can be measured specifically and accurately; moreover, one can ascertain, by the technique of enzyme inhibition, that the cross reaction is actually with the enzyme rather than a contaminating protein. Also, small differences in the catalytic properties of the same enzyme in different organisms provide a further method of structural comparison. Catalytic comparisons of enzymes, based for example, on their ability to discriminate between coenzyme analogs, are faster than any of the above methods. Finally, since catalysis is the biological function of an enzyme, one is presented with the possibility of explaining the differences or similarities observed in the catalytic properties in terms of selective advantages.

Since an animal contains many thousands of different proteins, a single protein can provide data about only a tiny frac-

tion of the genetic material. It is important to find out whether proteins tend to undergo parallel or coordinated changes in structure or whether each protein tends to change independently of other proteins in the genome. If the correlation between changes in structure of different proteins turns out to be poor, the study of systematics and evolution by protein methods will be difficult. In order to estimate the degree of genetic homology between any two species it will be necessary to compare the structures of many of their proteins. But if there is a strong correlation between changes in structure of different proteins, far fewer proteins will need to be compared. It also may be possible to select certain enzymes whose structural changes are closely correlated to the overall evolutionary changes in genetic structure.

To throw some light on this problem, we have prepared rabbit antibodies against several pure proteins, mostly enzymes, from the chicken. The antibodies were tested by the micro-complement fixation method against the corresponding proteins of a series of vertebrates ranging in taxonomic distance as far as the lamprey. The data indicate that the enzymes all evolve at similar rates but that ovalbumin (a non-enzymic protein) evolves several times faster. Since by far the majority of proteins are enzymes, it can be tentatively concluded that many proteins show a strong tendency to evolve in parallel. It appears likely that studies on as few as 5 to 10 pure proteins will suffice for the purpose of making estimates of overall degree of genetic homology.

The taxonomic range of the enzyme methods is considerable. Homologies can be detected between distantly related classes within the same phylum (e.g. Aves related to Agnatha; Crustacea related to Insecta) and even between phyla. On the other hand, small differences can easily be detected between genera of the sub-family Phasianinae (Aves).

Nucleic acids, as the actual carriers of genetic information, are the ideal materials with which to make estimates of degree of genetic homology. It has been possible in recent years to separate and re-unite the strands of bacterial DNA *in vitro*. The separated strands can also re-unite with homologous separated strands of DNA (or RNA) from related species. The observation of these phenomena used to depend on the technique of density gradient ultracentrifugation. A much simpler technique for producing and detecting strand recombination has been developed within the last few months by McCarthy and Bolton of the Carnegie Institution of Washington. This technique permits for the first time the detection *in vitro* of strand recombination in animal DNA. High molecular weight DNA is trapped in single-stranded form in agar. Low molecular weight DNA (or RNA), also in single-stranded form, from the same or another species is then passed through the agar matrix. Non-homologous incoming strands pass right through the matrix but homologous strands can undergo base-pairing with the trapped DNA molecules. Preliminary experiments by Hoyer, McCarthy and Bolton have revealed considerable homology between mouse and human DNA and between mouse and calf DNA but little

homology between mouse and salmon DNA; moreover, improvements in the technique's sensitivity can be expected. Our laboratory is also starting to explore the use of this technique. The way now seems open for the rapid solution of one important class of taxonomic problems, the measurement of degree of genetic homology.

ELECTROPHORETIC TECHNIQUES IN SYSTEMATICS. Herbert C. Dessauer and Wade Fox. Depts. of Biochemistry and Anatomy, Louisiana State Univ., School of Medicine, New Orleans, La.

Chemical similarities and differences between organisms offer the taxonomist sensitive means of characterizing organisms and estimating relationships (16, 25). Since the taxonomist needs to sample large numbers of individuals and frequently deals with very small species, he requires chemical methods that are simple, rapid, preferably economical and adaptable to a micro scale. Electrophoretic techniques and associated methods to be described here were assembled and developed with these requirements in mind.

Electrophoretic separation of proteins and other compounds results from their different rates of migration in an electric field (4,20). Migration rate may be studied in (1) an aqueous solution of buffer (free-solution electrophoresis); (2) buffer impregnated solids such as filter paper, cellulose acetate paper and starch grains (zone-electrophoresis); and (3) concentrated gels such as those formed of starch or polyacrylamide. All non-liquid media (20), and especially concentrated gels (26), create a barrier effect that retards migration. Thus two substances, equally charged, may separate if they differ in size or shape.

Free-solution electrophoresis (4) is of limited usefulness to taxonomists. Mobility measurements are reproducible, but the time required to analyze a single specimen is excessive. Spot test techniques cannot be utilized to localize enzymes, lipoproteins, etc. Free-solution electrophoresis cannot be combined with immunodiffusion methods. Only the slowest and fastest fractions are completely separated.

The resolving power of zone-electrophoresis (4, 20, 1) is as good as that of free-solution electrophoresis, and the cost of instrumentation is less than 10% of the latter. Several samples can be compared simultaneously and resulting electropherograms are available for chemical testing or immunodiffusion procedures. Untreated paper is a poor supporting medium for electrophoresis of proteins, especially in acid buffers, since protein adsorbs to the paper. Cellulose acetate (23) does not have this limitation, but such paper is more expensive than filter paper and is easily overloaded with sample.

Paper-electrophoresis with high voltages is useful for separating compounds of small molecular weight. Since these diffuse rapidly, they are best separated quickly in strong electric fields. High voltage electrophoresis (4) requires cooling and special measures to diminish electroosmotic flow. Effective instruments of simple design are available commercially or are easily constructed. For example, electrophoresis can be carried out on a sheet of thick filter paper (Whatman 3 mm) wetted with buffer and carefully spread on a flat surface of plexiglass cooled by the flow of ice water through a chamber built below its surface. This cassette rests horizontally between two plastic

electrode baths. Electrical contact between the paper supporting medium and the electrode baths is made by filter paper wicks. Three or four layers of dialysis membrane separate the wicks from the filter paper sheet. These membranes, an inovation of R. Weber (see 4), serve to diminish electroosmotic flow. Stainless steel rods make satisfactory electrodes. Power supplies, developed for ham radiotransmitters, make excellent high voltage sources.

Electrophoresis through concentrated gels (4, 7, 26) has exceptional resolving power. For example, in free-solution, paper and cellulose acetate electrophoresis only 5 to 7 fractions characterize the plasma protein electrophoretic patterns of each of 8 kinds of garter snake, but 12 to 15 fractions were resolved by means of starch gel electrophoresis using the same buffer (sodium barbital, pH 8.6, ionic strength 0.05). Many samples can be compared on parallel runs and electropherograms can be examined by a variety of chemical and immunological procedures. For details of starch gel methods see Smithies (26) and Bloemendal (5); for polyacrylamide gel electrophoresis see Burstone (6), Chang, et al. (7) or Raymond and Aurell (22). An effective apparatus, similar to that pictured in Bloemendal (5), is easily constructed. To eliminate evaporation of water from the surface of the gel during electrophoresis, Saran Wrap (Dow Chemical Co.) is as efficient as petroleum jelly and less messy. Only slits in the gels where sample is applied need be sealed with petroleum jelly.

Techniques are available for localizing the position of specific proteins on electropherograms. Methods of histochemistry (3, 5, 6, 15, 19, 20, 26) have been modified to detect lipoproteins, glycoproteins, copper and iron containing proteins and many hydrolytic and oxidative enzymes. Enzyme localization often depends upon reactions with synthetic substrates that result in the deposition of an insoluble dye at the site of the protein on the electropherogram. Radioactive tracer techniques are convenient for localizing iron binding proteins and other proteins involved in chelation reactions (8, 26). X-ray film packs (Kodak Royal Blue, Ready Pack) simplify autoradiography. Techniques of electrophoresis and immunodiffusion are combined to compare the serological correspondence of individual proteins (2,4,13,23,26).

Proteins and other charged molecules may be isolated for structural studies, bioassay or microchemical analysis. Hanging curtain, column and starch grain techniques (1,4) have high capacity; however, electrophoresis through concentrated gels yields fractions of greatest purity (26). The Hemmings filter (Standard Scientific Co., New York) is useful in eluting protein from starch grains or gels. For example, after starch gel electrophoresis, the section of the gel containing the protein desired is placed into one bottle of the Hemmings filter. The structure of the gel is broken by freezing and thawing. The metal unit of the filter is fitted with a ring of filter paper and screwed onto the bottle containing the starch. The second

bottle of the apparatus is screwed into place and the assembly, with the starch bottle nearest the rotor, is centrifuged at 2500 rev/min for 10 min. Protein, diluted with buffer, filters into the outer bottle.

Since proteins are diluted with buffer in most electrophoretic methods, these solutions must be concentrated and freed of buffer. Dry, cross linked dextrans (11) such as Sephadex G-25 (Pharmacia, Uppsala, Sweden) can be used to concentrate the protein without increasing the concentration of buffer. The Hemmings filter is useful in separating dextran from the protein solution. The glass suction apparatus (Schleicher and Schuell Co., Keene, N. H.), an ultrafiltration assembly that works from a water pump, is effective in removing both buffer and water. Buffer can be removed or its composition changed by passing the solution of protein through a column of Sephadex G-25 equilibrated with water or the desired buffer (21).

All of these electrophoretic methods have proved useful to varying degrees in characterizing specific organisms. Reviews of Sibley (24), Engle and Woods (10) and Dessauer and Fox (in 16) contain data and references on applications of electrophoretic methods to taxonomic problems. Since proteins, which largely form the structural basis for biological variation, occur in complex mixtures in tissues, the techniques which give the greatest resolution give the best picture of the genetic constitution of the organism. However, the total complement of proteins in tissue varies with the stage of development of the organism and to some extent with its physiological state (10, 16, 19). For example, remarkable changes in tissue proteins have been observed in mammals during embryogony and in insects, fish and amphibians during metamorphosis. Plasma vitellin appears during estrus in blood of vertebrates that produce yolky eggs; additional phosphatases and an oxytocinase appear during pregnancy in plasma of women. If such developmental and physiological variables are taken into account, a species can be characterized by the electrophoretic properties of its tissue proteins. Certain proteins of the tissue complement, frequently, point up individual variability in a population. For example, eleven different patterns of transferrins were found in plasmas of 13 specimens of the snake Coluber constrictor from southeastern Louisiana (8). Race or strain differences in certain proteins may be superimposed on a characteristic species pattern. For example, Chang, Srb and Steward (7) have demonstrated strain differences in soluble proteins of Neurospora. Jimenez-Porras (14) observed a varied distribution of L-amino acid dehydrogenases in venoms of fer-de-lance populations from different geographic areas of Costa Rica. He found that Bothrops nummifera and B. picadoi, which are difficult to distinguish morphologically, are clearly distinguishable by the electrophoretic properties of their venom proteins. Patterns of muscle proteins of fish may be used to detect improper labeling of fish fillets on the commercial market (17).

Electrophoresis has been applied less to analyses of rela-

tionships and evolution. Current trends in this direction are indicated by the following studies. Abramoff, et al. used immunoelectrophoresis of plasma proteins to estimate pathways taken by toads in populating islands of Lake Michigan (in 16). We found that transferrins, esterases and other plasma proteins gave a more sensitive measure of the degree of hybridization between two subspecies of lizard, Cnemidophorus tigris, than morphological characteristics (9). Electrophoretic properties of blood proteins (12) and venom steroids, indoles and amino acids (Wittliff in 16) served to identify hybrids between two species of toads, uncovered rare backcross individuals and indicated possibilities of gene transfer between species through hybridization. Sibley(24) has shown that among birds certain taxonomic units, such as the family, can be characterized by patterns of egg white proteins. Goodman (in 16), using immunoelectrophoresis, obtained evidence on the phylogenetic relationships of the primates. In other instances higher taxonomic units can be distinguished: most orders of Amphibia and Reptilia by characteristics of their blood proteins (Dessauer and Fox in 16) and orders of birds by their egg white proteins (24).

Electrophoretic properties of tissue proteins yield only a fraction of the data on relationships that are present in the protein (25). Two proteins often have identical migration rates but distinctly different amino acid arrangements. Obtaining complete amino acid sequences for a few proteins of even a few specimens would be unduly time consuming, but much useful data can be obtained in a reasonable time with methods based on "fingerprinting"(1). The table, which compares data on fingerprints of tryptic peptides of hemoglobins of the snake, Natrix taxispilota, with those of other reptiles of varying degrees of relationship, illustrates the potential of this technique(Sutton. and Dessauer, unpublished). Peptides were separated in one

Species	Member of same:	Tryptophane peptides		Histidine peptides		Arginine peptides	
		No.[a]	Diff.[b]	No.[a]	Diff.[b]	No.[a]	Diff.[b]
Natrix taxispilota	--	1	-	14	-	14	-
Natrix grahami	Genus	1	0	13	4	16	4
Thamnophis sauritus	Subfamily	1	0	14	5	15	4
Farancia abacura	Family	1	0	12	10	12	8
Iguana iguana	Order	1	0	17	19	15	13
Alligator miss.	Class	1	2	13	17	13	20
Pseudemys scripta	Class	3	2	13	19	8	17

[a]Number on fingerprint [b]Number of differences from test species

dimension by high voltage electrophoresis and in the second by chromatography. Resulting fingerprints were treated to localize and determine the number of tryptophane, histidine and arginine containing peptides in each hemoglobin. Positions of these peptides on fingerprints of each test species were compared to the position of those on fingerprints of N. taxispilota to estimate the number of differences between the hemoglobins of the test

and reference species. Allied species had very similar fingerprints; even Iguana which is in a different suborder had many peptides in common with snakes. Fingerprints of the alligator and turtle were greatly different.

The past few years have brought an ever-enlarging battery of techniques of possible usefulness to the taxonomist in solving problems of phylogenetic relationship and evolution. The refinements of analytical methods allow a closer and closer approach to the ultimate chemical characterization of the individual. Electrophoresis has a place of central importance in such comparative studies either alone or in conjunction with other methods.

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SERUM PROTEIN COMPARISONS BY TWO-DIMENSIONAL STARCH-GEL ELECTROPHORESIS AND IMMUNODIFFUSION PLATE REACTIONS APPLIED TO THE SYSTEMATICS OF PRIMATES AND OTHER MAMMALS. Morris Goodman. Department of Microbiology, Wayne State University College of Medicine, Detroit.

A cardinal tenet of post-Darwinian systematists is that organisms should be arranged in taxonomic schemes according to their propinquity of descent and degree of genotypic affinity. The problem is to discover the actual genotypic relationships existing among non-interbreeding organisms. When it is suspected that such relationships do not correspond to the recency of common ancestry of the organisms under comparison, systematists achieve their classifications by judging relationships in terms of morphological types and grades. For example the Crocodilia are placed in the class Reptilia despite considerable evidence that the time of phyletic separation is less between Crocodilia and Aves than between Crocodilia and the other contemporary reptiles. The assumption is that while the Crocodilia remained in evolution at the morphological grade of reptiles, the birds attained a new grade meriting taxonomic recognition at the class level. For similar reasons the Therapsida are kept in the class Reptilia although their position on the evolutionary tree is closer to contemporary mammals than to contemporary reptiles. It is generally recognized that grouping the Synapsida which contain the Therapsida with the Reptilia rather than Mammalia is an arbitrary convention used to express grade relationships. The acceptance of this convention by most systematists suggests that the pre-Darwinian concept of the scala naturae in which an animal series progresses from simple to morphologically complex organisms still operates in post-Darwinian systematics, particularly in the subdivision of those phyletic assemblages in which "lower" organisms coexist along side of "higher" organisms.

Among contemporary mammalian orders the Primates provide the best example of a morphological series which progresses from the "lower" to the "higher". It starts with tree shrews with their many primitive insectivore features, goes to lemurs, lorises, tarsiers, new world monkeys, old world monkeys, gibbons, large sized anthropoid apes, and finally ends in man. There seems little question that the placement of organisms in this scala naturae has guided systematists in classifying the members of the order. Within the Hominoidea man is invariably separated from the apes. He is placed in the family Hominidae as its only living member. In turn all living and extinct species of apes are usually grouped together in the family Pongidae. However it is quite possible that certain of the living apes have a more recent common ancestry and also more genetical affinities with man than with other living apes. There are a number of such questions concerning the phylogenetic relationships of various living Primates. Indeed even the boundaries of the order are in dispute with systematists undecided on whether to place the tree shrews in the Insectivora, or the Primates.

A truly evolutionary taxonomy of the Primates can only be

created after data are gathered which accurately reveal the genetical affinities existing among the various contemporary primates and related mammals. Ever since the work of Nuttall systematic serologists have demonstrated empirically that the phylogenetic relationships of organisms can be estimated by comparing the serological specificities of their proteins. The advances in protein chemistry and in genetics now provide a model which justifies on theoretical grounds descriptions of the genetical affinities of organisms in terms of protein specificities.

According to this model a protein is composed of one or more long polypeptide chains in which the sequence of amino acids is under strict genetic control such that the substitution of one amino acid for another can be related to a single gene mutation. The sequence of amino acids in the polypeptide chains guides the twisting and folding of these chains into globular molecules. A mutated amino acid sequence due to a mutated gene could change (and certainly would after a small number of such mutations had accumulated) the composition of one or more surface configurations of the globular protein. Since a polypeptide chain of average length contains as many as 150 amino acids of up to 20 different kinds, an astronomical number of permutations in the amino acid sequence and surface configurations of any single protein can be caused by gene mutations. Each protein has surface configurations which are responsible for a primary functional activity such as an enzymatic, antibody, or hormonal activity. A circulating protein in a higher metazoan would also have configurations which execute ancillary functions, e.g. configurations concerned with the protein's preferential transport across certain membrane barriers or its preferential uptake by certain cell types where it may then execute its primary functional activity. Furthermore the process of natural selection would tend to ensure that all the surface configurations of this protein were contoured stereochemically so as to not interfere with the specific functional activities and molecular associations of the other circulating proteins. It follows from this description that the serological specificity of any one protein distinguishes it from the other proteins in the organism and also exhibits to one or another degree the property of species specificity. In other words, a comparative analysis of the serological specificities of even a small proportion of the proteins found in a set of organisms can reveal the evolutionary relationships existing among the members of the set.

Two methods are being used in the author's laboratory to analyze the serological specificities of the serum proteins of primates and other mammals. The first is the two-dimensional starch-gel electrophoretic method of Poulik and Smithies in which serum is separated by electrophoresis on filter paper followed by electrophoresis in starch-gel. In the second dimension molecular size as well as electrophoretic charge affects mobility, and the arrangement of the serum proteins in the starch-gel after the

electrophoresis provides a complex pattern of 19 to 25 components. Since this method can detect mobility differences due to single gene mutations as in the polymorphisms of transferrin and haptoglobin, the lineages produced by phylogenetic branching need have only moderately divergent genotypes for striking differences to appear in their two-dimensional starch-gel electrophoretic serum patterns.

Serum and plasma specimens from over 1,000 animals including elephant shrews, hedgehogs, tree shrews, and many different primates have been analyzed by this technique. So far the data demonstrate that species belonging to the same genus have similar patterns whereas species belonging to different subfamilies have quite divergent patterns. Some groups such as the genus Lemur show a high degree of species specificity. For example, the positioning of a prealbumin permits us to distinguish Lemur fulvus, Lemur variegatus, and Lemur catta from each other. Otherwise the patterns are quite similar. In contrast, Propithecus verreauxi (belonging to a different family of the Lemuroidea) has a sharply different pattern. In the Lorisidae, Galago crassicaudatus and Galago senegalensis have essentially the same pattern, but Nycticebus and Perodicticus have different patterns.

When the two-dimensional starch-gel patterns of members of the Hominoidea (man, gorilla, chimpanzee, orang-utan, and gibbon) are compared with each other, the differences among the various hominoid types are much larger than those found among macaques, baboons, and green monkeys. Indeed, various species of the subfamily Cercopithecinae examined by two-dimensional starch-gel electrophoresis (five species of Macaca, one of Papio, and one of Cercopithecus) show a high degree of similarity. (However, a few differences, particularly between the genera, can be observed.) Although the starch-gel pattern of each hominoid type diverges sharply from the others, there is a constellation of about ten faster migrating proteins in Homo, Gorilla, and Pan, but not in Pongo or Hylobates. The faster migrating proteins of the orang-utan and gibbon present patterns in each case which are quite dissimilar from each other and from those of the other hominoids. Three samples of Pan paniscus (the pigmy chimpanzee) were also analyzed. These patterns are very similar to those of Pan troglodytes but do show a consistent difference with respect to several components. Using the generic separation of macaques, baboons, and green monkeys as a taxonomic yardstick we can deduce from the electrophoretic results that none of the five major groups of living hominoids (man, gorilla, chimpanzee, orang-utan, and gibbon) have sufficient genotypic similarity to be grouped together at the genus level or even at the subfamily level. In particular the electrophoretic findings do not support the convention of grouping Pan and Gorilla with Pongo in the subfamily Ponginae while placing Homo in a separate family, the Hominidae. Indeed the most divergent hominoid patterns were those of Pongo and Hylobates and the most similar were those of Pan, Gorilla, and Homo. Thus evidence is provided that the

phyletic line which branched to give rise to Gorilla, Homo, and Pan did so after it had separated from more ancient lines leading to Hylobates and Pongo.

The second method employed in this study for determining the degrees of genotypic relationship existing among different contemporary organisms is to measure the antigenic correspondence of protein homologues in these organisms by an immunodiffusion precipitin technique of the Ouchterlony type in highly sensitive comparative analysis plates (Goodman, 1962, Hum. Biol., 34: 104). A protein's antigenic specificity is determined by its unique constellation of surface configurations. As argued above such constellations reflect the genetic specificity of organisms. Thus the differences in antigenic specificity between a protein and its homologue in another organism tends to be greater the more distant the phyletic separation of the two organisms. The immunodiffusion plate reactions focus attention on the antigenic specificities of the individual proteins in a mixture in contrast to earlier serological work which did not separate the components of a mixture and often yielded erroneous results simply due to variations in the amounts of the protein homologues in different organisms.

The immunodiffusion plate reactions are being carried out with a variety of chicken, rabbit, and monkey antisera to human proteins (albumin, gamma globulin, transferrin, ceruloplasmin, alpha$_2$macroglobulin, orosmucoid, beta lipoprotein, and other serum antigens), also with antisera directed against serum antigens of the following animals: gorilla, chimpanzee, orang-utan, gibbon, rhesus monkey, cynomolgus monkey, langur, night monkey, squirrel monkey, capuchin monkey, spider monkey, woolly monkey, howler, titi, uakaris, lemur, loris, potto, tree shrew, and hedgehog. Antisera produced in monkeys to hominoid proteins react primarily with the more species specific configurations of these proteins and are proving particularly useful in investigating the interrelationships among members of the Hominoidea. Antisera produced in chickens to the proteins of various primates and other mammals are providing data on the affinities of the tree shrew within the class Mammalia. (For this purpose rabbit antisera would give a biased view since any mammalian group related to the lagomorphs would give a disproportionately smaller precipitin reaction.)

In support of the concept that the Tupaioidea became segregated very early in eutherian phylogenesis from all other surviving mammalian lineages, the anti-tupaiid precipitins failed to detect a close relationship of the tupaiids to any of the mammals tested (lemuroid, lorisoid, and various higher primates, insectivores such as hedgehog, tenrec, elephant shrew, and mole, and representatives of eleven other mammalian orders). However with respect to several serum antigens the tree shrew showed more affinity to hedgehog and bat (Myotis) than to the rest of the animals tested. On the other hand with chicken precipitins to human albumin and transferrin, man showed more affinities to the tree shrew than to all the non-primates tested. Indeed

human and tupaiid albumins showed more correspondence than did human and lemuroid albumins, suggesting that certain of the lemuroid genes have diverged more from the ancestral state than have the comparable tupaiid and human genes. Although evidence for a definative taxonomic assignment of the tree shrews has not yet been gathered, the serological data demonstrate that the tree shrews have affinities with the Primates. (Elephant shrews, placed by some taxonomists with tree shrews, do not show any primate or tupaiid affinities.)

The reactions of anti-potto and anti-lemur precipitins suggest that the Lemuroidea and Lorisoidea can be placed together as a separate group the Strepsirhini, for they showed more antigenic affinities to each other than to either the tupaiids (Urogale and Tupaia) or the Anthropoidea. With anti-ceboid precipitins human and macaque proteins developed larger reactions than lemur and galago proteins. From this it can be concluded that ceboids are more closely related to the catarrhine primates (hominoids and cercopithecoids) than to the lorisoid and lemuroid primates. Thus serology provides evidence that the suborder Anthropoidea is a valid phylogenetic taxon and not just an assemblage of organisms at a comparable morphological grade. However certain aspects of the data suggest that the phyletic separation of the ceboids from the catarrhine primates is almost as great as that of the Strepsirhini from the Anthropoidea.

The results obtained with the anti-rhesus and anti-cynomolgus precipitins, while showing a divergence of the Colobinae (langur) from Cercopithecinae clearly indicate that cercopithecines are more closely related to colobines than to hominoids and that these two groups (the cercopithecoids) are more closely related to hominoids than to ceboids. This relationship of the superfamilies Cercopithecoidea and Hominoidea within the Anthropoidea was confirmed by the reactions of anti-hominoid precipitins. In other words a subdivision of the Anthropoidea into Catarrhini and Platyrrhini is supported by serological evidence. However it was observed that cercopithecoids share some antigenic configurations with ceboids that hominoids lack, and similarly that hominoids share antigenic configurations with ceboids that cercopithecoids lack. This suggests that the catarrhine primates branched into the Cercopithecoidea and Hominoidea not long after the Catarrhini and Platyrrhini separated from each other.

Using anti-human precipitins, the proteins of human serum corresponded more to their homologues in chimpanzee and gorilla than in orang-utan or gibbon. The reactions of the anti-human precipitins were complemented by those of anti-chimpanzee and anti-gorilla precipitins. The key conclusions to be drawn from these analyzes is that the African apes have man as their closest relative, followed by the orang-utan and gibbon. Also it must be emphasized that there is no indication from the serological data of any closer relationship between chimpanzee and gorilla than between either of these apes and man. The reactions of the anti-orang-utan and anti-gibbon precipitins furnished evidence

that orang-utan and gibbon have man, chimpanzee, and gorilla as their closest relatives. There is also an indication from the data that the orang-utan may be somewhat more closely related to man and the African apes than to the gibbon. Nevertheless certain of the results suggest that soon after the primitive catarrhines branched into the Cercopithecoidea and Hominoidea branches leading to the gibbon and orang-utan separated from the remaining sequence of hominoid evolution.

Thus, although the serological findings support many established concepts of primate systematics, they do not support the taxonomic convention of having man be the sole living representative of the Hominidae while orang-utan, chimpanzee, and gorilla are grouped together as a subfamily of the Pongidae. The serological evidence would place Gorilla, Homo, and Pan in the Hominidae; Pongo would remain in the Pongidae, and Hylobates would be placed in the Hylobatidae.

It must be emphasized that zoological classifications are always tenative and subject to revision as knowledge of the actual phylogenetic relationships of organisms grows. In particular the premise that phylogenetic relationships can be accurately revealed by the genetic information expressed in the homologues of a relatively small number of protein types needs further testing by extending the immunochemical analysis to a larger spectrum of proteins. If this premise is correct, such an expanded analysis of hominoid proteins will not alter the main outlines of the picture of hominoid phylogeny already depicted, although it may add some details to the picture. Additional serological data might tell us, for instance, which two of the three genera (Gorilla, Pan, and Homo), have the greater degree of genetic resemblance. One might also be able to decide if as suggested by the current data these three genera have Pongo rather than Hylobates as their closest relative.

Initial results obtained with primate lens proteins (Maisel and Goodman, 1963, Fed. Proc., 22: 497) agree with the serum protein findings. With antisera to old world monkey lenses (cynomolgus monkey and green monkey) precipitin reactions decreased on going from cercopithecoids to hominoids to ceboids to lorisids to tupaiids to non-primate mammals. This set of data provides confirmatory evidence that cercopithecoids are more closely related to hominoids than to ceboids. Furthermore anti-human lens precipitins developed larger reactions with chimpanzee lens proteins than with those of old world monkeys.

The serological findings need to be expanded and integrated with the comparative anatomical and especially the paleontological evidence in order to determine a definative taxonomy of the Primates. (Supported by Grant G14152 of the National Science Foundation.)

THE STRUCTURE OF HEMOGLOBIN AND HEMERYTHRIN AS A SOURCE OF TAXONOMIC INFORMATION

Clyde Manwell

Dept. of Physiology and Biophysics, University of Illinois, Urbana, Illinois, U.S.A.

Several examples from studies by the author and colleagues have been chosen which illustrate the utility of chemical studies on respiratory pigments in contributing to biochemical systematics beyond the point of verifying the morphologically obvious.

During a survey of individuals for genetic variants of hemoglobin, it was noted that specimens of the sea cucumber Thyonella gemmata possessed either of two very different hemoglobin electrophoretic patterns. Normally in a genetic protein polymorphism, determined at a single locus, three patterns are found, the heterozygote possessing either both proteins characteristic of the homozygotes or an intermediate electrophoretic pattern, depending on the quartenary structure of the particular protein. Careful inspection of the "Thyonella gemmata" revealed two types which differ in certain minor characteristics observable only in living material. Survey of the hemoglobin patterns for 131 individuals revealed a perfect correspondence between hemoglobin pattern and "stout" vs. "thin" types. Further evidence for the existence of two sibling species with no gene flow between them is: (1) the existence of a typical genetic hemoglobin polymorphism in the "stout" race which never occurs in the "thin" population; and, (2) the esterases and a few of the otherwise identical organ-specific soluble proteins are consistently electrophoretically distinct in the two forms. So far as the investigations in the Alligator Harbor, Florida area are concerned, the two sibling species appear to be sympatric. Even after three years of investigations the author cannot differentiate the sibling species in preserved specimens; the spicule structures, upon which much of the taxonomy of holothurians depends, are identical, as are organ shape and color and the major morphological features. This example indicates that biochemical techniques can form a useful adjunct to speciation problems (Manwell, C. and C.M.A. Baker, "A sibling species of sea cucumber discovered by starch gel electrophoresis". Comp. Biochem. Physiol., in press.)

Because of the extensive fish hybridization studies of William Childers and other members of the Illinois State Natural History Survey, it has become possible to analyze the hemoglobin electrophoretic patterns and "fingerprints" from all possible hybrid combinations of the following centrarchid fishes: Chaenobryttus gulosus (warmouth sunfish), Lepomis cyanellus (green sunfish), L. macrochirus (bluegill), and L. microlophus (redear sunfish). In addition, hybrids between the black and white

crappies (Pomoxis nigromaculatus and P. annularis) and various F_2 hybrids and four-way crosses have been analyzed. These studies, based on sampling of over one thousand individuals, show certain generalizations which will be of use in the study of introgressive hybridization and related phenomena: (1) Each species and each type of F_1 hybrid has a hemoglobin pattern that is unique in starch gel electrophoresis, as well as a unique tryptic digest peptide pattern ("fingerprint"). (2) For a given species or F_1 hybrid there are no individual differences--no "abnormal" hemoglobins. (3) For most of the crosses the F_1 hybrids possess a hemoglobin electrophoretic pattern that is identical to that obtained by electrophoresis of simple mixtures of hemoglobin from the parental species. (4) However, the crossing of a warmouth sunfish with either a bluegill or a green sunfish results in hybrids in which there is, besides the parental hemoglobin types, the synthesis of 25-40% "hybrid hemoglobin"; this new protein type has improved blood gas transport properties over the hemoglobin of parent species or other hybrids. (5) F_2 hybrids from the mating of a green sunfish x warmouth bass, or from the reciprocal cross, possess three different electrophoretic patterns, corresponding to that for the green sunfish, the warmouth bass, and the F_1 hybrid, respectively. (6) Certain four-way crosses show quite a complexity of new hemoglobin electrophoretic patterns, thus suggesting that at least two different, probably unlinked genetic loci are involved.

Electrophoresis of serum proteins of hybrid sunfishes has so far indicated that the pattern of the hybrid is identical to that obtained upon mixing serum of the two parent species--no "hybrid proteins" in the serum. Studies on the serum proteins of two species of basses, Micropterus coosae (redeye bass) and M. punctulatus (spotted bass), show protein zones similar to those obtained by mixing sera from Micropterus dolomieu (smallmouth bass) and M. salmoides (largemouth bass). The hemoglobin electrophoretic patterns of redeye and spotted basses are almost identical and resemble that from a hypothetical hybrid--complete with "hybrid hemoglobin"--between largemouth and smallmouth basses. By polypeptide chain recombination studies with largemouth and smallmouth bass hemoglobins it has been possible to obtain a new hemoglobin electrophoretic pattern which is similar if not identical to that observed for hemoglobin from redeye and spotted basses. While such studies could be interpreted as indicating that evolution of smallmouth and largemouth basses has proceeded in exactly opposite ways from an ancestral type which evolved with much less modification to the present redeye and spotted bass species, it is much more likely that the results are indicative of introgressive hybridization between largemouth and smallmouth basses, followed by subsequent evolution of redeye and spotted basses from the introgressants (Manwell, C., C.M.A. Baker, and W. Childers, "Molecular genetics of hemoglobin in hybrids. I. A biochemical basis for 'hybrid vigor'." Comp. Biochem. Physiol., in press.)

"Fingerprint" studies on hemoglobin and hemerythrin primary structures have interesting possibilities from the standpoint of the taxonomy of higher systematic categories. Hemerythrin from the brachiopod Lingula has a similar number of tryptic or chymotryptic peptides when compared with the appropriate digest of various sipunculid hemerythrins (Manwell, C., "Genetic control of hemerythrin specificity in a marine worm." Science, 139:755-758, 1963.); in both cases each hemerythrin is now believed to be the product of two cistrons in spite of the observation of only one type of C- and one type of N-terminal amino acid. The similarities extend further in that certain histidine-rich peptides have similar chromatographic and electrophoretic properties for both brachiopod and sipunculid hemerythrins. This suggests that there are certain genes with rather similar structures in both sipunculids and brachiopods--phyla that are not usually considered closely related.

"Fingerprinting" of various vertebrate and invertebrate hemoglobins has shown that the peptide pattern for hagfish Myxine is different from that of lampreys, fishes, sharks, amphibians, reptiles, birds, and mammals. In fact, there are more similarities between lamprey hemoglobin and human hemoglobin than between Myxine hemoglobin and lemprey hemoglobin. There are also distinct similarities in the "fingerprint" patterns between holothurian and vertebrate hemoglobins, in contrast to the hemoglobin from other invertebrate representatives (annelids and molluscs). While other structural information besides number and distribution of peptides in the hemoglobin "fingerprints" must be considered in such evaluations, it would appear as if hagfishes and lampreys are not closely related and that the remainder of the vertebrate classes evolved from ancestors much more closely related to those which gave rise to the modern lampreys than to the line that has culminated in the hagfishes. This conclusion is also supported by new data on serum proteins, as well as by studies on osmoregulation and morphology (Manwell, C., "The blood proteins of cyclostomes: a study in phylogenetic and ontogenetic biochemistry." In The Biology of Myxine, A. Brodal and R. Fänge, eds., in press.)

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MIMICRY

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by

Lincoln P. Brower

Department of Biology
Amherst College
Amherst, Massachusetts

HISTORICAL BACKGROUNDS OF MIMICRY. Charles L. Remington, Department of Biology, Yale University, New Haven, Conn., U.S.A.

The term MIMICRY has its origin in a non-technical term used freely in most European languages and meaning "imitation." With such an origin, it is all too normal that its use in evolutionary biology has been so diverse that it is not ideal as a technical term. However, for this symposium we shall keep the word MIMICRY but restrict it to the sense of CLOSE RESEMBLANCE OF ONE ORGANISM TO ANOTHER WHICH, BECAUSE IT IS UNPALATABLE AND CONSPICUOUS, IS RECOGNIZED AND AVOIDED BY SOME PREDATORS AT SOME TIMES. This excludes instances of an organism having cryptic characteristics that help it to go unnoticed by potential predators.

While the many-faceted explanation of mimicry is an evolutionary concept, mimetic animals were recognized long before the establishment by Darwin and Wallace in 1858 and 1859 of the idea of evolutionary change regulated by natural selection. Kirby and Spence in 1817, for example, noted that certain Drone Flies strikingly resemble bumble-bees "in shape, clothing, and colour," and various 18th Century taxonomists called attention to the close resemblance of insects of different orders to each other by the species names they invented, such as various aegeriid moths named Spheciformis, Apiformis, Vespiformis, and Ichneumoniformis. Darwin did not deal with the area of adaptive coloration in the first edition of the Origin of Species, and it is H. W. Bates who, in 1862, first published a clear discussion of mimicry. Rössler had written the year before, in a brief general account of the significance of the shape and color patterns of the wings of Lepidoptera: "a further purpose of the appearance concerns the protective characteristics whereby they attempt to deceive the eye of the enemy through resemblance to other objects, to plant parts, and even to animals, as for example the sesiids, which Lepidoptera masquerade as species of bees and wasps (e.g., apiformis as the dangerous Hornet)" (translation). Bates in fact referred to Rössler's paper but may not have read it in full, because he mentioned only the point of resemblances to vegetable matter. No doubt there are other correct pre-Batesian statements of mimicry phenomena, and Rössler is worth mentioning merely to show in the historical context that Bates's important paper did not burst forth on a totally unprepared group of naturalists. Bates had recently returned from eleven years of field research in the Amazon region. There he had collected 94 species ("and many local varieties") of what he called "Heliconidae" belonging to two separate types which he named "Danaoid Heliconidae" (67 species) and "Acraeoid Heliconidae" (27 species). (They are now considered far apart phylogenetically: the Ithomiinae and Heliconiinae.) He found these butterflies exceedingly abundant, "although of slow flight, feeble structure, unfurnished with apparent means of defence, and living in places which are incessantly haunted by swarms of insectivorous birds." He was especially impressed with the presence in each locality of extremely rare Pieridae, Leptalis, which so exactly resembled certain

numerous "Heliconidae" that he wrote: "I was never able to distinguish [on the wing] the Leptalides from the species they imitated." Bates concluded that "Heliconids" are probably unpalatable to predators, that Leptalis is edible, and that its mimicry is "apparently its only means of escaping extermination by insectivorous animals." He explained the resemblance of Leptalis to "Heliconids" as being the result of a) the occurrence of small variations, among which those producing some resemblance to the unpalatable "Heliconids" were slowly accumulated b) through the agency of Darwinian selection whereby predators discriminated against non-imitative variations. He also tabulated whole complexes which variously included Leptalis and both types of "Heliconidae," plus unrelated butterflies and day-flying moths such as Papilionidae, Riodinidae, Castniidae, Pericopidae, and Dioptidae, all members of each complex presumably looking alike to potential predators. Bates was unable to explain the "imitative resemblances" between a distasteful "Acraeoid" and a distasteful "Danaoid," although he considered them to be the result of natural selection. He pointed out the widespread occurrence of mimicry in various parts of the world and in several groups of animals.

While working on a new synthetic volume on mimicry theory during the last several years, we have compiled a bibliography of nearly 1500 references. It is obviously impossible to cover any significant proportion of these publications for the purposes of our symposium. In this short discussion of the history of mimicry studies I shall proceed mainly chronologically but will sort out at each stage what I consider the major theoretical discoveries, which together make up the unified explanation of mimicry phenomena as we understand them today. In the course of this account I will refer to the serious counter-arguments which have been raised. The post-War research will be the subject of the other papers of this symposium.

Returning to Bates, we can credit him with the principal discovery of these factors of mimicry: 1) THERE ARE ANIMALS WHICH ARE UNACCEPTABLE TO PREDATORS DUE TO CHEMICAL AND/OR PHYSICAL CHARACTERISTICS; 2) THESE ANIMALS HAVE EVOLVED CONSPICUOUS ("WARNING") COLORATION AND BEHAVIOR WHICH MINIMIZE PREDATOR MISTAKES; 3) PALATABLE SPECIES HAVE EVOLVED AWAY FROM THEIR ANCESTRAL APPEARANCE AND COME TO RESEMBLE THE CONSPICUOUS, UNPALATABLE SPECIES SO CLOSELY THAT THEIR POTENTIAL PREDATORS ARE DECEIVED INTO LEAVING THEM ALONE; 4) THESE EDIBLE "MIMICS" TEND TO BE SCARCER THAN THEIR MODELS; AND 5) MIMICRY IS A WORLD-WIDE PHENOMENON BUT TENDS TO BE MOST EXTENSIVE IN THE NEW AND OLD WORLD TROPICS.

Soon afterward (1865), Wallace described mimicry complexes in the Indo-Malayan region, and these supported the basic points of Bates. He discussed the fact that 6) IN THE SAME POPULATION ONE SPECIES MAY HAVE TWO OR MORE VERY DIFFERENT FORMS, EACH OF WHICH MIMICS A DIFFERENT MODEL and that 7) THESE TEND TO BE POLYMORPHIC ONLY IN THE FEMALE.

During the late 19th Century the whole idea of evolution was under heavy, often emotional fire from philosophical and religious premises, but this was not of great concern to mimicry specialists. Much more harassing, and ultimately constructive, were the attacks of those biologists who accepted the idea of evolutionary change but rejected the Darwinian explanation that it proceeds by the action of natural selection on small, randomly occurring variations. The mechanism of heredity and therefore that of the origin of new hereditary material was still not understood, and a common view was the Neo-Lamarckian principle of induction by environmental stress of "needed" new heritable types. On this and other grounds a large group of antagonists to the mimicry hypotheses developed, and the debate in print has often been passionate and even insulting on both sides. The gist of the arguments of the antagonists was: 1) the putative selective agent disappears and so does the mimicry theory, because birds, lizards, et al. usually do not eat butterflies; 2) but even when they do, the so-called distasteful species are eaten as much as others; 3) the similarities attributed to mimicry are variously due to a) accidental resemblance, b) common ancestry of the similarities, or c) similar response by the several species to the similar physicochemical environment.

Fritz Müller, like Bates before him observing butterflies in the field in Brazil, deduced (1878) a solution to Bates's paradox of two or more distasteful but distantly related species looking alike. Müller reasoned that 8) PREDATORS MUST TEST UNPALATABLE SPECIES BEFORE LEARNING TO RECOGNIZE THEIR WARNING CHARACTERISTICS AND LET THEM ALONE, AND IN THE PROCESS OF PREDATOR EDUCATION A PORTION (n INDIVIDUALS) OF THE POPULATION OF EACH UNPALATABLE SPECIES MUST BE SACRIFICED; BUT IF THERE ARE TWO UNPALATABLE SPECIES (HAVING POPULATION SIZES OF a_1 AND a_2) THAT EVOLVE A SUFFICIENTLY SIMILAR APPEARANCE SO THAT PREDATORS DO NOT DISTINGUISH BETWEEN THEM, THE TWO SPECIES WILL SACRIFICE ONLY $\frac{a_1 n}{a_1+a_2}$ AND $\frac{a_2 n}{a_1+a_2}$ INDIVIDUALS, RESPECTIVELY.

Marshall (1908) argued against Müller's hypothesis on the ground that if the two species, A and B, are equally unpalatable and A is more numerous than B, any mutant in A causing resemblance to B will be less effective in escaping predation than was the normal type, and selection will therefore eliminate the B-like mutants in A. Extending this line, he showed that if A and B are equally numerous and unpalatable, no convergence will take place. Dixey (1909) took the opposite view, that mutants of A intermediate toward B would escape predators educated by A as well as by B. Fisher (1927) reasoned that variations, being random around the average type, are equally frequent in both directions and that the fate of B-like mutants in A must be compared with the fate of the exactly opposite B-unlike mutants. Since the two mutants of A are of equal deviation from the average, they will lose equally by not being recognized as A; but if the mutant toward B is sometimes mistaken for B, it will be favored over its

opposite, and the net result will be an approach toward B by A as well as an even more rapid approach toward A by B.

Wallace (1889), after summarizing the findings of Bates and Müller, proposed 9) AN EXTENSION OF MÜLLERIAN MIMICRY WHEREBY SEVERAL MEMBERS OF THE SAME UNPALATABLE GENUS LOOK ALIKE IN THE SAME LOCALITY (e.g., 4 or 5 Heliconius having a yellow-banded forewing and radiating red stripes on the hindwing).

This really is somewhat different from Müller's case of convergence of widely unrelated species. Modern speciation theory predicts that closely related species when sympatric will diverge in appearance, habits, and season due to rigorous selection for the two main speciation sequelae: anti-hybridization mechanisms and niche diversification (anti-competition). Wallace's Müllerian extension explains an important deviant. He also suggested the possibility of a still different sort of mimicry, in which 10) A SCARCE EDIBLE SPECIES CAN MINGLE WITH AND CLOSELY RESEMBLE AN ABUNDANT EDIBLE SPECIES AND THUS GAIN SOME FREEDOM FROM PREDATION. A similar but more elaborate hypothesis has recently come from van Someren and Jackson (1960).

Wallace, like many evolutionists of his day, thought that distastefulness and warning colors CAUSED the abundance of species like Danaus plexippus. This view has been rejected by various more recent writers, perhaps first in an explicit form by Nicholson (1927). The most valid explanation seems to be that: a) in the long run population size is not primarily regulated by predators and parasites, but rather by food; b) the competition in the evolution and stabilization of mimicry is intra-specific (mainly between possessors of one or a few pairs of alleles, as is usual in the operation of natural selection), and population size is little influenced. It needs to be noted, however, that at crash phases in the normal oscillation of population size, predation might eliminate the last few survivors of an edible non-mimic and bring about extinction.

Wallace discussed probable mimetic complexes based on venomous snakes in South America and South Africa and on distasteful birds in the East Indies. Since his day, although mimicry in the Lepidoptera has received the most attention, there has also been more or less sophisticated research on very many complexes based on lycid beetles, stinging Hymenoptera, ants, and Hemiptera. A few instances of supposed mimicry have been reported for a scattering of other groups.

Perhaps the most serious challenge to mimicry hypotheses has come in the area of the true role of the reputed predators (mimefactors). These detractors, such as Manders, McAtee, and Skinner, stimulated a great burst of studies aimed at gathering fuller evidence, leaders being Trimen, Weismann, Jordan, Eltringham, Haase, Swynnerton, Jones, and especially Poulton and Carpenter. One group of papers reports experiments with caged or tame birds, lizards, amphibians, and mantids. The most extensive tests include those of Marshall (e.g., 1909), Swynnerton (e.g., 1915), and Jones (e.g., 1932), Manders, Heikertinger, and others who questioned the validity of explaining natural behavior from

captive insectivores. McAtee and his staff examined the stomach contents of tens of thousands of wild North American birds and reported Lepidoptera almost entirely absent; on the other hand they found many stinging Hymenoptera and supposedly toxic Coleoptera. The other major criticism of edibility "experiments" has been that until recently few have been carried out by the critical methods standard in experimental biology (e.g., adequate controls, replicates, minimizing of variables, and statistical treatment of data).

A second source of data on predator response in the wild to Lepidoptera has been the presence of beak marks on wings of preserved specimens. Carpenter has been especially prominent here (e.g., 1933). It is probably valid to conclude that nearly all crisp beak marks indicate that the bird or lizard caught the insect in a dash when careful visual examination had not been possible, that it immediately recognized the prey as a species to be rejected (either due to having learned its unpalatability and color pattern or to mistaking it for its unpalatable model) and released it unharmed. And the beak-mark records do show regularities which correlate well with palatability data from other sources and yet extend these data to new material and suggest important groups to be studied experimentally.

A remaining sector of mimicry problems is the genetics of mimicry. Genetics of polymorphic species of Papilio and Limenitis were studied fragmentarily very soon after the establishment of the field of genetics around the last century, notably by Fryer, Jacobson and de Meijere, Lamborn, Rogers, and Platt. The polymorphs in general are controlled as simple Mendelian factors.

THE GENETICS OF MIMICRY. P.M. Sheppard, Department of Genetics, The University, Liverpool, England.

The primary importance of genetic studies of mimicry resides in the help thus given to the understanding of the evolution of the phenomenon and of evolution in general. Unfortunately, only in butterflies are enough genetic data available for useful conclusions to be drawn on the evolution of mimicry; consequently I shall confine my remarks to them.

Most of what is known about the mode of inheritance of the morphs of polymorphic mimetic butterflies can be found in the reviews by Carpenter and Ford, 1933; Ford, 1937, 1953; Sheppard, 1961, 1962. I am very grateful to Dr. E.B. Ford, F.R.S., Dr. C. A. Clarke, and Mr. J.R.G. Turner for reading the ms. and for their very helpful suggestions.

Batesian Mimicry. Fisher and Ford proposed that in the evolution of Batesian mimicry the mimetic resemblance first appears in a very imperfect form as the result of a mutation. If the resemblance is sufficiently good to give the mutant a slight advantage it will increase in frequency. Variation in the rest of the gene-complex which alters the effect of the new mutant towards more perfect mimicry will be selected and thus the effect of the gene will be changed gradually until the very good resemblances observed in nature are obtained. Sheppard, 1958, has suggested that the initial change, in order to produce an advantageous resemblance to the model, may have to be large and therefore that the main difference between monomorphic mimetic and non-mimetic species will often be controlled by a single gene. Moreover, in polymorphic species the original mutants will act as switch genes (Mather, 1955).

Genetic investigations do indicate that monomorphic mimetic races and species differ from their non-mimetic relatives by one or at the most two major genes (Clarke and Sheppard, 1955, 1956; Remington, 1958) and that major genes do act as switch genes in polymorphic species (see Ford, 1953).

The main genetic evidence to support the hypothesis that the mimicry arises in an imperfect form and is then improved by the selection of modifiers comes from work on the highly polymorphic butterfly Papilio dardanus (Clarke and Sheppard, 1959, 1960a, b, 1962a, b.). This species is polymorphic in the female but the males are monomorphic, non-mimetic and tailed. Females are monomorphic and like the males in Madagascar, but both male-like and mimetic forms are found in Abyssinia, although the mimetic forms, unlike their models, are tailed. Over the rest of Africa the females are polymorphic, and unlike the males in colour-pattern. They are always tailless, like the models, although not all are mimetic. No one form of the female is found in all races of the species.

Clarke and Sheppard have shown that the sympatric female forms are controlled by a multiple allelomorphic series of at least twelve allelomorphs (or perhaps a small number of closely linked

genes whose linkage has been evolved, thus forming a super-gene), sex-limited to the female. They further found that in any one area the difference between the sympatric forms is controlled by single genes. However, allopatric forms show no such simple inheritance, but the nature of the complication differs from example to example.

The genetic difference between mimics of different sub-species of one model.

The mimics of Amauris niavius. The allopatric forms hippocoonides, hippocoon and niavioides mimic the sub-species dominicanus, niavius and aethiops of A. niavius respectively. Hippocoonides and hippocoon are good mimics of their respective models and differ from one another polygenically. The Abyssinian form, niavioides, which is a much poorer mimic of its model, differs from the other two polygenically and also by a major gene which controls the presence of the tails on the hind wings (see below). Thus, unlike the situation found with sympatric forms, these rather similar allopatric mimics do not differ from one another on a unifactorial basis.

The mimics of Amauris echeria. The form cenea of P. dardanus mimics Amauris albimaculata and A. echeria and is found along the east coast of Africa from South Africa to Abyssinia inclusive and as far west as Lake Victoria. However, in Northern Kenya on Mount Marsabit and Mount Kulal, cenea is absent and is replaced by a distinct allopatric form ochracea, mimicking the local model A. e. septentrionalis. Cenea and ochracea differ from one another by a single allelomorph. However, the particular allelomorph responsible for the ochracea pattern produces a pattern indistinguishable from that of cenea in most gene-complexes. In the populations on Mount Kulal and Mount Marsabit there are special modifiers present which affect the ochracea allelomorph H^o but not that for cenea, H^c, to produce the ochrous-spotted mimic (Clarke and Sheppard, 1962a).

In other parts of Kenya where the models are rare, there are at least two and probably more than two allelomorphs producing a cenea-like pattern. It therefore seems probable that in the evolution of ochracea special modifiers, affecting only the local "cenea allelomorph" H^o, have been selected. If this interpretation be true then the difference between ochracea and an ancestral cenea-like form is multifactorial, thus paralleling the evolution of the difference between hippocoon and hippocoonides.

The effect of major genes in different gene-complexes. In P. dardanus the allelomorphs h, H^c, H^o and H^T control the mimetic forms hippocoonides, cenea, ochracea and trophonius (the latter mimicking Danaus chrysippus) and each produces good mimicry in the gene-complexes of the populations in which the allelomorph is found. However, on crossing one of these forms to a race that does not possess the allelomorph in question, poor mimicry is obtained (Clarke and Sheppard, 1960c and in press). Thus the allelomorphs producing good mimicry do so only in the gene-complexes of those races in which they occur and not in others where there

has been no opportunity to evolve a gene pool co-adapted to them.

Disruptive selection on a metrical character. Although mimetic forms of Papilio dardanus are found in Abyssinia they are less perfect than elsewhere (with the possible exception of parts of Kenya where the models are rare) because all the females have tails, whereas the models are tailless. If tails are advantageous in the male-like females, as they probably are, but disadvantageous in the mimics, there will be disruptive selection in Abyssinia, tending to reduce tail length in the mimetic females but not in the male-like ones (see Mather, 1953).

Thoday's experimental investigation in Drosophila melanogaster (Thoday, 1959, 1960; Thoday and Boam, 1959) suggests that disruptive selection on a metrical character will increase the variance of that character and may lead to a bimodal distribution of the character. This is apparently what has happened in Abyssinia (Clarke and Sheppard, 1962c). The variance of tail length in the Abyssinian females is greater than in males and in the monomorphic non-mimetic male-like females of Madagascar. Moreover, the mean tail length of the mimetic females is less than that of the non-mimetic male-like forms in Abyssinia and this difference is polygenically controlled.

As Dr. J.V.Z. Brower once pointed out to me, these findings are particularly relevant to the situation in Limenitis archippus in Florida. The local form floridensis mimics Danaus gilippus berenice and differs from archippus mimicking Danaus plexippus by two major genes (Remington, 1958) and probably a number of modifiers. However, inspection of the Florida material shows that floridensis is a much poorer mimic of its model than is archippus of its model and moreover, is far more variable in colour-pattern. Now both the models are found in Florida although D. plexippus is much the rarer in summer (Brower, 1961). This suggests that there is disruptive selection acting on L. a. floridensis which would account for its variability. It would be particularly instructive to investigate this variability to see if there is any sign of a bimodality.

The data available indicate that Batesian mimicry in butterflies is controlled by major genes, but that good mimicry depends on the rest of the genotype becoming co-adapted to these major genes. There is also evidence that non-mimetic selection is acting. It seems probable that the polymorphisms, once maintained by the mimicry, have evolved stability based on the selective advantage of heterozygotes.

The genetics of Müllerian Mimicry

Although in theory Müllerian mimicry should lead to monomorphism there are a number of species which are polymorphic, notably in the genus Heliconius in South America. What is particularly striking in this group is that when a Müllerian mimetic species is polymorphic the several morphs are often either mimics of other monomorphic species or they are mimicked by similar morphs in other species. Thus Heliconius numata is a member of that huge group of Müllerian mimics which have a black, brown and

yellow "tiger pattern"; a group which includes several Heliconids as well as butterflies in other families, for example the Danaids Lycorea and Mechanitis. H. numata is usually monomorphic but in Trinidad, besides the usual brown form mimicking the local Lycorea ceres and Heliconius isabella among other species, there is also another form which has a yellow instead of a brown stripe on the hind wing. This yellow form is very rare or absent from mainland populations. The yellow form is not a very good mimic of Lycorea but comes much closer to the pattern of the common rather yellow Trinidad sub-species megara of Tithorea harmonia, although it is not as good a mimic of that species as is the brown form a mimic of Lycorea ceres. The yellow form in H. numata is dominant and controlled by one or perhaps two major genes (Crane and Sheppard, in preparation).

The very closely related Heliconius melpomene has an entirely different pattern from H. numata. The species is monomorphic in Trinidad and is so close in appearance to another species, Heliconius erato that only an expert can tell them apart. The pattern of these species in Trinidad is black with a broad red sub-apical band on the forewing. In Surinam both species are highly polymorphic and show remarkable parallel variation in their morphs (Beebe, 1955; Turner and Crane, 1962). A random sample from Surinam (Table 1) indicates that the broad-banded form, the only one in Trinidad, is the commonest form. Moreover,

TABLE I

Random samples of H. melpomene and H. erato collected as eggs, larvae and adult butterflies at Moengo, Surinam from July 21st to August 3rd, 1962.

Phenotype	H. melpomene	H. erato
Broad-banded	115	49
Narrow-banded	15	8
Radiate broad-banded	7	10
Radiate narrow-banded	0	6
Dennis broad-banded	11	0
Dennis narrow-banded	1	0
	149	73

Assuming that dennis and radiate are controlled by allelomorphs their gene frequencies are estimated at 0.042 and 0.024 in H. melpomene and 0.000, 0.116 in H. erato respectively. Assuming that all the narrow banded individuals in the sample of H. melpomene are dominant, the estimated gene frequencies are 0.055 for H. melpomene and 0.101 for H. erato.

the relative frequencies of the corresponding morphs in the two species in Surinam compare well with each other (correlation coefficient $r = 0.817$ $P < 0.05$) although there are notable differences. The nature of the genetic control of the morphs in the two species also shows similarities (see below).

In H. erato, besides the broad-banded form (the only one in Trinidad) there is another, "dennis," in which there is a red

area at the base of the forewing. In a third form, "radiate," besides the red base to the forewing, there are red ray-like marks on the hind wing. A fourth form "narrow banded" has the red forewing sub-apical band invaded by black. This is a rather variable character and can be found in combination with dennis, with radiate or on its own. The dennis pattern has not been investigated genetically but the radiate form (which incorporates the dennis markings) is dominant to the non-radiate one. The invasion of black pigment into the red sub-apical bar is apparently controlled by a single gene dominant in effect. It was suggested (Turner and Crane, 1962) that this gene was linked to the locus producing the dominant rayed pattern, but more recent evidence suggests that the two are not closely linked (Crane and Sheppard, in preparation).

In H. melpomene there are again several forms in Surinam: 1) broad-banded, the Trinidad form with black wings except for the broad red sub-apical bar, 2) the "dennis" form with a red area at the base of the forewings and, in this species but not in erato, a small red basal patch on the hindwings and 3) the radiate pattern, in which there are rays on the hindwings, and which in Surinam always incorporates the dennis pattern. The rays are different from those in erato both in form and distribution. The other pattern - "narrow-banded" (as in erato found in combination with dennis, with radiate or on its own) has the sub-apical red bar much reduced but this is achieved by a narrowing of the band not by an invasion of black pigment into the bar as in erato. The dennis pattern is dominant to non-dennis as is the radiate pattern to non-radiate although the latter could be semi-dominant. Turner and Crane, 1962, showed that narrow-banded is recessive to broad-banded and controlled by a gene closely linked to the locus controlling dennis. Subsequent investigation, however, (Crane and Sheppard, in preparation) has shown that there is a second gene semi-dominant in effect and unlinked with that controlling dennis, which also produces a narrow banded form which, in the heterozygote is indistinguishable morphologically from the homozygote of the recessive one. Thus in these two species the patterns look much alike but differ morphologically in detail. Moreover, the genetic control of the pattern shows considerable similarities except that the equivalent of the recessive narrow-banded form of H. melpomene has not yet been found in H. erato.

It is important to note from the point of view of the evolution of Müllerian mimicry that unlike the situation in Papilio dardanus which is a Batesian mimic, in both H. melpomene and H. erato the crossing of the morphs from Surinam with the monomorphic race from Trinidad produces no apparent breakdown in the mimicry. However, the Surinam population may itself be subject to much gene-flow from the monomorphic populations to the west.

Although there is a reasonable explanation for the maintenance of a polymorphism in Batesian mimics and evidence that once a mimicry has arisen it is improved by the selection of modifiers

affecting the expression of the major genes, in Müllerian mimicry there is as yet no adequate explanation for the maintenance of a polymorphism and the data such as they are give no evidence that modifiers affecting the pattern have been selected. It is perhaps pertinent that similar radiate patterns to those found in H. erato and H. melpomene are also found in other members of the genus, some rather distantly related. Thus, Heliconius doris, which is itself polymorphic and in which one morph mimics several monomorphic species (e.g., H. sara and H. wallacei) there is also a red form both in Trinidad and Surinam which may be a mimic of H. ricini and is controlled by a gene probably dominant in effect (Crane and Sheppard, in preparation). This form has red rays on the hindwing and a red patch on the base of the forewing, thus, in this respect, being very similar to the rayed pattern of both erato and melpomene. Moreover, in Surinam where the red on the sub-apical bar of the forewing is sometimes partially or completely converted to yellow in H. erato and H. melpomene (a feature not discussed here) the red rayed form of H. doris is often quite a good mimic of radiate individuals in the other two species and appears to be rather commoner than it is in Trinidad. The red form of doris is also a fine mimic of Heliconius burneyi form burneyi which is found in the "Guianas" but is apparently more abundant further east at the mouth of the Amazon.

Conclusions. Although genetic investigations are producing data in agreement with previous hypotheses on the evolution of Batesian mimicry, the rather scanty information on Müllerian mimicry suggests that existing hypotheses on the evolution of the mimicry need some revision. There is an indication, however, that the frequency of the forms in polymorphic species is being influenced to some extent by the presence of similar forms in other species; that is to say the frequencies are not wholly dependent on non-visual selection. It might be argued that the correlation between the presence and frequency of similar morphs, such as it is, is due to similar climatic or other non-mimetic selection, coupled with gene flow between populations. But this does not explain all the polymorphisms for if Robertson (1962) be correct, the frequency of some of the genes responsible for the Müllerian mimicry is too low for the polymorphism to be maintained by the advantage of heterozygotes alone.

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EXPERIMENTAL STUDIES AND NEW EVIDENCE ON THE EVOLUTION OF MIMICRY IN BUTTERFLIES. Jane Van Zandt Brower, Biology Laboratory, Amherst College, Amherst, Massachusetts, U.S.A.

A vast observational literature exists on mimicry, which has been reviewed by Carpenter and Ford (1933). The theoretical aspects have been discussed by Fisher (1930), Ford (1936), Goldschmidt (1945), Nicholson (1927), Punnet (1915), deRuiter (1958), and Sheppard (1959), but experimental evidence has been very incomplete. Direct demonstration of mimicry in nature represents a problem of the utmost difficulty because of the complexity and rarity of the events concerned. The most that one could hope to see would be an occasional predator in the act of eating or rejecting a model or mimic insect. To witness a sequence of experiences in the wild in which the same predator was observed to

learn that a model species is unpalatable, to reject it at a later encounter, and still later refuse the mimic, would be extremely unlikely. Indirect evidence for mimicry, as will be seen below, can sometimes be inferred from natural populations of models and mimics. However, the most reasonable approach to the problem of actually testing its effectiveness, in lieu of natural observations, is the use of caged predators in controlled experiments designed to give results that can be subjected to statistical analysis. By the use of this method, an attempt is being made to shed light on the abilities of predators to act as selective agents in bringing about the evolution and maintenance of mimicry. A brief account of the work to date will be followed by a consideration of the effect of one aspect of predator behavior on the evolution of mimicry.

In the initial experiments caged scrub jays were used as predators of three complexes of North American mimetic butterflies (J. Brower, 1958a, b, c). In order to test for mimicry in each, a group of four experimental birds was trained on the model butterfly which they learned was unpalatable and came to reject on sight. This is a necessary feature for the evolution of mimicry, for if every individual insect had to be tried before it was rejected, mimetic advantage would never be gained for a palatable mimic. Following experience with the model, each bird was then given the mimic, and this too was refused on sight in most trials. A group of four control birds, which were not given experience with the models, found the mimics to be relatively palatable, and thus Batesian. A highly significant statistical difference was obtained in the number of mimics not touched vs. those pecked, killed, or eaten by the experienced experimental animals vs. the inexperienced controls (J. Brower, 1958a, b). The higher rejection of the mimics by the experimental birds was attributed to their association of the mimetic color-patterns with those of the respective models.

Throughout the tests, all the birds made occasional errors in which they pecked a model which had already been recognized as unpalatable in a series of rejections. This behavior has been described by Swynnerton (1915) and theoretically expanded by Fisher (1930). The occurrence of errors is of undoubted selective value to the predator in the instance of Batesian mimicry, because should the frequency of the mimic increase relative to the model, a bird would readily become aware of the altered situation. It could then incorporate into its diet insects of a color-pattern which now represented an edible food item. Should the mimic become preyed upon in this way, one would predict that the reduced or absent selective advantage for mimicry might bring about variability or breakdown of the mimetic color-pattern. Such a change is based upon the assumption that the mimetic state is favorable to some alternative one only under conditions of model abundance, and that other factors, probably related to sexual behavior, would tend to favor the non-mimetic condition (see L. Brower, this volume). That breakdown of mimetic color-pattern does in fact occur

in nature when a model is scarce relative to its mimic has been demonstrated for African butterflies by Carpenter (1920), Ford (1936), and reviewed by Sheppard (1959). Brower and Brower (1962b) have shown that the frequencies of the mimetic and non-mimetic female forms of the tiger swallowtail butterfly Papilio glaucus are adjusted to the relative abundance of the model, Battus philenor. However, even if the model is rare, the mimic may still have a selective advantage. Experiments with caged starlings as test predators showed that artificial model insects made highly unpalatable with quinine conferred a 17% advantage upon visually identical artificial mimics when the latter were as high as 90% of the combined laboratory population of models and mimics (J. Brower, 1960). This result indicated that substantial mimetic advantage can still occur when the mimic greatly outnumbers its model, although at model frequencies of 50% or greater, the mimetic advantage was 80% or more.

In addition to mimicry in butterflies, the phenomenon has been studied in Hymenoptera and Diptera by Mostler (1935). More recently, the resemblance of robberflies to bumblebees, and of droneflies to honeybees, has been tested (Brower, Brower, and Westcott, 1960; Brower and Brower, 1962a). The same basic design described above was used and caged toads proved to be excellent subjects. In both instances the flies were effective Batesian mimics, and the noxious quality of the bees was shown to be the sting, for individuals with the sting removed were fully palatable to the toads. One finding of particular interest to mimicry theory emerged from these experiments. For the first time, it has been possible to show that the relative degree of noxiousness of a model affects (1) the number of times it is attacked, and (2) the number of mimics protected from predation. Table 1 shows that the bumblebee models, which were far more noxious than honeybees to judge from the reactions of stung toads, were attacked less than 20% of the time, while honeybees were subject to much more frequent attack. Furthermore, the percentage of robberfly mimics not eaten was much higher than that for droneflies. Thus two levels of noxiousness conferred corresponding degrees of protection upon the respective mimics.

Table 1

The numbers of live intact bumblebees and honeybees attacked by learning experimental toads and the numbers of respective mimics subsequently not eaten by them.

	Bumblebees & Robberflies	Honeybees & Droneflies	
		Expt. 1	Expt. 2
No. Bees Attacked	9/51 (18%)[1]	36/80 (45%)[2]	77/110 (70%)[3]
No. Mimics Not Eaten	28/30 (93%)	34/60 (57%)	13/22 (59%)
No. Toads	3	5	22

1 from Brower, Brower & Westcott, 1960; 2 from unpublished data;

3 from Brower & Brower, 1962a.

In all of our experiments, non-mimetic insects of known palatability and of similar size to the test insects, but of different color-pattern and/or shape, were given to the caged predators in random sequence before or after experimental models or mimics. By this procedure, each predator showed discrimination by memory and not by comparison between an edible food item which was always eaten as a criterion for further experimentation, and an unpalatable model or its mimic, which might be rejected. The scrub jays, starlings, and toads all learned to make this distinction. However, scrub jays trained on the model butterfly Danaus plexippus which is orange with black and white, learned to reject it and then confused with the model its relatively palatable mimic, Limenitis a. archippus. But they also failed to recognize as different the mahogany-colored race, L. a. floridensis, and even its model, D. berenice which bears only a general resemblance to D. plexippus. This tendency of birds to generalize from experience with one model to refuse a mimic and model of a darker color suggested that even a broad similarity may be of protective advantage and that an incipient mimic would thus be favored over its non-mimetic variants.

The works of Mühlmann (1934), Schmidt (1958), and Sexton (1960) with variously contrived artificial Batesian mimics, and with birds, chicks, and lizards, respectively, as predators, have also given evidence that generalization takes place. The results showed that although closer mimicry gave more protection, a quite remote resemblance of mimic to model allowed some reduction in the amount of predation on the mimic. However, in all instances of effective mimicry at least one color component of the model was present also in the artificial Batesian mimics.

In Müllerian mimicry, as Fisher has emphasized (1930), one would predict that generalization would occur because all of the mimicking species are unpalatable and there would be strong selective advantage for rejection of the color-pattern on sight by predators, excepting under conditions of food scarcity. However, there are indications that even in a Batesian situation a model can confer protection upon a generalized mimic. One would predict that if this is so, then still greater diversity in appearance of model and mimic should be effective where all the mimicking species are Müllerian. Let us consider two extreme conditions in which a pair of unpalatable insects (1) have no color components in common but are similar in shape and morphology; or (2) have no similarity in shape or morphology, but do share one or more color components. Can Müllerian advantage accrue in either or both?

There is a striking example of the second alternative which was investigated by Morgan (1900) and by Windecker (1939). They found that inexperienced birds would initially attack the yellow and black banded caterpillars of the Cinnabar moth, but learned to reject them because of their noxious hairs. These same birds would thereafter refuse any insect with similar yellow and black color-pattern, even though its shape and form were very different, e.g.,

an adult wasp. Recent studies of Müllerian mimicry among the Heliconiine butterflies of Trinidad showed that they are indeed unpalatable, as well as effective mimics, to caged, wild-caught silverbeak tanagers (Brower and Brower and Collins, in press). Palatable Satyrid butterflies were eaten by all birds throughout the experiment. Evidence was also obtained which lends support to the idea that Müllerian advantage can occur between 2 species of butterflies which share a similar shape and form, but no color components. As part of a much larger investigation, 11 birds were trained on a monocolored (orange) unpalatable butterfly, which they learned to reject on sight. After 20 presentations of this model, the birds received its Müllerian mimic, also orange, which they refused to take. In a reciprocal test, this model served as the mimic, and vice versa. At the end of both of these tests, a single butterfly of bicolored blue and yellow pattern, but of similar form and shape, was offered to each bird to test for generalization. These were not touched on sight (table 2).

Table 2

The number and frequency of birds which rejected first models compared to the same birds' subsequent rejection of first mimics and generalization butterfly.

MODELS	MIMICS	GENERALIZATION BUTTERFLY	No. and frequencies of birds not touching First Model	First Mimic	Generalization butterfly
Mono-colored, orange	Similar to model	Bi-colored, blue & yellow	5/19 (.26)	10/10* (1.00)	10/10 (1.00)

* Discrepancy due to death of 9 birds.

Now these results were compared with the reactions of another group of birds to the bicolored blue and yellow butterflies, offered as the model at the beginning of an experiment (table 3).

Table 3

Reactions of birds to bicolored blue and yellow butterflies as first models compared to the reactions of other birds to them as generalization butterfly, after experiencing monocolored orange butterflies.

Category of Reaction	Reaction of Birds to Bicolored blue and yellow butterflies as first model	The same as generalization butterfly	Totals
Not Touch	3 (18%)	10 (100%)	13
Peck, Kill, or Eat	14	0	14
	17	10	27

Exact chi square = 13.96; d.f. = 1; $P < .001$

Thus the bicolored butterflies were refused significantly more by the birds after they had experienced the orange monocolored ones. It appears likely that these distasteful monocolored Heliconiines in some way recalled to the birds prior experience with bicolored Heliconius spp. which they had learned in nature are highly unpalatable. This shows that a similarity in form and shape alone, with accompanying unpalatability, can result in a selective advantage by provoking generalized rejection responses in birds. The first mutation towards Müllerian mimicry need not be great; even a remote resemblance of an unpalatable species to a Müllerian model-mimic pair could lead to its inclusion within the complex as an incipient mimic. It will be interesting to design an experiment to compare the amount of divergence in appearance of a mimic from its model which can be supported in a Batesian situation vs. a Müllerian one.

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QUELQUES ASPECTS ZOOGÉOGRAPHIQUES DU MIMÉTISME CHEZ LES LÉPIDOPTÈRES. G. Bernardi, Muséum National D'histoire Naturelle, Entomologie, Paris, France.

Le mimétisme comporte de nombreux aspects zoogéographiques: problèmes de corrélation entre répartition des mimes et modèles, variation géographique du mimétisme, fréquence du mimétisme selon les domaines zoogéographiques, relations entre coloris locaux et mimétisme. Dans les limites de la place disponible nous examinerons les deux premiers aspects cités.

I. Problèmes de collélation entre la répartition des mimes et

modèles. A. Polymorphisme apparemment mimétique mal équilibré par la pression mimétique. Le meilleur exemple est celui des ♀ d'Hypolimnas misippus, mimes de Danaus chrysippus (cf. Poulton, 1898; Eltringham, 1910; Stride, 1957). J'ai cherché à dégager une vue d'ensemble des "anomalies" en Afrique au sud du Sahara: en négligeant la présence exceptionnelle d'une morphe dans une zone donnée on peut distinguer 6 combinaisons de l'occurence des morphes de D. chrysippus (cf. tableau) tandis que dans cette même région l'H. misippus présente partout les mph. ♀ misippus ("mime" de chrysippus), inaria ("mime" de dorippus)et alcippoides ("mime" d'alcippus), inaria étant particulièrement fréquent au sud de l'Afrique (zone 6) et alcippoides toujours rare.

Zones	1) ouest-afr.	2) Gabon N.W. Congo	3) Ouganda W. Ethiopie E. Congo
Modèles: D chrysippus	-- -- alcippus	chrysippus -- alcippus	chrysippus dorippus alcippus
Mimes: ♀ d'H. misippus	misippus inaria alcippoides	misippus inaria alcippoides	misippus inaria alcippoides

Zones	4) Ethiopie Kenya	5) Somalie	6) Madagascar Sud Afr.
Modèles: D chrysippus	chrysippus dorippus --	-- dorippus —	chrysippus -- --
Mimes: ♀ d'H. misippus	misippus inaria alcippoides	misippus inaria alcippoides	misippus inaria alcippoides

Il y a donc "anomalie" (Cf. caractères gras) dans toutes les zones sauf la zone 3. Je ne vois pas d'explication globale satisfaisante, notamment les intéressants travaux de Stride ("inhibition au blanc" des ♂ d'H.misippus) expliquent l'anomalie seulement dans la zone 1. N'est-il pas plus simple d'admettre que le polymorphisme des ♀ d'H. misippus est indépendant de la pression mimétique? Cela pose le problème de l'apparition de ce polymorphisme car on conçoit mal une pression mimétique donnant naissance à un polymorphisme dont l'équilibre lui échappe ensuite si totalement.

B. Phénomène du relachement de la ressemblance mimétique et mimes à populations non mimétiques. Le phénomène du relâchement mimétique a été étudié chez les Pseudacraea eurytus des îles du Lac Victoria, Papilio dardanus polytrophus du Kenya et les

Hypolimnus dubia drucei de Madagascar et des Comores (cf.Bernardi, 1960; Carpenter, 1949; Ford, 1936, 1940, 1953; Poulton, 1912a, b; Sheppard, 1959) et brièvement signalé chez Papilio troilus (Brower et Brower, 1962). Il est bien connu qu'il consiste en un accroissement de la variabilité conduisant à l'apparition de mimes imparfaits mais il me semble nécessaire de serrer d'un peu plus près ses aspects morphologiques avant de tenter de l'interpréter.

Notons donc qu'il ne s'agit pas d'un phénomène homogène puisqu'il porte sur des individus: a) soit étroitement apparentés à un seul mime (cas 1), b) soit sans parenté étroite avec aucun mime (cas II), c) soit intermédiaires entre deux mimes (cas III), d) soit intermédiaires entre un mime et un individu non mimétique du cas II (cas IV).

L'importance du cas III est très nette chez H. dubia (cf. Bernardi, 1960) où les formes intermédiaires diffusa et antelmi sont communes aux Comores et à Madagascar tandis que les formes intermédiaires daemona, daemonides et millari sont très rares sur le continent africain, par contre le cas I ne joue qu'un rôle restreint (présence simultanée de drucei et de bewsheri à Madagascar). Il en est de même chez P. eurytus dont Carpenter (1949) exprime le relâchement mimétique en chiffrant les individus intermédiaires entre formes mimetiques ("terra-tirikensis," "tirikensis poggeoides," etc... etc...), soit des exemplaires du cas III.

Une situation très différente s'observe chez P. dardanus polytrophus où l'on rencontre soit des formes non mimétiques très variables (cas II) telles que poultoni conduisant à dorippoides, lamborni conduisant à jeanneli, trimeni conduisant à dionysoides soit des formes intermédiaires entre celles-ci et des formes mimétiques (cas IV) telles que "brown cenea" et "orange cenea."

A l'exception de quelques précieuses données de Clarke et Sheppard sur P. dardanus polytrophus le mécanisme génétique du relâchement est inconnu, on peut cependant se demander s'il ne met pas en jeu les mécanismes suivants: a) apparition de mutations ni éliminées ni stabilisées par la pression mimétique (cf. Clarke et Sheppard 1960 a 76, 78). b) traces d'hybridation non éliminées par la position mimétique (cf. Clarke et Sheppard (1960 b: 177) à propos de P. dardanus et Bernardi (1960: 155) à propos d'H. dubia). c) présence ou disparition d'une dominance nette chez les hétérozygotes en l'absence de pression mimétique (cf. Sheppard,1959) à propos de P. eurytus. d) dans le cas du polymorphisme mimétique limité à un sexe contrôle par la pression mimétique de la variation géographique du dimorphisme sexuel.

Ce dernier mécanisme sera certainement jugé comme "hérétique" et doit donc être quelque peu défendu. Je crois que les ♀ andromorphes non mimétiques (telles que la ♀ cyrus de Papilio polytes et les ♀ "yellow" de P. dardanus y compris les ♀ trimeni et dionysoides) habituellement interprétées comme des ♀ n'ayant pas encore été soumises à la pression mimétique sont au contraire des ♀ ayant été plus ou moins libérées de cette pression et repré-

sentant ainsi la phase finale de l'évolution des ♀ de ces espèces. Cette opinion est basée sur les remarques suivantes: a) l'existence de ♀ andromorphes est un phénomène très général (cf. les ♀ de Lycaenidae, de Colias ou de Parasemia) indépendant du mimétisme et interprété d'une manière exactement inverse quand le mimétisme n'est pas en cause, b) le dessin des ♀ mimétiques de P. dardanus ou de P. polytes est plus proche de celui du "prototype" des lépidoptères de Suffert et de Schwanwitsch tandis que les ♀ andromorphes de ces espèces montrent les dessins les plus éloignés de ce "prototype." Je considère donc les mimes à populations ♀ non mimétiques comme la phase finale du relâchement mimétique.

L'ensemble des phénomènes étudiés au B constitue ainsi, à mon avis, une excellente démonstration du role essentiel de la pression mimétique non pour l'apparition mais pour le maintien d'une ressemblance mimétique.

C. Ressemblances superficielles allopatriques (pseudomimétisme d'Heikertinger, 1954). Il me semble que beaucoup d'exemples de ce phénomène sont peu pertinents (Heikertinger cite Urania leilus et Armandia lidderdali!)

Mais il existe des "pseudocycles" troublants tels que Hypolimnas misippus ♂ et Limenitis albomaculata (asiatiques), Chloripe et Adelpha (sud-américains) et Limenitis zayla (asiatique), cycle d'Amauris echeria (africain) et ♀ de Catonephele chromis (sud-américain), Pinacopteryx eriphia (africain) et ♀ de Catonephele nyctimus (sud-américain). Notons que dès 1912 Dixey admettait une "ressemblance accidentelle" entre H. misippus et Cybdelis mnasylus.

Malgré l'absence de statistiques, je crois cependant que le mimétisme entre formes sympatriques est plus fréquent et souvent plus parfait que les ressemblances entre formes allopatriques. Mais il me semble que le pseudomimétisme nous oblige à admettre qu'une ressemblance superficielle peut être obtenue en l'absence de pression mimétique mais qu'elle n'est généralement maintenue, voire améliorée, que grâce à cette pression.

II. Variation géographique du mimétisme. J'ai longuement abordé l'étude de ce phénomène (Bernardi, 1962) et je crois avoir montré qu'il ne constitue pas toujours une démonstration de la pression mimétique comme cela a été admis jusqu'ici (cf. p.e. Huxley, 1955). Par contre, l'étude des anomalies vis-à-vis de la règle écogéographique de Gloger chez les espèces mimétiques et non mimétiques peut constituer, à mon avis, une nouvelle méthode d'approche du mimétisme s'il se confirme que les anomalies sont plus fréquentes chez les espèces mimétiques. On notera que dans les exemples africains cités en-dessous la règle exige une extension des dessins foncés dans les parties les plus humides de l'aire d'extension d'une espèce (soit < clair dans l'ouest africain et > clair dans l'est africain).

A. Variation géographique coincidant avec la règle de Gloger. Si la variation géographique d'une espèce mimétique coincide avec la règle de Gloger elle peut être interprétée 1°) soit comme le

résultat de l'action des facteurs macroclimatiques du milieu-2°) soit comme le résultat de la copie (a) de modèles cospécifiques dont la variation géographique vérifie également la règle de Gloger ou (b) de modèles appartenant à des espèces différentes mais à habitus présentant une analogie fortuite avec la règle.

		ouest africain (< clair)	est africain (> clair)
a)	Modèle	Amauris n. niavius (< clair)	A.n.dominicanus (> clair)
	Mime	Papilio dardanus	P.dardanus
		♀ hippocoon (< clair)	♀ hippocoonides (> clair)
b)	Modèle	cycle d'Amauris tartarea(<clair)	A. ochlea (> clair)
	Mime	Pseudacraea l. lucretia (<clair)	P.l.expansa(> clair)

La copie d'A. niavius dominicanus (ou d'A.ochlea) dans l'est africain au lieu d'A.n.niavius (ou d'A.tartarea) de l'ouest africain doit tendre à l'extension des espaces clairs des mimes dans l'est africain par rapport aux mimes de l'ouest africain mais cette extension des espaces clairs peut être prévue d'après la règle de Gloger même si les modèles n'existaient pas. Ici l'action des facteurs macroclimatiques et la pression mimétique oeuvrent dans le même sens et ne sont pas discernables.

B. Variation géographique présentant une anomalie vis-à-vis de la règle Gloger. Si la variation géographique d'une espèce mimétique présente une anomalie vis-à-vis de la règle de Gloger il est séduisant de considérer que cette anomalie provient précisemment de l'antagonisme entre l'action des facteurs macroclimatiques du milieu et la copie (c) de modèles cospécifiques présentant déjà une anomalie vis-à-vis de la règle de Gloger ou (d) de la copie de modèles appartenant à des espèces différentes dont l'habitus ne coincide pas avec la direction géographique de la règle de Gloger.

		ouest africain (< clair)	est africain (> clair)
c)	Modèle	Bematistes e. epaea(> clair)	B.e. paragea (< clair)
	Mime	♀ Papilio c.cynorta(> clair)	♀P.e.peculiaris (< clair)
d)	Modèle	cycle d'Amauris tartarea(>clair)	cycle d'A.echeria(<clair)
	Mime	Hipolimnas d.dinarcha (> clair)	H.d. grandis (< clair)

Selon la règle de Gloger l'H.dinarcha ou la ♀ de P.cynorta devraient présenter des espaces clairs plus étendus à l'est qu'à l'ouest de leurs aires de répartition. Or on observe le phénomène inverse, ce que l'on peut interpréter comme le résultat de la copie de modèles à espaces clairs plus réduits dans l'est africain que dans l'ouest africain. Ici l'action des facteurs macroclimatiques et la pression mimétique oeuvrent dans des sens différents et sont donc discernables.

III.Zoogéographie et théories explicatives du mimétisme. Il me semble que les faits dégagés ci-dessus suggèrent que la pression mimétique n'est pas indispensable pour l'apparition des ressemblances superficielles mais joue un rôle essentiel pour le maintien de ces ressemblances. En d'autres termes, je suis tenté

de ne pas admettre obligatoirement une même théorie explicative pour l'acquisition et pour le maintien d'une ressemblance mimétique et de penser que dans bien des cas de mimétisme entre lépidoptères l'acquisition de la ressemblance relève de la théorie dite des "causes internes" (plus exactement de la loi des séries homologues de Vavilov, 1922 ou de la "conservation des types anciens" comme l'écrivait Verity, 1929) tandis qu'il faut revenir à la théorie classique du mimétisme pour expliquer le maintien de la ressemblance entre formes sympatriques. Ainsi loin d'accélerer l'évolution, le mimétisme doit la freiner dans bien des cas.

Ajoutons que les données zoogéographiques du mimétisme ont été à peine exploitées jusqu'ici. Les tâches les plus urgentes consistent à mon avis a) à dresser des aréotypes précis de tous les cycles mimétiques en vérifiant s'il y a ou non strict inclusion de l'aire du mime dans celle du modèle b) à étudier le mécanisme génétique des cas connus de relâchement mimétique c) à dresser une statistique des cas d'anomalie vis-à-vis de la règle de Gloger entre espèces mimétiques et non mimétiques.

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MIMICRY AND PREDATOR BEHAVIOR. C. S. Holling, Forest Insect Laboratory, Sault Ste. Marie, Ontario, Canada.

A small mountain of information has been collected about mimicry since Bates in 1862 reasoned that resemblances between species had ecological significance. Now that the fact of Batesian mimicry and its ecological significance has been clearly established, it seems logical to begin a detailed analysis of the structure of mimicry and in particular to analyze the role predator behavior plays in making predation a selective force of a potency to mould such astonishing resemblances as occur in nature.

Biological processes such as predation are distinctively complex. First, the processes are often discontinuous. The rela-

tively small number of contacts between animals, the specific generation time, and diurnal and seasonal changes all contribute to the discontinuity. Second, thresholds and limits are pervasive features of biological systems -- a nerve impulse is propagated only when the intensity of a stimulus is above a certain value; a predator attacks only when its hunger exceeds a certain value; a population of animals persists in an area only when its density exceeds a certain value. Finally, the historical character of biological processes is perhaps the most dominant feature of all, for what happens at any moment depends upon conditions in the past as well as in the present. This has long been a recognized part of evolution and genetics but it is just as true of other processes such as predation.

Biological theory must retain these prime features if it is to be sufficiently realistic to be meaningful. Fortunately, new techniques are available that hold dramatic promise of mastering the type of complexity facing the biologist. These techniques have developed around computers and the languages used to program them, and their peculiar suitability arises in part because the digital computer operates in very much the same way as a living organism. Fortran, one of the computer languages developed for I.B.M. computers, has proved to be remarkably well-suited to handle discontinuous processes, thresholds and limits, and the historical character of biological events. It allows the biologist to think in terms scarcely considered before and to analyze complex processes without simplifying them unrealistically.

But a well-designed language is not enough. We must have something worthwhile to say in that language. Again the complexity of the processes demands a rigorous analytical approach. Man's imagination is so limited that it must be given detailed direction through an intimate union between theory and experiment, with experiments dictating theory and theory suggesting experiments in successive steps. Part of predation, the attack process, has just been analyzed in this way and the analysis provides a detailed explanation of attack that can be incorporated in a mathematical model. This attack model provides a powerful tool to explore various aspects of predation and it forms the basis for the present analysis of mimicry.

A general description of the experiments and model has already been published and a more detailed account is now being prepared for publication. It is sufficient here to give a brief outline with only enough detail to convince the reader that the model does not simply represent a flight of mathematical fancy but a biologically realistic and meaningful explanation derived from detailed experiments.

The study was designed to analyze attack of prey present at different densities in a system affected by four main components i.e. searching, the time predator and prey are exposed, the time spent handling prey (e.g. pursuit, eating) and hunger. These seem to be the only components affecting attack by most invertebrate predators. Their precise action and interaction were

demonstrated experimentally and a series of equations was devised to describe the relations. These equations were then synthesized in a single attack model that generates attacks successively. A predator that attacks only during daylight, for example, starts the day with a relatively high level of hunger as a result of a foodless night. As time passes the hunger increases until a prey is captured and eaten. The model reproduces the change of hunger in this interval and the changes in the other relevant parameters such as searching rate. On this basis it can then predict the length of time required to locate, to successfully pursue and capture, and to eat that prey. The hunger is lowered as the prey is consumed and provides a new level of hunger for the generation of the next cycle. Hence capture cycles are generated one after another, thus preserving both the discontinuous and historical features of attack.

Each of the twenty-two parameters of the model is associated with some biologically meaningful feature of attack. One, for example, is the rate of digestion, another represents speed of movement of the predator and still another measures the predator's success in capturing a prey. The cryptic and defensive qualities of the prey, the size and shape of the predator's reactive field, the maximum capacity of the gut and the rate of pursuit and eating are each represented by one or more parameters. When values for each parameter were independently measured in a mantid - housefly system and were then introduced into the model, it accurately predicted the actual sequence of attacks at various prey densities and times. It seems, therefore, to provide an accurate, meaningful explanation of this specific attack process and, hopefully, others as well. By assigning a range of values to the parameters of the model the spectrum of attack types can be explored and the importance of each parameter judged.

But something more is needed if the attack model is to be useful as an aid to understanding mimicry. It now only includes one prey species whereas Batesian mimicry demands at least two - an unpalatable prey, the model, and a palatable prey, the mimic, that resembles the model. An additional, more basic revision is also necessary, however. The ability of vertebrate predators to avoid distasteful models before physical contact is apparently a learned behaviour rather than an innate avoidance of a particular color or pattern. No learning component appears in the model since learning did not play a significant role in attack by praying mantids. A learning component therefore must be added.

Ideally the model should be expanded to include learning by applying the same rigorous experimental analysis that dictated the explanation of the action of the other components. This represents a very major effort and until it is completed an alternative approach can be of some advantage. This approach has been called "mathematical experimentation" by Bellman and requires relatively little information concerning cause and effect relations -- just enough information, in fact, to suggest a variety of different hypotheses, any one of which might be appropriate.

These hypotheses are then tested by comparing predicted features with those actually observed. When any one set of hypotheses seems to produce reasonable predictions it can be further expanded or modified to increase its predictive accuracy. The digital computer again serves as an essential tool, for it permits us to test rapidly a large number of different hypotheses and to handle equations that are otherwise intractable. Without the computer we are forced to accept hypotheses so simple and naive that they are grossly unrealistic.

Fortunately there is a considerable body of data available concerning the learning process and vertebrate predation, enough data to yield a model that will at least provide sufficient insight to suggest potentially profitable lines of research. The essential features can be briefly summarized. 1. Vertebrate predators show an initial generalized responsiveness to any unfamiliar stimulus that appears in the environment. 2. The responsiveness to a specific stimulus increases if the stimulus is associated with palatable prey and decreases if it is associated with unpalatable prey. 3. Learning is selective. That is, information from different stimuli can be separately channelled so that a predator can simultaneously avoid one prey while accepting a different one. 4. The learned association disappears if it is not regularly reinforced.

These four features of learning must now be expressed in more precise terms for integration with the existing attack model. Recent experiments have shown that of the 22 parameters involved in the attack model the one most likely to be affected by selective learning is the hunger level that just initiates attack. When the predator's hunger is below this level it will not attack but as the hunger exceeds the attack threshold the predator becomes progressively more responsive since the reaction distance of the predation for a prey is directly proportional to the difference between its hunger level and the attack threshold. Changes in this threshold induced by exposure to a particular prey, would therefore change the responsiveness of the predator to the prey concerned.

A level of generalized responsiveness to unfamiliar stimuli is provided by a threshold of a moderate level. Each separate encounter with a particular prey would establish a specific threshold for that prey by changing the level of the generalized threshold and by cueing it to the visual and other stimuli of the prey. If the prey is unpalatable, the threshold is raised so that the predator must be hungrier before it will attack. Conversely, if the prey is palatable the threshold is lowered. The actual magnitude of the effect of each exposure to a single prey presumably depends on how familiar it is. That is, one exposure to a completely unfamiliar prey is likely to have a greater effect than one exposure to a completely familiar one. Hunger, too, may modify the effect, for a distasteful prey will probably inhibit a hungry predator less than a satiated predator. These ideas were incorporated in an equation that expressed the effect on

the attack threshold of each exposure as a function of hunger and threshold level.

A "forgetting" component must also be a part of the learning process. Once the threshold deviates from the stable level appropriate for an unfamiliar stimulus it begins to return to that level. Experiments with small mammals have shown that this decay closely resembles the familiar die-away curve and can be accurately described by the appropriate exponential equation.

These two equations - the first expressing the amount of change of the threshold with exposure to each prey and the second expressing the decay of the threshold, form the basis for introducing a learning component into the attack model. Since the original model generates attacks successively one after another, the learning component can be readily added, for at each prey capture the attack threshold will be raised or lowered according to the first equation and between captures it will decay according to the second equation.

The final revised attack model was constructed to simulate a system in which a predator attacks three prey species - an unpalatable model, a palatable mimic that is identical to the mode in appearance, and an edible alternate prey totally dissimilar in appearance from the model and mimic and present at a very high density. The three prey species affect only two attack thresholds. Since the mimic and model are presumed to be identical in appearance there is no way for the predator to separate them before contact. Hence the two species affect only one threshold which is raised whenever an unpalatable model is attacked and lowered whenever a palatable mimic is attacked. The edible alternate prey is presumed to be totally dissimilar in appearance so that a second, completely separate threshold is provided.

To be acceptable the attack model must be capable of accurately reproducing the appropriate known features of associative learning and vertebrate attack. The significant features that have been clearly established in the literature can be summarized as follows.

1. The detection of an unfamiliar stimulus in a familiar environment immediately elicits an attack response. 2. Progressively more prey are attacked in each succeeding unit of time if the unfamiliar stimulus is associated with a palatable reward. The reverse is true if the stimulus is associated with an unpalatable prey. 3. Removal of the prey results in a gradual extinction of the learned response. 4. As the density of an edible prey increases, the number of prey attacked also increases to produce an S-shaped curve. The attack responses of invertebrate predators to prey density are distinctively different, for the response curves rise at a regularly decreasing rate up to a sustained maximum. 5. Vertebrate predators rarely consume one prey exclusively in nature, even when that prey is vastly more abundant than others. 6. Batesian mimics are attacked less frequently in the presence of their unpalatable models. This protection can persist even when there is a higher proportion of

the palatable mimics.

Each of these six prime features of vertebrate predation were compared with output generated by the revised model. The values assigned to those parameters of the model that were present before it was expanded to include learning, were the ones measured in a mantid - housefly attack system. The additional parameters required for the learning component were assigned values that seemed reasonable in the light of a series of experiments involving the attack by small mammals of sawfly prepupae. The output from the model faithfully reproduced the six prime features of vertebrate predation listed above. Therefore the revised model effectively transforms the simulated mantid from a typical invertebrate predator to a vertebrate predator. It provides an effective tool for exploring various dimensions of mimicry, for by assigning various values to the parameters a wide array of situations can be simulated.

The discipline of developing the model and the simulated cases have supported or expanded a number of ideas concerning vertebrate attack and mimicry. In the space available these can only be briefly summarized.

1. Any animal in nature is confronted with a bewildering array of stimuli that must be reduced to those with survival value if the species is to persist. Hence many invertebrate predators have evolved feeding responses that are triggered only by a small class of stimuli, stimuli that efficiently guide the animal to food. Vertebrate predators, on the other hand, have retained a general responsiveness to many stimuli and are able to concentrate on those possessing some survival value by evolving the ability to learn selectively. The learned associations, however, are not permanent for they decay if they are not continually reinforced. Hence the ability to forget and to remain responsive to unfamiliar stimuli affords great flexibility, for the predator can concentrate on few stimuli and yet still retain the ability to exploit changes in the environment. 2. Experimental studies of mimicry emphasize the importance of prey rejection in assessing the effectiveness of mimicry. In many of the cases simulated, however, mimics received highly effective protection in the presence of their unpalatable models even though the predator did not totally reject them. Rejection is the most extreme case in a graded scale of lowered responsiveness. Since changes in responsiveness and not just acceptance or rejection are involved, predator action exerts a graded selective effect in the evolution of mimicry. Even slight similarities between model and mimic will have a selective advantage and even a slight degree of unpalatability in a prey will be sufficient for it to act as a model. As a result, the image of Batesian mimicry as typically involving models that are markedly distasteful and mimics that are dramatically similar is an extreme view. It is more likely that mimicry is an extremely pervasive feature of life involving not only extreme visual similarities but also subtle similarities of position, behavior, sound, odour and visual appearance. 3. An essential feature of

mimicry theory is a mechanism that allows the predator occasionally to sample the model - mimic complex so that changes in frequency of models and mimics can be detected. The stochastic element of learning (incidentally not included in the mathematical model) offers one such mechanism, since the predator, after learning to avoid unpalatable models, may make an occasional error. The present analysis suggests two other mechanisms, as well. First, attack of a model - mimic complex need not result in complete avoidance but just in a drastically reduced responsiveness. Hence models and mimics are inevitably sampled occasionally. Second, even when complete avoidance does occur, the forgetting component of learning assures an occasional sampling of the models and mimics. 4. Even when the frequency of unpalatable models is low, the mimic still receives some protection. The proportion of models required to raise the protection significantly depends primarily on the magnitude and relative palatabilities of the model and mimic, (compare curves A and B, Fig. 1), on the rate of forgetting and to some extent on the density of models and mimics. 5. The presence or absence and quality of prey species other than the model and mimic, has a powerful effect on mimicry. In the absence of alternate edible prey, for example, the effectiveness of mimicry is drastically lowered (compare curves B and C, Fig. 1). A similar effect occurs if the palatability of the alternate food is lowered. Moreover, the protection the mimic receives is directly related not only to its degree of similarity to the model but also to its <u>dissimilarity</u> to the alternate prey. The more dissimilar, the greater the effectiveness of mimicry. Thus alternate prey that are not part of the model - mimic complex have a significant effect on mimicry and should be considered to be an essential part of mimicry theory. 6. The mathematical model can also generate data to explore Müllerian mimicry. An example of typical output is shown in Fig. 2. As expected, the number of each species attacked during the first five days decreases as the number of unpalatable prey species increases. When there are many species of distasteful Müllerian mimics, the number of prey required to teach the predator that all are distasteful can be spread among more species, and the learning can be accomplished more efficiently because of the high total density.

The foregoing analysis can do no more than provide an interim explanation. A detailed and satisfactory mathematical model can only be developed from an extensive and intensive experimental program. Until this information is available, the foregoing attack model at least substantiates many concepts and suggests others that deserve consideration.

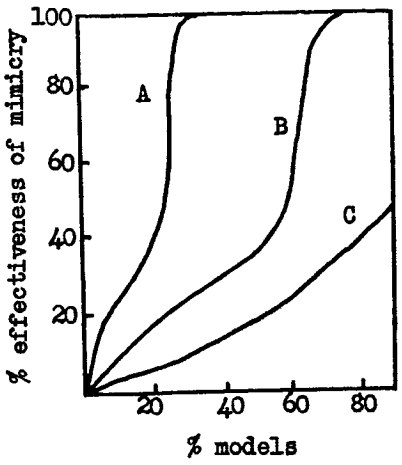

FIG. I

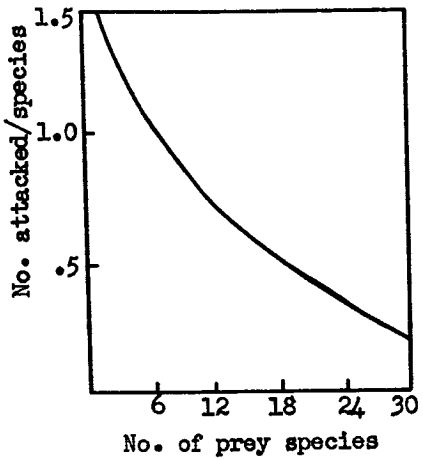

FIG. 2

Fig. 1. Effect of proportion of models on the effectiveness of mimicry. A: highly unpalatable models, edible alternate prey present. B: moderately unpalatable models, edible alternate prey present. C: moderately unpalatable models, no edible alternate prey.

Fig. 2. Typical output generated to demonstrate Müllerian mimicry. The density of each species is held constant.

THE EVOLUTION OF SEX-LIMITED MIMICRY IN BUTTERFLIES. Lincoln Pierson Brower, Department of Biology, Amherst College, Amherst, Mass., U.S.A.

Introduction. An unsolved problem in evolutionary biology is the phenomenon of sex-limited mimicry in butterflies. Commonly in these insects, the female alone is mimetic (with modifications in color, pattern, size, form, and even behavior), while the male retains the ancestral characteristics of the taxonomic group to which the species belongs. This abstract will briefly review the theories put forth to explain its evolution and will weave together the diverse lines of evidence including some new experimental data which all point conclusively, I think, to the idea that it is the result of differential mate selection mediated by the visual sense of the females. This study has been generously supported by the United States National Science Foundation (Grants 8707 and 20152) in cooperation with the Archbold Biological Station. I am also grateful to Drs. E. B. Ford, F. R. S., and P. M. Sheppard for much stimulating discussion.

History. Sex-limited mimicry (S.L.M.) was first discussed by Wallace (1865) who attributed its origin to two causes. (1) First, female butterflies tend to show greater variability than their males. Fisher and Ford (1928) statistically examined this

hypothesis in the light of Mendelian genetics. They pointed out that the observed greater female variability in Lepidoptera is indeed to be expected both on the basis that the female is the heterogametic sex in this order of insects and on the nature of sex-controlled inheritance. However, female variation and the complete inhibition of mimicry in males occurs in excess of that predicted on genetic bases. Consequently they rejected the explanation and concluded that S.L.M. must be the product of a selective process. Wallace's second point (2) was that the sex limitation could evolve because it would be especially advantageous for the females which are heavily laden with eggs, fly more slowly than males, and expose themselves to attack repeatedly throughout their lives while ovipositing. Darwin (1871) rejected Wallace's female protection principle by pointing out that although mimicry in that sex might in fact be of greater advantage, "it would surely not have been in any way injurious to each individual male to have partaken by inheritance of the protective colors of the female, and thus have had a better chance of escaping detection." (p. 400-401.) He concluded by saying that if the "variation was not...originally limited, the process would be extremely difficult perhaps impossible." (p. 276) Darwin himself accounted for S.L.M. as (3) a by-product of the hereditary process, rather than as the outcome of a selective process acting on the sex which most required the protection. Belt (1874) agreed with Darwin that the mimicry would be of advantage to the male and made the important new suggestion (4) that its absence in this sex might be explained on its being disadvantageous through sexual selection "on the supposition that the female had a choice of mates and preferred those that retained the primordial appearance of the group" (p. 295) Belt thus originated the principle that sex-limitation can evolve as the result of a balance of selective forces opposed in their operation, i.e., the mimetic characters are of net positive advantage to the female because of increased protection from predators, and net negative advantage to the male because of reduced mateability. Wallace (1877) rejected the idea of sexual selection but responded to Darwin's criticism and admitted the necessity of a balance of selective factors as suggested by Belt. To achieve this completely within the context of protective selection, he formulated the principle (5) that mimetic variations increase protection in the female but decrease it in the male. This was held to be true because in South America the males congregated on river banks in the absence of the models, whereas the females occurred together with them in the forests. Thus while mimetic variations would be advantageous to the female, they would tend to make males more conspicuous than their brethren, and so be living targets for predators. Elsewhere Brower and Brower (1962) have provided evidence that males of *Papilio glaucus* flock together with males of the species which serves as the model for the *P. glaucus* females, and so Wallace's idea based on the principle that only the females overlap the habitat of the models, must be rejected

as a general explanation.

A sixth explanation (6), also based on the observation that the sexes of the mimetic species tend to occupy different habitats, was put forward by Bates (1866). He maintained that in Amazonian species, the females and males led different modes of life, the latter flying out in the sun, while the females were active in the forest. In this way, the male had become adapted through selection by the abiotic environment, whereas the female had become mimetic through selection by the biotic influences, i.e., selection by predators. Darwin (1871) summarized the evidence and rejected this as a general cause and evidence to date has not contradicted him. (Wynne-Edwards, 1962, has recently put forth an unlikely explanation (7) which can not be discussed here.) Fisher (1930) presented theoretical reasons supporting the idea that sex-limitation of characters can evolve gradually and Gordon and Gordon (1939) produced a sex-limited difference in palp color of Drosophila, thereby experimentally confirming the hypothesis.

It has already been said that Fisher and Ford (1928) concluded that S.L.M. must be the result of a selective process which they also thought to be sexual selection. In their words, "One of the chief functions of the male is to stimulate the female to copulation; this is most frequently performed by sight or smell, sometimes by touch and hearing; but the displays of an insect often combine several of these types... It is probable therefore, that in certain cases selection in favour of keeping the males to a relatively constant type in that their sex should be recognized by the female and cause her the appropriate stimulation, outstrips any advantage which they might derive from mimicry..." (pp. 377-378). Ford maintained this view (Carpenter and Ford, 1933; Ford, 1937, 1953) with an increasing emphasis on sight as the principle sense involved in the female's mate choice. Subsequent investigators have tended to accept this hypothesis without further evidence in direct support of it (Hogben, 1940; de Ruiter, 1958; Sheppard, 1959; 1960 a, b; 1961).

Synthesis. In order for sexual selection to result in the S.L.M., two major conditions must be met. First (A.) there must be a breeding system that can result in selection against the males which depart from the primitive pattern, and secondly, (B.) the female butterflies must be able to mate selectively based on information received via their visual senses. It is not necessary to invoke the idea of conscious or aesthetic choice on the part of the female (see Huxley, 1938 a, b) because certain colors or patterns may simply stimulate her visually to a greater degree than others, and if these happen to be the primordial ones, differential mating will result as required.

A. Elsewhere Brower (ms. in prep.) has summarized the vast evidence that the breeding system is eminently suited in butterflies of several families for assortative mating. Briefly, this is reflected in the fact that most collections of butterflies have a predominance of males, even though when reared, the sex ratio is

near equality. This means that the male butterflies (and collectors) frequent one type of habitat, while the females occur in another most of the time. The well known phenomena whereby males congregate on hill tops, fly along streams, assemble at moist places, are attracted to certain lures, occur in numbers in wood clearings, and congregate in semi-shaded islands in open fields, all attest to this idea. Detailed observations on the Queen butterfly Danaus gilippus berenice (Brower and Cranston, 1962) indicated that whenever a female flew into one of these congregations of males, she would be courted by several and thus have abundant opportunity for mate choice.

B. To find an answer to the question of whether visual selection by the female occurs, it is necessary to consider the general role of vision in the courtship of butterflies. Two facts are evident: the compound eye of these insects is significantly large and they have color vision. On the basis of the extensive literature (Brower, summary in prep., Brower and Brower, 1962; see also Magnus, 1963, in this symposium), there is no doubt whatsoever that the color and pattern of the female butterfly is an important visual stimulus to the male in his initial approach towards her. However, with one exception, even as Wallace (1877) pointed out long ago, there is still "literally not one particle of evidence that the female is influenced by color or even that she has any power of choice" (p. 401). Even though this is true, lack of evidence for something should not be taken to deny its existence. The principal reason for this lacuna in our knowledge is the technical difficulties of carrying out the necessary experiments. With the exception of Stride (1958) no one has ever attempted to modify the color of the males to see what the influence on the females' receptivity would be, although many reciprocal experiments have been done indicating the importance of color to the male. It is therefore of the very greatest interest that Stride's preliminary study of the Nymphaline Hypolimnas misippus showed that experimental males rendered colorless were less attractive to the females than normal control males. However, the experiment did not conclusively differentiate between color and scent, since the wing scales were removed to make the experimentals colorless.

A general review of courtship of butterflies indicates that in all instances where color and/or pattern has been shown to play a major role, the butterflies studied belonged to the Papilionidae (excluding the Pharmacophagous groups), the Pieridae, the Nymphalinae, or the Satyridae. Now these butterflies in fact are the groups which contain most of the Batesian mimics. In contrast, most of the butterflies which are involved in Müllerian mimicry belong to the Danaidae, Ithomiidae, Heliconiinae, and the Pharmacophagous groups of the Papilionidae. A comparison of Batesian and Müllerian mimicry indicates two very important differences. First, S.L.M. is a characteristic only of Batesian species; there is no instance known to me where mimicry occurs in the female only of a Müllerian species. Second, it is an outstanding fact that

the males of all the species in these Müllerian groups are endowed with elaborate scent disseminating organs including extrusible hairpencils, abdominal glands, wing folds filled with loose scales, wing pouches, or large areas of scent scales on the wing surfaces. It is my contention that the first of these facts is a consequence of the second. In other words, the occurrence of mimicry in the males of Müllerian species is made possible because scent plays a more important role in the courtship than sight, although sight probably is involved to a certain extent as shown by Crane (1955) for Heliconius erato. According to this thesis, the visual selection exerted by the female against the mimicry in the male is either absent or not strong enough to overcome the Müllerian advantage, and so both sexes become mimetic. It is surprising that no one has made this correlation, for as early as 1907, Poulton pointed out that another sense than sight must play a major role in Müllerian mimics in order to reduce the chaos of interspecific courtship that would result in such groups as the "tiger-pattern" complex in South America involving many species of Ithomiidae, Heliconiinae, and Lycoreinae. More generally, it seems probable that Müllerian convergences and scent-oriented courtships in butterflies evolved in an interacting system similar to that of the hand and brain of Homo sapiens, an increase in complexity of the first requiring an increase of the second which in turn set the stage for the next round of interaction.

New Experimental Evidence. Brower and Cranston (ms. in prep.) carried out a large scale experiment in the field on the role of color of the female in the courtship of the Queen butterfly. The study involved releasing laboratory-reared 2 day old, virgin females to wild males in their natural environment, verbally recording the ensuing behavior, and analysing it by quantitative statistical methods. (The procedure is shown in detail in our film, Brower and Cranston, 1962).

Two major experiments were conducted. The first increased the amount of white in the discal area of the upper and under sides of the forewings, and the second eliminated white spotting on both surfaces of all wings. "Flo-Paque" paint (see Crane, 1955) was used to mask or increase the areas of natural colors. White, black, and a mixture resembling the mahogany color of the butterfly were employed. Over the range of 400-700 mu, the mahogany did not vary at any wave length more than 10% from the spectral reflectance characteristics of 6 regions of the wings. Controls were handled and painted to exactly the same extent. In the first experiment these had a mahogany colored blotch in the same place the white was applied in the painted experimentals. For controls in the second experiment, paint was put adjacent to the white spots, not over them as in the experimentals. Thus the only variable tested was color. Normal, unpainted females were used as an additional control.

It can be seen in the table that these color modifications of the females made no difference in their mating success. However,

there is a tendency for the painted butterflies to be less successful than the unpainted ones. In addition, we released several brightly painted females (blue or yellow blotched, large white polka-dotted, yellow, blue, and green parallel striped, and the same colors in concentric circles) as well as painted controls, and there was again no difference. On the other hand, these Danaids have elaborate scented hair-pencils which are always used in successful courtship (Brower and Cranston, 1962; ms. in prep.). The conclusion is that scent plays a more important role than color in the courtship of the Danaidae.

EFFECT OF MODIFICATION OF FEMALE COLOR
ON SEXUAL RESPONSE OF MALE.*

Experiment 1: increase of white in discal area of forewing.

(outcome of 181 courtships of 68 females)

Outcome of Courtship	Normal	Painted Control	Painted Experimental	Totals
Successful**	18(30%)	11 (18%)	12 (20%)	41
Unsuccessful	43	49	48	140
Totals	61	60	60	181

(x^2 = 2.05, P> .30; normals vs. p. controls x^2 = 2.09, P>.10)

Experiment 2: removal of white spots from all wings.

(outcome of 100 courtships of 45 females)

Outcome of Courtship	Normal	Painted Control	Painted Experimental	Totals
Successful	8 (30%)	6 (17%)	7 (19%)	21
Unsuccessful	19	30	30	79
Totals	27	36	37	100

(x^2 = 1.71, P> .30; normals vs. p. controls x^2 = 1.50, P>.20)

* All females = 2 day old virgins, courted ≤ 3 times; males wild.
** Successful = terminated in copulation.

Summary. What direct evidence there is does not contradict the hypothesis that mimicry limited to the females in many butterfly species is the result of female mate choice mediated by a visual preference for the males which retain the primitive ancestral color-pattern. The general importance of color in courtship is greater in those butterflies which exhibit sex-limited mimicry than in those which do not, and these occur, respectively, in the Batesian and Müllerian groups. There is, in other words, a non-random occurrence of sex-limited mimicry. It seems most probable that the strength of female visual selection is great enough in Batesian groups to prevent mimicry in the male, but in Müllerian groups the increased importance of scent has lowered the visual disadvantage sufficiently so that it is outweighed by the benefits accrued from mimicry.

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SEX LIMITED MIMICRY II - VISUAL SELECTION IN THE MATE CHOICE OF BUTTERFLIES. Dietrich B. E. Magnus, Zoologisches Institut der Technischen Hochschule, Darmstadt, Germany. (Translated from the German by D. O. White, and edited by L. P. and J. V.Z. Brower.)

In all butterflies whose mating habits have been examined, optical stimuli appear to play a large part and in all probability are a fundamental component of their mating behavior. The mating activity itself appears to occur according to a single scheme in all species. The male is always the active partner who introduces the mating activity by looking for females or waiting for them. For this to occur, the male must be in a situation of sexual mood or appetite. In the seeking behavior the male usually flies around looking in the habitat in a very typical zig zag flight, as for example in the Nymphalids, Argynnis paphia, Mesoacidalia charlotta, and Fabriciana adippe (Magnus, 1950, 1958), and the Pierids, Pieris napi and P. bryoniae (Petersen, 1952, 1954) and Gonepteryx rhamni (Magnus, unpublished). In the waiting behavior the male sits at a certain place waiting for the females to fly by. This can be observed in the Satyrids, Hipparchia semele (Tinbergen et al., 1941, 1942) and Pararge aegeria (Magnus, unpublished), in the Papilionid, Iphiclides podalirius,

and several Hesperids (Magnus, unpublished).

The male approaches all optical stimuli which appear to him to be sexually positive by a direct flight either from his seeking flight or from his waiting place. This reaction is always and exclusively caused and directed by optical, distance stimuli. Female olfactory stimuli certainly do not play a directing part in this approach flight because they cannot be perceived by the males at a distance. This makes the seeking behavior in the mating of butterflies substantially different from that of many moths, as for example, Bombycids.

The effective optical stimuli are either only light-dark contrast or color stimuli and the way in which the male reacts to these is dependent on the hereditary predisposition according to his species. The optical stimulus pattern produced by the female of the same species is not necessarily the optimal one. In species that have polymorphic females some female stimulus patterns are preferred by the males and others are ignored. As a rule, they prefer the females that are most like themselves. In Argynnis paphia the male is colored yellowish brown and one female form is very similar to the male. A second female form, valesina, is blackish green, does not have the yellowish color, and is a far less effective optical stimulus for the males than the normal form (Magnus, 1958). In Pieris bryoniae all females are considerably darker than the bright, white males. Preferred optical stimuli for the males are males of the same species and the white males and females of Pieris napi, if they occur at the same time and at the same place, which is so in the marginal areas of the habitat of both species. The dark bryoniae females are clearly at a disadvantage during the optical mating search of their males (Petersen, 1952, 1954). With P. napi, in addition to the pure white females there are also yellow females. The males prefer the pure white females and white males of the same species to the yellow females (Petersen, 1952, 1954).

In order to bring about the approach flight reaction it is not absolutely necessary that a color-pattern similar to his own be shown. This can be seen in the following example: the males of Hipparchia semele prefer sharply contrasting black and also red dummies to the bright brown color of the males and females of the same species (Tinbergen, 1941, 1942). The males of Argynnis paphia prefer colored paper dummies more strongly reflecting yellow brown to the normal wing color of the males and females of the same species (Magnus, 1955, 1958).

Further the color stimulus of dummies can be made "super normal" by changing the size and by changing the frequency of its exposition per unit time. The natural size of females is by no means optimal as a stimulus. Larger dummies produce a better effect than those of normal size especially when exposed at a greater distance. Also the more frequently the optical stimulus is exposed, the more effective it is. The upper limit is not reached until the physiological limit of resolution of the eye for the quick changes in stimuli is reached. This is much higher than the

normal speed of movement of the female wings. The same reaction can be produced by various combinations of surface size, intensity of color, or intensity of contrast effect and by the speed of changing of the stimulus (Magnus, 1958). For this reason, a female whose color-pattern is not optimal for the male can compensate by a more rapid change of stimulus, for example, by extremely rapid movements of the wings and thus can cause an approach flight by the male.

Whereas in general a single copulation is sufficient to fertilize all eggs in a female butterfly, the males can copulate successfully several times and are sexually active during their whole lives. As they grow older, and if they have performed mistaken approach flights to females or males of different species, or to males of the same species, or to other false optical stimuli, their stimulus threshold is reduced as a consequence of sexual overexcitement and they then will react more frequently to non-optimal optical stimuli. For this reason experiments with old males are inadequate to analyze the normal optimal optical stimulus patterns of these animals. To young Argynnis paphia males, the dark valesina females are not attractive at all when not moving and if moving, only slightly so. But their optical stimulus value is increased for old males that are highly motivated. Despite the fact that the valesina mutation has practically no effect as an optical stimulus on the normal searching flight behavior of the males, in the wild every old valesina female is mated with and lays fertilized eggs. This is brought about by the abnormal behavior of the old males and also by the fact that the optical stimuli of the females function especially as distance stimuli which make it easier for the sexes to find each other from greater distances. Therefore this is of importance only for those species of butterflies that live in a larger habitat area and are few in number and therefore meet each other less often. In all species that live in small populations and in small habitats and stay in a limited area, the optical stimulus of the females does not play a large role at all, or in any case no decisive role. At short distances the male and female butterflies act in the very same way as the moths; that is to say, they react to intra-specific sexual olfactory stimuli. It is these olfactory stimuli that in reality play the decisive role in the mating of butterflies. They show the male whether the goal to which he is flying is a female of the same species or not. They show him whether the female is ready to be fertilized or whether she has already been fertilized and they indicate to the female whether the partner which has just arrived is a male of her own species. The female sexual odor usually appears to be perceptible to the male not more than a few centimeters from her. For this reason, the male often seeks to make antennal contact with the partner while in flight. It is only by this odor test that he can find out whether he has been successful or has erred. He then immediately turns away from males of the same species or different species, or from females of different species and as a rule, also from females of the same species that have already been

fertilized. The sexual odor of fertilized females is no longer attractive. The males begin mating activities only with females that have not been fertilized.

The results of my own investigations with Argynnis paphia and other species leave no doubt that the specific optical stimuli which have led to the finding of the female no longer play any role in the mating itself (Magnus, 1950, 1955, 1958). The valesina females that are optically unattractive or only slightly so are mated with the same energy as soon as the males have noticed their sexual odor. But this also occurs with leaves, pieces of wood, stones, and fingers of the experimenter, or a paper dummy, if female sexual odor of the same species is placed on these objects. In such cases the male will try with a great deal of activity to copulate with the object. It is only after several futile efforts that he gives up. During the mating act the male gives off his own sexual olfactory substance. The mode of secretion is species-specific. Frequently the scent glands have openings on the upper sides of the wings via special scent scales. In such cases, the male moves the wings very intensively against the female and secretes a cloud of scent in her direction. Such male glands have been found and investigated, for example, in the Nymphalids, Argynnis paphia, Mesoacidalia charlotta, and Fabriciana adippe, in many Hesperids and Lycaenids and in Pierids (Barth, 1944, 1949, 1952). Tinbergen (1941, 1942) investigated the effect of the male scent on the females of Hipparchia semele. Male olfactory stimulation of the female is generally supplemented by mechanical stimuli, such as striking with the wings, or the legs or pushing with the palps or with the entire head. A female which is not ready for mating or which has already mated, flees from the male so suddenly and quickly that as a rule he is no longer able to pursue her. Usually the female then drops to the ground and waits motionless with folded wings. Thus she loses her effect as a color stimulus and the olfactory stimulus is also no longer perceptible to him. On the other hand a receptive female normally reacts positively to the approach of a male of the same species characteristically either by remaining in the same place and fluttering her wings or by flying around the male. It is typical in this situation that she not only remains visible but offers her stimuli in coquettish fashion. She is then pursued by the male and both stimulate each other until excited to such an extent that copulation can be performed or until one partner interrupts the mating. The mating activity now no longer serves for the selection of a partner but only to bring both partners to the same condition of sexual excitement.

The phenomenon whereby many species of butterflies exhibit polymorphism only in the females is doubtless to be explained primarily on a genetic basis, since the females are heterogametic. There is no proof to date for the assumption that any visual sexual selection in favor of normally colored males takes place by butterfly females. This assumes that the females, either by ability or by intent, do not allow males of abnormal coloring to

copulate with them. In experiments in the natural habitat and in cages it is always only the males and never the females that fly toward optical stimuli such as dummies or living butterflies. Males which refuse or ignore their own females from a distance, which thus have no optical stimuli, will immediately attempt to mate with them if they can perceive their sexual scent; when close, the optical stimulus is no longer necessary. Even if the females did react to optical stimuli, they, like the males when close by, would have to be guided by olfactory stimuli only and thus would no longer refuse optically negative males. To be sure, the optical stimulus and the reaction to it occur at the beginning of mating but it is often only a relatively secondary part of the total mating activity. It is, so to speak, an additional bit of assurance supplementing the many other measures that insure success, but it is not an absolute prerequisite for copulation or for finding a partner. Sexual selection is very definitely performed by the males since they do not find or only infrequently find mutant females which differ optically from the norm to too great a degree. This occurs, for example, when the populations are small or dispersed. But in species which are rich in individuals that stay within a limited habitat, as for example, Argynnis paphia, the visual disadvantage of the abnormal female form will be of no practical consequence. At short distances the males meet the females often enough by accident, for example on blossoms, and recognize them by their sexual odor.

In butterfly species that have polymorphic females, female forms can exist that differ markedly from the normal optical stimulus pattern of the species if their positive ecological selective value is equal to or higher than their negative selective value with respect to mate choice; that is, when the following conditions are met: 1) As key optical sexual stimuli these females are just within the reaction range of the males of the same species. 2) These females no longer represent a key optical stimulus for the males. However, because of fortuitous ecological reasons, the sexes meet just often enough so that the males find and recognize them solely on the basis of their sexual odor. 3) Thus the males find only a few of these females, and these by accident. However, because of mimicry or some other ecological advantage, these females have a much better chance for survival than the normally colored ones, and the disadvantage of the male selection is thereby compensated.

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MIMICRY. E.B. Ford, F.R.S., Genetics Laboratories, Dept. of Zoology, Oxford.

We are fortunate in being able to start this Symposium with an historical survey of mimicry contributed by Dr. Remington. He brings into relief certain former misconceptions and errors whose repercussions have not yet died away. One may, in particular, mention the significance of selective advantage, first analysed successfully by Nicholson. A mimic will be maintained relative to its model at the equilibrium point when its mimicry confers neither advantage nor disadvantage however perfect or imperfect it may be. Nevertheless selection will favour the improvement of the adaptations involved. Remington rightly draws attention to the challenge to mimicry hypotheses based upon the misconception that birds rarely destroy Lepidoptera selectively: an error due partly to the difficulty of detecting their remains in the stomach and, as now demonstrated by Kettlewell in moths also, to the fact that the act of capture is exceedingly rapid. A former criticism, that edibility-tests in the laboratory have not been conducted by precise methods has now been met by such studies as those of the Browers.

Their work is here reviewed by Jane van Z. Brower. They showed that caged Florida Scrub Jays could learn to avoid a distasteful model and extend that recognition to its mimic, while control birds without experience of the model found the mimic palatable. The occasional errors made by the experienced birds are of importance since they give predators an opportunity to detect the increase of a Batesian mimic relative to its model. As the Browers point out, should a mimic then come to be preyed upon, relaxed selection for the deceptive qualities should result in the breakdown of the mimetic colour-pattern: a situation often encountered in regions where a model is rare. Jane Brower has, however, shown that mimicry may be effective when the models are much outnumbered by a Batesian mimic. Turning to the Mullerian situation, the Browers have demonstrated that effective mimicry can develop both between species similar in shape and morphology but with no colour components in common and the reverse: thus this form of deception has a wider scope than heretofore recognised.

The evolution of mimicry cannot be understood without a knowledge of its genetics. This has been supplied by C.A. Clarke and P.M. Sheppard, and the latter has reviewed the subject here. Working in part with the polymorphic *Papilio dardanus*, these authors have established that Batesian mimicry is controlled by a number of super-genes, simulating multiple alleles, the effect of which has been gradually perfected by selection operating on the gene-complex. Thus mimicry, even when controlled by a single switch-gene (or super-gene), breaks down in crosses between allopatric forms. Clarke and Sheppard have also obtained evidence that, as expected in polymorphism, heterozygous advantage is often evolved in respect of the genetic switch units, so that mimetic phases, though imperfect, may be maintained in areas where their mimicry is ineffective. Sheppard points out that though Mullerian mimicry should lead to monomorphism a number of species, especially in *Heliconius*, are polymorphic; moreover, allopatric crosses do not lead to a breakdown in such mimicry. Thus current theories on the evolution of the Batesian situation need adjustment for the Mullerian one. However, it may be relevant, as Sheppard suggests, that patterns similar to those of the mimetic forms occur widely in *Heliconius*.

Heterozygous advantage is doubtless responsible for the situation discussed by G. Bernardi, who points out that in Africa the three mimetic forms of Hypolimnas misippus are often all present in regions where one or two of their models are absent. Indeed the available bred families indicate a deficiency of homozygous dominants (Ford, 1953); further information on this subject is badly needed. We require to know, moreover, in the instances to which Bernardi draws attention if the frequency of a mimic declines in the absence of its model and if its mimicry then becomes imperfect. Bernardi points out that selection for adjustment to climate may work in the same direction in a model and its mimic, so facilitating the mimicry. In view of the information which he has collected, Bernardi suggests that an accurate resemblance is not responsible for the appearance of mimicry but is necessary for its maintenance. Certainly, as indicated by Sheppard's results, mimicry may not be precise at first but may be improved later. Doubtless heterozygous advantage may evolve when a controlling gene spreads, as in non-mimetic polymorphism.

C.S. Holling has considered the programming of computors so as to analyse mimicry phenomena. He tested a mathematical model of attack against experimental data derived from the reaction of Mantids and houseflies. Four main components were included, and when each was independently measured the sequence of attacks at various densities and times was accurately predicted. Consequently methods employing this type of programme may provide an important tool in understanding aspects of mimicry. These, however, involve the inclusion of more than one species of prey, at least model and mimic; and, in the concept here developed, of an alternative and abundant edible form distinct from them. As a condition of the test, it was considered that mimic and model evoke a single type of attack-reaction since the resemblance between them is treated as perfect: a situation which is in fact unrealistic. Various refinements must be added to the mathematical structure, using as data the attack of a Mantis on houseflies, if this is to throw light upon mimicry. These must include certain fundamental aspects of learning and forgetting. To be effective, that is to say, the programming must take cognizance of known relevant features of vertebrate attacks. These were analysed under six components each of which were compared with the output generated by the mathematical model developed to include them. Such additional parameters were assigned from experiments involving small mammals predating Saw-fly pupae. The computor correctly reproduced these features: thus it transformed the reactions of an invertebrate predator into those of a vertebrate one. Consequently it provides a tool for analysing those features of mimicry which involve predation.

Dr. Magnus has investigated mating behaviour in butterflies with polymorphic females and monomorphic males. In the species studied, he finds that the sexual reactions can be divided into two groups. First there is an approach-flight in which the male is optically stimulated by the colour-pattern and movements of the female, also by other similar species and inanimate objects. These may be much stronger in one polymorphic form than another: thus in Argynnis paphia the dark valezina females are unattractive compared with the common male-like type. In such circumstances, the mating disadvantage must be counter-balanced by other qualities, physiological or mimetic. Secondly he finds that at short distances the stimuli leading to copulation are olfactory. Two types of scent stimulation must be considered: that produced by the

virgin female, which indicates to the male that his approach-flight has succeeded, and that produced by the male to stimulate the female during mating. Magnus has so far obtained no proof that normal male coloration exerts any visual stimulus upon the female. Here he meets a difficulty: the fact that butterfly polymorphism, both mimetic and non-mimetic, is generally restricted to the female, never to the male. His attempt to explain that situation fails, for he interprets it on the basis that the females are heterogametic. Much information is now available on the genetics of such polymorphism, and its control proves almost always to be autosomal. Thus its evolution must be selective.

This impasse appears to be resolved, at least in part, by Dr. L. Brower's contribution. He finds that the general importance of colour in courtship is greater in butterflies which exhibit mimicry that is sex limited than in those where both sexes are affected. The latter includes all known Mullerian instances, comprising genera especially noted for scent production. Moreover in the list of species studied by Magnus, none is mimetic. Brower suggests that the strength of female visual selection in Batesian mimicry is great enough to prevent male mimicry. In the Mullerian groups, on the other hand, the increased significance of scent has so lowered the importance of visual stimuli that it is outweighed by the benefits of male mimicry. It is to be noticed, however, that Argynnis paphia is a species with (non-mimetic) polymorphism restricted to the female in which, though fully studied by Magnus, no indication of visual selection by the female could be detected. In truth, we now require further investigation to resolve this matter along the lines carried out by Dr. Brower upon colour and courtship in the Queen butterfly. These could perhaps be transferred to the male now that we have his information for the female. That is to say, we need to know whether the monomorphic males of polymorphic females mate with average success when painted so as to look abnormal or to resemble the mimetic females.

This Symposium has shown that, using experimental methods, great advances have recently been made in the study of mimicry. We may enquire what aspects of the subject now demand special attention. The papers presented here provide some answers to that question. I will suggest a few others. We require to establish more widely and decisively the existence of heterozygous advantage in mimetic polymorphism. For that purpose, extensive breeding of F_2 families is needed. Any differential viability should be accentuated by rearing the broods in unfavourable conditions: a technique which has proved successful in the past (Ford, 1940). Studies on the super-gene control of the phases, begun by Clarke and Sheppard, need to be extended: if possible by the selective modification of crossing over, as undertaken by Detlefsen and Roberts in 1921 and Parsons (1958). It is important to ascertain if genes controlling polymorphism for colour-pattern influence also habits of flight, as strongly suggested for the mima and wahlbergi forms of Hypolimnas dubius. Such mimicry in behaviour may be of great importance: so also may be sound and scent mimicry, in the analysis of which a beginning only has been made (Gaul, 1952; Rothschild, 1961). Work on the latter subject may well throw further light on some of the problems so ably discussed in this Symposium.

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BIOLOGY OF BLOOD PROTOZOA

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by

William Trager

The Rockefeller Institute
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THE CHEMICAL COMPOSITION OF TRYPANOSOMES. James Williamson, National Institute for Medical Research, Mill Hill, London.

A series of analytical studies on trypanosome constitution, begun in the West African Institute for Trypanosomiasis Research and subsequently continued in London, was designed (a) to detect differences between normal and drug-resistant strains of trypanosomes, (b) to indicate or reinforce taxonomic differences, and (c) to assist in the location of fractions with antigenic activity. The initial work in West Africa was done with Professor R. S. Desowitz, and the analysis and localization of antigens has been a joint venture with Mr. K. N. Brown, who was engaged in a study of antigenic variation in trypanosomes. As a reference standard throughout, an old monomorphic laboratory strain (N) of Trypanosoma rhodesiense has been used, together with its stilbamidine-resistant offshoot (S) which has a wide cross-resistance spectrum. In the first stage of this investigation, amino acid and sugar analyses by paper chromatography were made on these strains and on a variety of related trypanosomes: T. cruzi and T. lewisi (Group A trypanosomes; Hoare and Coutelen, 1933), T. equinum and T. vivax (as Group B animal trypanosomes outside the T. brucei sub-group), and a monomorphic T. brucei, a fly-transmissible polymorphic T. rhodesiense, and a monomorphic T. gambiense (as Group B trypanosomes of animals and man, inside the T. brucei sub-group); a free-living trypanosomatid flagellate Strigomonas oncopelti was also included for comparison. The species and strains chosen thus covered, within a group of related organisms, differences in type of existence, in host and tissue preference, in mode of development in an intermediate host, in drug sensitivity and virulence, as well as in morphology; these differences vary widely in kind as well as in degree.

The results (Williamson and Desowitz, 1961) showed that the amino acid constitution of trypanosome protein conforms to the general pattern of the majority of known proteins and is closely similar to that of bovine plasma albumin, from which it differs mainly in having a higher proportion of glycine and a lower proportion of cysteine/cystine. Comparison with published figures for the constitution of bacteria and of a ciliate protozoon, Tetrahymena geleii, shows that the content of basic amino acids and the leucines is generally higher than in bacteria or T. geleii, though the dicarboxylic amino acid content is lower than that of T. geleii. The amino acid constitution shows little variation among the species and strains examined, and only two deviations of any significance ($P < 0.01 > 0.001$) was found between the alanine content of T. cruzi (19.1%) and T. lewisi (8.4%); this may well be related to the aberrantly low level of free amino acids in T. lewisi and to the preponderance of alanine which has been found in the free amino acid pool of trypanosomes. A

difference of borderline significance was found between the combined lysine/arginine content of normal and stilbamidine-resistant strains of T. rhodesiense. An increase in basic amino acids in a strain predominantly resistant to cationic trypanocides would be expected from Schueler's (1947) hypothesis of a shift in parasite protein isoelectric point associated with resistance to ionized drugs, and it is of interest to note that the ratio of cationic (lysine, arginine, histidine), to anionic (tyrosine, glutamic and aspartic acids) protein constituents is 0.99 in the normal strain and 1.4 in the resistant strain. This result must be considered suggestive rather than established, as the experimental error is large and a mixture of at least five proteins is involved. It is possible that such a difference may be more clearly established by analysis of individual protein constituents.

Well-washed trypanosomes contain intracellular free amino acids, liberated only on cell rupture, and present in amount equivalent to about 2-3% (w/w) of cell protein. This level in trypanosomes, which are Gram-negative organisms, is comparable to that in gram-negative bacteria, such as Escherichia coli (0.25 - 1.5% of dry weight), but is rather lower than the levels in Gram-positive bacteria such as Staphylococcus aureus, in T. gelei or in cultured human cells. Assuming that protein accounts for approximately 20% of the intact trypanosome, the intracellular level of free amino acids would be about 500 mg./100 ml whole trypanosome. An average free amino acid level of human post-absorptive plasma is 30-40 mg./100 ml. and the corresponding level in the rat is somewhat higher. It would seem therefore that trypanosomes actively maintain their intracellular free amino acids at a level considerably higher than that of the mammalian host plasma. The dependence of this mechanism on an energy source is implied by in vitro experiments where the stimulating effect of amino acids on the motility of T. equiperdum and T. rhodesiense in blood-free media, is apparent only in the presence of glucose. In the case of African trypanosomes which rapidly produce massive septicaemic infections in laboratory rodents, it is unlikely that cell protein synthesis is fed primarily from the products of rapid trypanosomal glucose catabolism. Amino acid assimilation from the host plasma is likely to be a major source, but few or no experimental data on this or other aspects of trypanosomal protein synthesis are available. The individual free amino acids in trypanosomes have not been estimated quantitatively, except for alanine which accounts for about 50% of the total. Most of the amino acids in cell protein were present, but aspartic acid was often absent, and generally much reduced in amount. In T. rhodesiense, Grant and Fulton (1957) have shown that only alanine and aspartic acid in trypanosome protein show relatively high radioactivity after incubation of trypanosomes with (U-^{14}C) glucose. This implies that both amino acids can be synthesised by the trypanosome;

glutamic acid was only slightly labelled, and may therefore be nutritionally essential. Alanine and aspartic acid are probably formed by transamination, as high levels of transaminase activity are demonstrable in T. cruzi, (Bash-Lewinson and Grossowicz, 1957), and in African trypanosomes (Stephen and Gray, 1960). On a comparable basis, the glutamic-pyruvic transaminase activity of T. rhodesiense is more than fifty times higher than the normal serum enzyme level in the uninfected host; glutamic-oxaloacetate transaminase activity is not so marked (A.R. Gray, unpublished results). Elevated serum glutamic-pyruvic transaminase activity has been observed in trypanosome-infected guinea pigs (Lippi and Sebastiani, 1938) and in cattle and sheep (Stephen and Gray, 1960); this may be partly or wholly due to the enzyme activity of parasites in the course of destruction by the host. As pyruvate is a major product of the incomplete catabolism of glucose by African trypanosomes, the high concentration of free alanine in trypanosomes probably arises from transamination between this keto acid and glutamic acid. Oxaloacetate-glutamate transamination in T. rhodesiense is less active, as might be expected from the much lower levels of oxalo-acetate likely to be involved in pyruvate metabolism (Grant and Fulton, 1957), and an intracellular surplus of aspartic acid from this source would be improbable. A significant pointer to the importance of intracellular amino acids in trypanosome protein synthesis is the low free amino acid content of T. lewisi, the only flagellate among those examined which was not in an active state of division. The only carbohydrate material detectable in African trypanosome hydrolysates was glucosamine which was present only in small amounts (0.2 - 0.3 µg./mg. protein). Other sugars were present in T. cruzi and T. lewisi but were not examined in detail.

The soluble proteins of T. rhodesiense strains were examined more intensively by physical methods in order to detect isoelectric point changes associated with drug-resistance, and to provide a basis for the characterization of trypanosome antigens which have subsequently proved to be almost exclusively unconjugated proteins of fairly low molecular weight (Brown and Williamson, 1962). Ultracentrifugal analysis of homogenates freed from large particles and cell debris demarcated two main groups of macromolecules. Three heavy components representing about 1/5 of the total sedimenting material, had $S_{20,w}$ values (71.5, 53.8 and 42.8) which were typical of nucleic acids and nucleoprotein; the 71.5 S component predominated. Two light constituents ($S_{20,w}$ 4.06 and 1.01) were also present. The former was predominant and the latter present only in small amounts; the corresponding molecular weights are 4-8 x 10^4 and 1-2 x 10^4 respectively. Electrophoretic separation on paper at pH 8.6 showed that the immobile residue consisted of the heavy 43S - 72S constituents (microsomes) and that the mobile fraction consisted of the light components (cell sap

proteins). The size distribution found here for the nucleoprotein particles of T. rhodesiense cytoplasm (43S, 54S and 72S) may be compared with the 30S, 50S, 70S and 100S groups which seem to occur regularly in cells from a variety of animals and micro-organisms, bearing in mind the sensitivity of association of the size groups to Mg^{++} ion concentration, pH and ionic strength and in yeasts, to nutritional state. The existence of typical microsomes and ribosomes in T. rhodesiense has been demonstrated by differential ultracentrifugation of homogenates in sucrose solutions and by electron microscopic examination (Brown and Williamson, 1962). The 4S protein unit shows occasional doublet formation on paper electrophoresis, and a triplet appearance on starch gel; [redacted] at least 4 separate antigens have subsequently been found in this protein group. Isoelectric point estimates for the 4S unit by zone and moving boundary electrophoresis gave values (μ,0.05) of pH 4.8 and 4.4 respectively; the mobility of the unit at pH 8.6 was -2.1 x 10^{-5} cm^2/sec/volt. No differences between normal and stilbamidine-resistant preparations were detectable by this method nor by analysis of comparative acid-base titration curves in water, 0.5 MKCl and 8M urea, using pure bovine plasma albumin (BPA) as reference standard. Detailed interpretation of the trypanosomal protein dissociation curves in terms of amino acid composition was limited by the heterogeneity of the preparation, but the parasite material was less susceptible to urea action than BPA; in both cases the most susceptible locus appeared to be the imidazolium groups of histidine. Detailed examination of T. rhodesiense homogenate non-protein components separable by electrophoresis indicated the presence of free ribonucleic acid demonstrable most readily by "crossing" or "spot-line" electrophoresis with bands of the fluorescent trypanocide ethidium bromide. In trypanosomes taken from infected rats after treatment with this drug, localized binding of the drug to microsomes can be shown by the fluorescence of these constituents when separated by paper electrophoresis of homogenate preparations. It seems likely that these structures will be found to represent in trypanosomes, as in bacterial and mammalian cells, an important locus of drug action.

Some further insight into the nature of the soluble proteins of trypanosomes has been gained in a study of antigenic constitution. The Ouchterlony agar gel diffusion method for detection of the precipitin reaction has been used as a convenient indicator of antigenic activity in trypanosome preparations pre-treated or fractionated by a variety of procedures designed to characterize specific constituents. The task of initial antigenic analysis was simplified by the finding that two widely different strains of Brucei trypanosomes (the old monomorphic T. rhodesiense (N) strain and a fresh polymorphic fly-transmissible strain) and a number of serological variants derived from them, all showed the same basic precipitin pattern.

Of the cytoplasmic macromolecules, only the 4S and 1S protein groups were antigenic. These variable trypanosome antigens, both in their response to various pre-treatment procedures and on chemical and physical fractionation appeared to be proteins devoid of carbohydrate, lipid or nucleic acid haptenic components. The antigens are sensitive to heat, acid, alkali, urea denaturation and to protease action; they precipitate wholly or partly with TCA and saturated ammonium sulphate, dissolve in water, physiological saline and in phenol but are insoluble in non-aqueous organic solvents, exhibit U.V. absorption at 210 and 280 mμ, react with the biuret and Folin-Ciocalteu reagents and with protein stains, are resistant to carbohydrases, nucleases and phosphatases, and are adsorbed by activated charcoal and carboxymethylcellulose. Two groups may be distinguished by a different physical and chemical reactivity, the larger of which has an estimated molecular weight by gel diffusion of $4\text{-}16 \times 10^4$ and is non-dialysable and completely precipitable by trichloroacetic acid (TCA). The smaller group has an estimated molecular weight of 10^4 and is diffusible through cellophane and incompletely precipitable by TCA. The concurrence of these typically protein properties and weight estimates with those of protein components separable electrophoretically and in the ultracentrifuge, together with the results of immunoelectrophoresis, strongly suggest that the antigenicity of the Brucei trypanosomes is associated with at least two groups of soluble unconjugated proteins of molecular weight 10^4 and $4\text{-}16 \times 10^4$ approximately, principally with the latter; these antigen groups are therefore referred to for convenience as the 1S and 4S antigens respectively.

An attempt was made to learn something of the chemical nature of these antigens by treating the crude antigen with reagents used in histochemical procedures and in non-denaturant methods for indicating specific reactive groups in enzymes. The 4S antigen may be completely inactivated by four chemical procedures: acetylation, esterification (propylene oxide), coupling (diazobenzenesulphonic acid) and periodate oxidation. The only group which is likely to be attacked by all four reagents is the phenolic residue of tyrosine, an amino acid which appears to be closely associated with antigenicity in other contexts.

The reactive structures of the 1S antigen are less easy to discern. It was initially differentiated from the 4S antigen by being susceptible only to the more vigorous types of pretreatment, and is more resistant to heat and protease treatment, to acetylation and to periodate oxidation, than the 4S antigen; free carboxyl and guanidyl groups may be susceptible loci. The molecular weight of this protein (10^4) is within the range of proteins which are not precipitated in the normal manner by TCA and are diffusible through cellophane. Water-swollen cellulose film may have a pore size of up to 60Å and Visking cellulose dialysis tubing has been shown to be able to pass proteins of molecular weight up to 4.5×10^4. Present observations on the

TCA precipitability and diffusibility of the 1S antigen are thus compatible with the estimates of its molecular weight.

The antigenicity of both protein groups appears to depend on the integrity of tertiary hydrogen-bonded structures (perhaps involving tyrosine) as both groups are susceptible to 4M urea action. The antigenic structure of the 4S protein is more easily attacked by pepsin and chymotrypsin than that of the 1S protein, and this differential susceptibility may indicate a difference in peptide configuration related to aromatic amino acid residues. Separation of protease hydrolytic products strongly suggests that antigenicity is associated only with whole or partially hydrolysed protein, but further quantitative analysis of unhydrolysed or partially hydrolysed protein residues will be required in order to establish the minimum molecular size necessary for antigenic activity. The common antigens, which were detected only in the later stages of this investigation, have not been characterized.

Intracellular localization of the variable antigens was attempted by centrifugal fractionation of T. rhodesiense homogenates in aqueous sucrose media. The best structural and chemical differentiation of conventional nuclear, "mitochondrial" microsomal and cell sap fractions was obtained in 0.25M sucrose-5mM magnesium sulphate. The cell sap was found to contain the highest concentration of antigens. The "mitochondrial" fraction contained mainly kinetoplasts and flagella; this is of interest in view of electron micrographic evidence showing that although the nucleus, cell membrane, flagellum and endoplasmic reticulum in Brucei trypanosomes appear to have a conventional ultrastructure, the kinetoplast, as in some other flagellates, is a unique combination of nucleus and mitochondrion (Mühlpfordt and Bayer, 1961; Vickerman, 1962); metazoan-type mitochondria with well-developed cristae seem to be absent. Some of the lacunae in normal cell metabolism shown by the bloodstream forms of African trypanosomes on transfer from the tsetse to the mammalian host may well be explicable in terms of changes or loss of mitochondrial function (Williamson, 1962; Vickerman, 1962).// In a preliminary survey with Mr. C. D. Ginger, the lipid constitution of a number of representative parasitic and free-living protozoa has been examined qualitatively and semi-quantitatively by thin-layer chromatography, with initial emphasis on the nature of the sterols present (cf. Aaronson and Baker, 1961). Ultraviolet absorption spectrophotometry of free and esterified sterols, and the Fieser selenium dioxide colour reagent have been used to differentiate cholesterol and ergosterol, following reports of occurrence of the latter in T. cruzi and in Leishmania tropica (Halevy, 1962). No ergosterol has yet been detected in the following parasites examined here: T. rhodesiense (bloodstream (B) and culture (C) forms), T. lewisi (B,C), T. cruzi (C), L. donovani (C), Strigomonas oncopelti (C), Plasmodium knowlesi (B) and Spirochaeta recurrentis (B), all of which appear to contain

both free and esterified cholesterol. The culture forms of T. rhodesiense appear to have a very markedly higher content of glycolipid than the bloodstream forms, and in this respect they have been found to resemble the Group A trypanosomes, T. lewisi and T. cruzi.

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THE IMMUNOLOGY OF TRYPANOSOMA CRUZI INFECTIONS. Tulio Pizzi, Dept. of General Pathology, Faculty of Medicine, University of Chile, Santiago, Chile.

Immunological mechanisms in Chagas' disease are poorly understood. In the last years some progress has been achieved through experimental studies of the acute stage of the disease. On the other hand very little is known on the immunology of the chronic disease and on the mechanisms underlying the development of chronic myocarditis.

During the acute stage, phagocytosis of parasites seems to be an important defensive mechanisms. Phagocytosis occurs mainly in inflammatory macrophages in the interstitial connective tissue of several organs and is coincident with a rise in antibody titers. It is very likely that the phagocytic activity is assisted by the action of non-lytic or opsonin-like antibodies. Lysins active in vivo against blood stages of Trypanosoma cruzi apparently do not develop during the acute phase.

The inability to demonstrate any lytic activity in vivo, and the general participation of macrophages in defense, with the probable cooperation of opsonic antibodies, are at variance with the main defensive mechanisms observed on other trypanosome infections, which have been found to be essentially humoral. Therefore, immunological mechanisms of Chagas' disease seems to be similar to those observed in some Plasmodium infections. Nevertheless, in T. cruzi infections, the spleen is not an important site of parasitic destruction, as in malaria; on the contrary the phagocytic activity occurs mostly in inflammatory macrophages in the connective tissue or different organs.

Parasitic destruction is very often associated with focal inflammatory reactions that likely represent a defensive mechanism Diffuse and late inflammation, without topographic relationship to the parasites probably represents a hypersensitivity reaction.

Immunological conditions of the host would influence the degree of phagocytosis, the site where it occurs, the type of inflammatory reaction and the general reactions phenomena in the lymphoid organs. Thus, in hosts with a low degree of innate and a low ability to develop acquired immunity, phagocytosis of inoculated parasites is not prevalent, there is not marked inflammatory reaction and in the lymphatic organs there is a tendency to depletion and lymphorrhexis. In host with a high degree of innate immunity which are able to develop a strongacquired immunity, there is a marked phagocytic activity in inflammatory foci that occur in the connective tissue of different organs and the lymphatic system shows marked hyperplastic changes.

Finally in hosts with strong innate immunity or with acquired immunity previously established due to prior infection most of the parasites are destroyed at the site of inoculation.

In the chronic stages, it is very likely that humoral defensive mechanisms may be prevalent. It is also possible that autoimmune mechanisms might explain the development of chronic myocarditis.

The degree of the immune response in *T. cruzi* infections is regulated by many factors, some of which have been experimentally studied. The influence of the genetic constitution of the host, age, sex and some environmental factors will be discussed. Attention will also be paid to several factors that depress the immune reaction such as cortisona, X-rays, irradiation and certain drugs.

LEISHMANIASIS. Leslie A. Stauber, Dept. of Zoology and Bureau of Biological Research, Rutgers-the State University, New Brunswick, New Jersey.

Parasites with heteroxenous life cycles are fitted for growth or survival in uniquely different physiological situations. The malaria parasite probably illustrates this best. The change from sporozoite to preerythrocytic meront involves not only a change from poikilothermic to a warm-blooded host but also from an organism suspended in mosquito salivary gland juice to a state of intracellular parasitism in the cells of visceral organs (liver, spleen) of the vertebrate host. Two further changes are observable in the vertebrate host: intraerythrocytic merogony and beginning gamogony ending with the formation of the differentiated gametocytes. In the mosquito midgut, completion of gametogenesis, fertilization and penetration of the midgut wall occurs and is followed by sporogony. The differentiated sporozoite later reaches the salivary glands.

Rogers (1962) in "The Nature of Parasitism" emphasizes, for many parasitic nematodes, the importance of the infective stages in the life cycles. He stresses that these often appear as though they were "resting stages" incapable of further growth and differentiation without a restarting stimulus furnished by the conditions found in the host. He also refers to this as "an event in the process of infection....which seems critical." Examined from this viewpoint the gametocyte and sporozoite of the malaria life cycle clearly are similar infective stages. We have long recognized exflagellation in the mosquito gut as host initiated and essentially a restarting of the new developmental cycle in the mosquito. Huff (1953) has emphasized what is probably an equally profound physiological change from free sporozoite to intracellular cryptozoite though the mechanisms involved in restarting growth and development are unknown. Such a view of the malaria life cycle might lead to profitable research directions.

The Leishmania have a much simpler life cycle and, although it does not involve so many important changes for the parasite as in malaria, there still are the critical shifts between vertebrate and invertebrate hosts with stage changes which might be interpreted along the lines of Rogers' hypothesis. The present discussion will attempt to consider the Leishmania as two ways of life, leptomonad and leishmaniform, and review some of the morphological, cultural, serological and other evidence for clues to the meaning of these two ways of life. There is, however, at least one other interpretation of the life cycle which I have at times shared with other workers and which should be noted here. This would visualize the leishmaniform stage as a "cyst"-like stage, semi-resistant perhaps, to homologize it with events in the lower Trypanosomidae where passage of such a stage effects transmission from one invertebrate host to the next in simple homoxenous life cycles. In any case, the changes from leptomonad to leishmaniform stage and vice versa are important events in the life cycle and, of course, somewhat more complicated in the heteroxenous genus Leishmania where two quite different kinds of hosts are a part of that life cycle.

Morphological. The electron micrographic studies of the Leishmania, especially those of Rudzinska, D'Alesandro and Trager (1962), show important similarities as well as differences between the leptomonad and leishmaniform stages

(Ld bodies, Lds). The nuclei appear the same. The flagellar apparatus is complete except for a free flagellum but the "Rhizoplast" of earlier descriptions is not an intracytoplasmic portion of the flagellum as in the sense of Giardia nor like the marginal filament of an undulating membrane but more like the flagellum of Peranema passing up through a "pocket-like invagination"--already a free flagellum as it leaves the area of the blepharoplast or basal body. Sauer (1935) has emphasized the discarding of the flagellum of Euglena in the presence of antiserum. It would be interesting to learn where the break occurs in Euglena and whether there is an analogy which may be of some importance to us here in the transformation of leptomonad to Ld body.

Two important differences between leptomonad and Ld body have been reported by Rudzinska, D'Alesandro and Trager (1962). The first concerns the presence of two membranes around the Ld body. Whether the outer one, often much thickened at one side of the Ld body, is host-secreted or parasite-secreted material is not definitely known but it is lost from the leishmaniform stage soon after transfer to in vitro culture medium. With the loss of this external membrane, the differentiating Ld body appears less distinct in outline but even later when the new leptomonad shows a sharply-outlined limiting membrane it is single not double as in the Ld body. The second difference involves the elongate mitochondria of the leptomonad, extensions of the mitochondrial part of the kinetoplast. This material, except for the part always associated with the kinetoplast, is much reduced in amount in the Ld body. The elaboration of the elongate mitochondria is described as part of the differentiation which occurs when Ld bodies are placed in culture medium. The reverse, loss of this material in change from leptomonad to Ld body, has not yet been studied. It is important to know the conditions for this latter change. Many workers have reported on the low infectivity of culture leptomonads (see e.g. Adler, 1962) implying that loss of virulence is the more usual occurrence. Our studies would indicate that, even in the highly susceptible hamster by intracardial inoculation, leptomonads have such a low order of infectivity that in many cases it would appear that only one or a few organisms survive the transition from leptomonad to intracellular Ld body. Where large numbers of organisms are injected we have shown that it takes 30 to 1,000 or more times as many first passage culture leptomonads as splenic leishmaniform stages to produce similar infections. It also takes about 1000 times as many leptomonads intraperitoneally as splenic Lds intracardially to produce similar infections in the hamster.

Trager has suggested that the presence of the elongated mitochondria denotes synthetic capacities lacking in the Ld body. It might be that log phase leptomonads in full synthetic activity are less infective than stationary phase organisms. This could be tested easily. Adler, Theodor and Wittenberg (1938) noted differences in the appearance of flagellates in infections of L. tropica in P. sergenti but were not then able to explain the significance of their finding. If all leptomonads are not equally infective, then perhaps there is another interpretation for the raisin (Smith, Halder and Ahmed, 1940) or salt (Adler and Ber, 1941) stimulation of infectivity in the sandfly; not only increased numbers of organisms and "blocking" of the anterior alimentary tract but perhaps physiological

differentiation into a "metacyclic" leptomonad, a non-reproducing "resting" stage in Rogers' interpretation.

I am impressed by the discrepancy between our findings noted above (the need to inject relatively enormous numbers of leptomonads to produce infection) and the report of Hoogstraal and Dietlein (1963) who report "heavy" infections in flies as those with as few as 5000 leptomonads. Obviously, the numbers of leptomonads introduced by the fly even under the best conditions are several orders of magnitude different, yet successful in natural transmissions.

Vickerman's (1962) studies on Trypanosoma cruzi also show mitochondrial elaboration as a function of change from blood trypanosome to the crithidial form in culture. The appearance of the metacyclic trypanosome in the hindgut of the reduvid and the transformations from metacyclic stage to intracellular Ld body and from Ld body to blood trypanosome are yet to be reported for whatever light they may shed on this general problem among these flagellates.

Cultural. Leptomonads and Ld bodies behave differently in culture also. It has long been known that in vitro cultivation of Leishmania can be easily obtained. Most suitable conditions are with blood-containing media kept at temperatures around 25° to 28°C. Trager (1957) showed that Leishmania donovani would not grow on the chemically defined medium suitable for L. tarentolae and that it evidently requires the additional substances supplied by the blood usually used in their cultivation. Leishmaniform stages have been grown in vitro only in tissue culture at 37°C where they are found in the intracellular position (Hawking, 1948; Pipkin and Coles, 1960). Hawking (1948) showed that transformation of Lds (escaping from host cells) to leptomonads could occur under these conditions. He also showed that under the conditions of tissue culture medium with host tissue still present the leptomonads could grow and reproduce. In addition he found that when culture flagellates were added to tissue cultures of hamster spleen some entered host cells and transformed to Lds while others multiplied as leptomonads in the fluid portion of the medium. Conditions are satisfactory to both states at 37°C in a sufficiently rich medium (see also Pipkin and Coles, 1960). Trager (1953) observed "intermediate" forms of Leishmania in modified Chang's (1947) culture medium at 37°C and showed that they had more exacting requirements than did leptomonads grown at 28°C. He also showed that the intermediate forms and typical leptomonads were differentially affected by nicotinamide in the medium. It is presumed that the typical Ld body is still more fastidious and can grow and reproduce at 37°C only when furnished with substances made available to it in the intracellular environment of a host cell (Trager, 1963).

One of the principal arguments against the viewpoint that the Ld body is a resistant, "cyst-like" stage, it seems to me, is implied by the finding that it reproduces in that stage in the intracellular position. It is true, of course, that many protozoa reproduce in cysts (amoebae, coccidia, etc.) but true binary fission of the whole Ld body as commonly described does not fit the picture of a reproductive cyst. Furthermore, the rates of multiplication of log-phase leptomonads and splenic Lds are very similar according to our findings /about 20 hours in modified Tobie's medium (Evans; see also Chang and Negherborn, 1947) and only slightly longer in the hamster liver

or spleen (Stauber, 1955)_7. One might argue that the rate of reproduction should be greater at the higher in vivo temperature although other rate limiting factors may be involved. At any rate, Ld bodies in vivo, although possibly inhibited in part, are still capable of metabolizing and reproducing.

The survival of Lds in refrigerated whole spleen at 4°C for at least 30 days without loss of infectivity (Heyneman and Mansour, 1962a) is matched by the persistence of live leptomonads at 25°C for many days (as long as 224 with L. donovani) in some diphasic media (e.g. 30% sheep blood agar, Heyneman and Mansour, 1962b). Neither of these items can be interpreted as evidence for "resting" stages in the life cycle of the parasite though both are interesting points of departure for further studies.

The most meaningful extensions of the influence of ambient temperature on growth and reproduction of protozoa can be obtained by referring to the work of Hutner et al. (1959) and Scherbaum and Zeuthen (1954). In the former case exposure of Ochromonas malhamensis to different environmental temperatures showed that, as the temperature was raised, the organism became more fastidious in requirements. Not only were greater concentrations of essential ingredients required but whole new blocks of requirements appeared. Small temperature increments at critical points led to profound changes in needs. Finally at the highest temperature used, in addition to a very complex medium, a suspension of autoclaved Ochromonas cells grown at more favorable temperatures was required to maintain growth. Obviously, catabolic and degradative processes and losses of synthetic abilities were exceeding the limits of the remaining anabolic and synthetic capacities of the organisms. In the second case, with Tetrahymena, exposures of the ciliates in suitable culture medium to alternating 6 hour intervals of high and low temperatures, while not causing cessation of feeding or growth throughout the experiment, did so interrupt reproduction that the ciliates finally were all inhibited in the same phase. When returned to optimal temperature conditions synchronous reproduction of the ciliates occurred. Since the ciliates had grown but not reproduced up to this time giantism was strikingly evident among them.

Application of such findings to the problem of the Leishmania might suggest that leptomonads with larger synthetic capacities can grow in vitro at room temperatures. The leishmaniform stage can only be achieved and reproduce in the presence of a much richer environment (host cell). These host cells are normally found in living vertebrates with body temperatures above 35°C, which environment itself may imply greater needs to be supplied to the organism. A decade ago (Stauber, 1953) we tried to influence the course of infection of leishmaniasis in the golden hamster (Mesocricetus auratus) by manipulating the hamster in ways which might be presumed to increase its body temperature. Exposure to high environmental temperatures, injections of bacterial pyrogens and injections of dinitrophenol were procedures used. The results were mostly equivocal except that a few animals surviving exposure to high temperatures were "cured." At that time our methods for producing and studying the infection were rather crude and inherent variability of the host response made evaluation of the data difficult. In addition, the chronic, though insidious, nature of the infection

meant long term exposures subject to a variety of intercurrent events deleterious to a successful completion of an experiment.

Recently we have returned to this problem using the elements of the sensitive 8-day drug-screening method previously described by us (Stauber, Franchino and Grun, 1958). In this test the almost exponential growth of the parasite in spleen or liver over the first 8 days after intracardial inoculation of Ld bodies is used as the comparative slope of the reproductive rate of the parasites. Exposure of the host to conditions ultimately unfavorable to the parasite will result in a decreased slope for the same period. In practice we put uninfected hamsters under the high environmental temperatures a week or more prior to infection and hold them there until sacrifice 8 or more days after infection. Controls at room temperature are infected and sacrificed at similar intervals. When the maximum temperature in the Hotpak is only 33°C or lower (relative humidity 40%) the parasites reproduce at comparable rates in animals at ordinary animal room or at Hotpak temperatures. When, however, the hamsters are held at a higher temperature (maximum 36°C; range usually from 35-36°C) repeated experiments have shown an almost complete cessation of reproduction of the parasites. In some experiments parasites were not seen in some of the Hotpak-exposed animals after only 8 days. At longer intervals after inoculation, parasites were more regularly not seen (this does not necessarily mean "cure" but at least reduction of numbers below the threshold for finding parasites on impression smears). A typical experiment is shown in Table I ("Total" Lds as defined in Stauber, Franchino and Grun, 1958).*

Table I. Numbers of L. donovani in liver of hamsters exposed to cage environments of 25°C or 36°C. Each animal inoculated with approx. 20 million splenic Ld bodies.

Time after Inoculation	Exposure Temperature	No. of Animals in Group	"Total" Lds in Liver (Group Median)
1 hour	25°C	8	61
1 hour	36°C	5	25
8 day	25°C	8	2650
8 day	36°C	8	5
21 day	36°C	8	<2 (5 of 8 animals negative)

When parasites were found in the impression smears of the organs of animals exposed to high temperature it was observed that the parasites were abnormal in appearance--enlarged, vacuolated, degenerating and clearly suggestive of a poor host cell environment for the parasite. An additional noteworthy finding was that the animals exposed to the high temperatures ate significantly smaller amounts of food per day than those animals held at room temperatures and lost weight. In a

*I am indebted to Robert Herman, Jack Blaine and Stuart Beeber for the technical assistance in conducting this experiment.

typical experiment daily food consumption was 1.9 gm vs. 5.0 gm for Hotpak and ordinary animal room groups respectively. Since Actor (1960) has already shown the profound effects of dietary manipulation on leishmaniasis in the mouse and hamster it may well be that the effects on the parasite of exposure of the host to high temperature are related to the problems of nutritional requirements noted above. The high temperatures may increase the needs of the parasite, or, even if host body temperature is not increased (not measured yet), decrease the availability of nutrients ordinarily furnished by the host cell to the parasite in the host kept at room temperature. This line of investigation is presently under study.

Serological. Both Adler and Adler (1955) and also D'Alesandro (1954) have shown that antisera made either to leptomonads or Lds show little cross reactivity (measured as agglutinin titers) to the heterologous state of the parasite. This is in spite of evidence (Wegener and Koch, 1926) for at least as much cross reactivity between leptomonads of different species of the genus Leishmania. In the one case where D'Alesandro did get a heterologous reaction with anti-leptomonad serum the Ld antigen from spleen homogenates was not preserved until after several hours at room temperature. These are the conditions which Rudzinska, D'Alesandro and Trager show by electron microscopy to lead to the loss of the outer membrane by the Ld body recently freed from the host cell and beginning differentiating into a leptomonad. Adler (1961) has expressed the opinion that perhaps Lds are coated by antibody in the host cell thus possibly explaining their lack of reactivity in the presence of heterologous antiserum. The sharpness of the outer membrane in the electron micrograph does not suggest that this is an antigen-antibody coat, nor would its later loss during change to leptomonad be expected to leave a parasite with a poorly-defined limiting membrane. On the other hand though not necessarily related to the above, an antibody coat might play a role in the availability of nutrients to the parasite within the host cell but again intracellular multiplication of Ld bodies suggests that this might not be so; Adler and Adler (1956) showed significant inhibitory effects on flagellar formation and differentiation of leishmania in cultures containing antiserum as an ingredient of the medium. None of these observations shed any light on whether any of the stages of the parasite can be considered "resting stages" in Rogers' sense though application of more sophisticated immunological procedures might yet permit detection of differences among the leptomonads.

Although this discussion has been largely speculative, it is hoped that it may stimulate further discussion and work on the significance of the 2 life stages in the Leishmania.

Summary

By morphological, cultural and serological methods the importance of the distinctions between leptomonad and leishmaniform stages of the Leishmania is recognized. The possible significance of these differences is discussed in terms of the life cycle of the parasite in vertebrate and invertebrate host.

PINOCYTOSIS IN INTRAERYTHROCYTIC PARASITES.* Maria A. Rudzinska, William Trager and R.S. Bray, The Rockefeller Institute, New York, N.Y., and London School of Hygiene and Tropical Medicine, London, England.

Intracellular parasites have been supposed to feed by the diffusion of nutrients across their cell membrane. This supposition has been supported by little experimental evidence, a situation which is perhaps not remarkable when we consider the small size of the organisms and the difficulties of physiological studies with them. Malaria parasites, living within their host erythrocytes, are known to digest hemoglobin with the formation of a brown degradation product, hemozoin. Since the granules of hemozoin lie within the parasite it seemed necessary to assume that hemoglobin, a rather large molecule, could diffuse through the cell membrane of the parasite.

The need for this assumption was quickly dispelled when electron microscopy showed clearly that malarial parasites feed by a process of intracellular phagotrophy or pinocytosis (1). The first intimations of this came unexpectedly in the course of a study of the fine structure of the bird malarial parasite *Plasmodium lophurae*. Electron micrographs of thin sections of infected duck erythrocytes showed, as the most conspicuous structures within the parasites, relatively large oval bodies having the density and general appearance of the erythrocyte cytoplasm within which the parasites lay. Further study made it clear that these bodies did in fact consist of erythrocyte cytoplasm, and partly formed bodies were found still connected by a wider or narrower channel to the surrounding erythrocyte material. These bodies were evidently food vacuoles, formed by the pinocytotic ingestion by the parasite of globules of red cell cytoplasm. This conclusion was soon confirmed and greatly strengthened by altogether similar findings made with the rodent malaria *P. berghei* in rat reticulocytes (2). Some electron micrographs of *P. berghei* showed that the ring stage of malaria, so often exhibited by young parasites soon after they have invaded red cells, results from a cup shape assumed by the parasite as it ingests into a food vacuole its first large portion of host cytoplasm. This pinocytotic method of feeding has now been found in all species of malarial parasites examined, *P. falciparum* (3), *P. vivax*, *P. ovale*, *P. gonderi* (3), and *P. knowlesi* (4). Furthermore, it has been found in an unrelated intraerythrocytic parasite, *Babesia rodhaini* of the mouse(5).

The general fine structure of all of these erythrocytic parasites is a relatively simple one. All are surrounded by two unit membranes (except *Babesia* which has one membrane) lying in close contact with the surrounding host cytoplasm. The parasite cyto-

*Supported by a grant (E-1407) from the National Institute of Allergy and Infectious Diseases, U.S.P.H.S.

plasm contains granules, which are probably ribonucleoprotein particles, and poorly developed endoplasmic reticulum. Golgi material was rarely seen. In P. lophurae and probably also in the human parasite P. falciparum a few mitochondria with villi projecting internally occur. In all the other species of malaria studied and also in Babesia typical mitochondria are lacking. Instead one finds structures composed of 2 to 5 concentric double membranes. These structures seem to arise from the plasma membrane. They resemble the mitochondria described in Helix pomatia during certain stages of spermatogenesis (6,7). In P. berghei and in Babesia there is also present a single elongate vacuole-like organelle bounded by a double membrane. Perhaps it serves as a reservoir of fluid.

The nucleus of Babesia as well as of the malaria parasites has the structure found in higher organisms: it is bounded by two membranes with pores and the nucleoplasm is differentiated into chromatin and nucleolar material. Often the nucleus lies adjacent to and even partially surrounds a recently formed food vacuole.

Although the process of pinocytosis of host cytoplasm is the same in all erythrocytic parasites thus far studied, the further fate of the ingested material may take one of three courses. In the bird parasite P. lophurae, digestion of hemoglobin and the concomitant formation of coarse granules of the pigment hemozoin occur within the food vacuole. Food vacuoles with pigment invariably have a matrix of low density, whereas food vacuoles with a matrix as dense as the red cell cytoplasm have little or no pigment, and intermediate stages of decreasing density of matrix and increasing amount of pigment occur. This same situation exists with the human parasite P. falciparum.

In P. berghei, however, the large food vacuoles always have a matrix as dense as the erythrocyte cytoplasm, and they never contain pigment. Surrounding some of the food vacuoles are minute vesicles pinching off from it. Within each of these one to two very fine pigment granules are found as the hemoglobin is digested. These fine granules, lying within tiny vesicles, become dispersed through the cytoplasm of the parasite. This difference in the digestion process accounts for the contrast between the coarse pigment of P. lophurae and P. falciparum and the very fine pigment hardly visible by light microscopy, of P. berghei. In P. vivax and P. ovale in chimpanzees and in P. gonderi in the mangabey monkey digestion takes the same path as in P. berghei but the separate pigment grains found are somewhat larger. In Babesia digestion must follow yet a third course, for here no pigment grains whatever are found.

Recent studies show that pinocytosis is a quite common

process. It was first observed by Edwards (8) and emphasized by Lewis (9) who introduced the term pinocytosis. Lewis made his detailed observations on macrophages and other cells in tissue culture stressing the possible significance and universality of pinocytosis. Electron microscope studies support fully Lewis' supposition and show in addition that pinocytosis is not limited to pseudopodia-forming cells like amoebae and macrophages but that it also occurs in cells of relatively fixed forms.

Since the first observations on pinocytosis it was known that this process may be induced by certain salts and proteins (8,10, 11). Studies on the mechanism of pinocytosis (12,13) strongly suggest that inducers act directly on the plasma membrane resulting in its invagination. Support for this hypothesis comes from studies on the ferritin cycle (14). Electron micrographs show that ferritin is incorporated into erythroblasts by pinocytosis and that formation of pinocytotic invaginations is preceded by the attachment of ferritin molecules to the plasma membrane of the erythroblast. Here ferritin acts as an inducer.

It seems most probable that similar mechanisms are involved in the formation of food vacuoles in malarial parasites and in *Babesia*. It has to be stressed that *Plasmodium* and *Babesia* are not closely related forms. The same mode of feeding in both is most likely connected with the same type of medium they live in. Both are intraerythrocytic parasites and the surrounding medium consists of erythrocyte cytoplasm. It is therefore reasonable to assume that in erythrocyte cytoplasm some proteins and salts are present which act as inducers and are responsible for invaginations of the parasite's plasma membrane. Droplets of host cytoplasm are trapped into these invaginations with the subsequent formation of food vacuoles. This type of pinocytosis, though not differing from pinocytosis in other cells, is of special nature in that it results in the uptake of the living cytoplasm of the host cell which forms the immediate environment of the intracellular parasite.

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STUDIES IN THE ACQUISITION OF IMMUNITY TO P. FALCIPARUM INFECTIONS IN AFRICA. I. A. McGregor, Medical Research Council Laboratories, Gambia, West Africa.

An account is given of clinical investigations made in Africa to elucidate the intimate relationship between malarial immunity and gamma globulin.

1. The acquisition of immunity in young African children is associated with a marked and significant rise in serum gamma globulin concentration.

2. The high serum gamma globulin concentration of adult "immune" Africans living in hyperendemic malarious circumstances can be markedly and significantly reduced by prolonged malarial chemoprophylaxis.

3. The maintenance of malarial immunity appears to require greatly enhanced rates of synthesis and catabolism of gamma globulin. Metabolic studies using isotopes of iodine have indicated that adult "immune" Africans living unprotected in malarious circumstances synthesised gamma globulin at a daily rate that was almost twice that of African adults resident in the same area but protected by chemoprophylaxis and approximately seven times that of Europeans resident in England.

4. In the Gambia passive transfer of malarial immunity has been achieved. Young children suffering from acute P. falciparum infections showed a satisfactory therapeutic response following inoculation of gamma globulin prepared from "immune" adult African serum. "Gamma-free" serum from "immune" adults and gamma globulin from the serum of non-immunes did not produce a marked therapeutic response.

5. The protective value of immune serum has been shown to reside in the 7S gamma globulin fraction.

6. 7S gamma globulin prepared from the serum of "immune" West African adults has been shown to have a marked therapeutic effect in children acutely infected with P. falciparum in East Africa.

7. Morphological changes in P. falciparum parasites were not observed during 7S gamma globulin therapy. Growth and maturation of trophozoites appeared to progress unimpeded until preschizogonal withdrawal occurred. Following schizogony a new generation of parasites appeared usually, although not invariably, in greatly reduced numbers. These young parasites again continued to grow in apparently normal fashion until withdrawal. From exacerbation of clinical illness a second schizogony was deduced to occur but this was not followed by the reappearance of parasites in the peripheral blood in high

density.

Fully formed gametocytes showed no change in morphology nor did "immune" gamma globulin appear to have any effect in reducing their numbers. In two instances termination of asexual parasitaemia was followed by a wave of gametocytaemia.

Discussion:

The results of the various investigations described indicate that acquired malarial immunity, to P. falciparum infections at least, is predominantly serological in nature. They further indicate that it is specifically associated with the 7S fraction of serum gamma globulin. As antibodies associated with this fraction are known to cross the placenta and enter the foetal circulation, the passive transfer of malarial immunity from mother to offspring thought by many workers to occur in hyper-endemic regions may be explained.

Immune 7S gamma globulin appears to exert its protective action against the late stage intra-erythrocytic asexual parasite or, more likely perhaps, against the extracellular, liberated merozoite. It appears to have little effect upon gametocytes. Evidence already exists which indicates that in primates naturally acquired immunity is developed towards erythrocytic parasitaemia but not towards the sporozoite or the pre- or exo-erythrocytic forms. These facts suggest that the antigenicity of the plasmodium is not identical at all phases of its life cycle, and that consequently in a clinically immune individual infection will proceed until the parasite reaches the requisite antigenicity to permit antibody-antigen interaction. Thus immunity acquired to P. falciparum infections may effectively act against late stage asexual erythrocytic parasites, yet afford no protection against sporozoites, pre-erythrocytic schizonts or the early stages of the first generation of erythrocytic asexual parasites.

No evidence at present exists to support a belief that all other developmental phases of the plasmodium are antigenically inferior to asexual erythrocytic forms. It is likely therefore that in nature immunity is acquired to this latter stage because of the numerical preponderance it achieves. Antigenic constancy and numerical density are probably extremely important factors in the stimulation of immunity by parasites.

That a passively acquired immunity, strong enough to curtail drastically asexual reproduction, had little obvious effect on gametocytes is of interest, and suggests that gametocytes and perhaps the merozoites that give rise to them may be serologically distinct from their asexual counterparts. Such serological differences could well explain the known importance of "immune"

adults as a reservoir of mosquito infection in regions of high malarial transmission. Moreover, it would also imply that the dense gametocytaemia of young children in hyperendemic regions may be more an index of recent heavy asexual parasitaemia than evidence of lack of malarial immunity at the time of examination.

If, as seems likely, naturally acquired immunity is specifically orientated towards the asexual erythrocytic parasite, its development is probably profoundly affected by the density and duration of such parasitaemia; prolonged dense infections are likely to constitute stronger stimuli than light transient ones. The efficiency of an established immunity to subsequent homologous infection may be expected to vary with the magnitude of the asexual erythrocytic challenge, the amount of antibody in the blood at the time of segmentation and the ability of the host to synthesise further quantities of antibody rapidly. Heavy infection with viable sporozoites will always constitute a severe test of acquired immunity. In view of the great reproductive capacity of the pre-erythrocytic schizont (x30,000 in P. falciparum) and because of the fact that it is apparently unaffected by the immune mechanism, such sporozoite infection may be expected to give rise to an abrupt erythrocytic parasitaemia of a magnitude sufficient to break all but the strongest immunity.

The clinician, engaged in the study of induced malaria, and the epidemiologist have often stressed the apparent existence of serologically distinct strains of a single species of plasmodium. Thus the impression is gained that an efficient immunity acquired in one part of the world is likely to be the product of local experience and to carry no guarantee of protection against infection with the same species in other endemic areas. The therapeutic response to West African "immune" gamma globulin of P. falciparum infections in East Africa is therefore of interest for it suggests a pronounced antigenic similarity of the strains (if more than one exists) of this parasite in two areas some 4,000 miles apart. This interest is increased by the recent demonstration in fluorescent antibody studies that several species of mammalian plasmodia possess common antigens to a surprising degree.

Further research is required into factors capable of interfering with antibody response to malarial infection. Some evidence exists to suggest that active malarial parasitaemia "crowds out" antibody response to tetanus toxoid inoculation in children. Does this indicate that infections with organisms capable of evoking a strong serological response may suppress or embarrass antibody response to malarial infection? Again the effect of pregnancy on immune status is poorly understood. Exacerbation of parasitaemia in pregnancy has been described in Africans, while a close association between relapse of L. simondi

parasitaemia and the onset of reproductive activity in ducks is known. Does the profound hormonal change that occurs in periods of reproductive activity interfere with antibody synthesis or antigen-antibody reactions?

The persistence of low grade parasitaemia in otherwise efficiently immune individuals is perplexing. In hyperendemic regions of the world this may be partly explained if first generation erythrocytic parasites can develop without hindrance. However, the persistence of P. falciparum parasitaemia in individuals resident in non-malarious circumstances can hardly be attributed to the persistence of pre-erythrocytic schizogony in the host. Does, therefore, an exo-erythrocytic cycle of infection develop in P. falciparum infections or can, as has been suggested in P. berghei infections in white mice, biological variations and selection by immune influences result in the appearance of plasmodial variants capable of thriving in the immune host?

The results of studies herein reported suggest that immune mechanisms in malarial and bacterial infections have much in common.

INSECT DEVELOPMENT AND ENDOCRINOLOGY

A symposium organized for the
XVI International Congress of Zoology

by

Hans Laufer

Mergenthaler Laboratory for Biology
The Johns Hopkins University
Baltimore, Maryland

HORMONES AND THE DEVELOPMENT OF INSECTS. Hans Laufer, Dept. of Biology, The Johns Hopkins University, Baltimore, Maryland.

It is generally accepted that embryonic and post-embryonic development are under genetic control. One view is that developmental processes consist of sequences of genic activations and inactivations, which result in alterations in the metabolism of nucleic acids, proteins, lipids, and carbohydrates and other molecular species. The organism and its constituent subunits is of course composed of combinations of these molecules. Alterations in the production of macromolecules constitute a major portion of the processes of differentiation. Thus, development is considered to be the organized expression of genetic capacities; the orderly progression of the processes that regulate and interact with genes lead to the emergence of new form.

Post-embryonic development of insects is regulated by hormones. The cells are capable of developing along alternative pathways. The developmental course chosen depends upon the environment presented to the cells and often is influenced by hormonal stimuli. The nature of this control and the molecular interactions which bring about development are the subject of inquiry leading to the discussions to be presented here at this symposium.

A number of hormones significant to development have been purified, isolated, and crystallized, and tentative structures have been proposed for some (see the contributions of Karlson, Kobayashi, and Schneiderman). Fractions with brain hormone activity have been prepared. Ecdysone, the molting hormone, was crystallized by Butenandt and Karlson (1954). The juvenile hormone, elaborated by the corpus allatum, has been highly purified (Williams, 1956; Schneiderman and Gilbert, 1959). Several pheromones have also been identified and characterized (Butenandt *et al.*, 1959; Jacobson *et al.*, 1960); these include the sex attractants in several species of insects.

A model system of development

It is helpful to select one example of development as a model for our consideration of the hypothesis of differential gene activation and inactivation. By this method we can pin-point the various ways in which hormones may act and the sites at which they can function during development. The material we use in our laboratory is the larval salivary gland of the Dipteran, *Chironomus thummi*; a similar system is studied in a number of laboratories. Several of the speakers at this symposium will refer to work with this tissue.

The Dipteran salivary gland is a tissue composed of comparatively uniform type of secretory cells. It is a transitory larval structure with a small number of cells (about 33 in *C.thummi*) derived from an ectodermal invagination at the anterior of the embryo. The salivary gland of the adult differentiates from an imaginal disc and therefore is an entirely unique tissue with an origin different from that of the larval gland. The latter gland,

the one with which we are concerned, grows most rapidly at the closing phases of larval life. Growth is accomplished by an increase in cell size and mass. The increase in protein and nucleic acid is extensive, but the number of cells remains constant.

Early in pupation, the salivary gland disintegrates completely. Bodenstein (1943) working with Drosophila, has shown that the growth and final destruction of the gland are under hormonal control. He has also pointed out that the gland is most susceptible to hormonal influences when it reaches a critical age of competence, that is, glands from young animals cannot degenerate in response to the hormonal stimuli in the same manner as do the older glands.

The gland possesses giant polytene chromosomes which contain morphologically distinct regions termed "puffs," and even more greatly enlarged prominences, called "Balbiani rings." These puffs occur in precise locations along the chromosomes, that is all homologous salivary gland chromosomes exhibit the same pattern of puff at a particular time of development. Some puffs are tissue specific. A particular pattern is characteristic of the chromosomes of a given tissue; in other tissues different patterns are present. Some puffs may exhibit stage specificity, arising or regressing at particular times during development (Beerman, 1952). Such tissue and stage specificity suggests that particular functions may be attributable to these sites on the chromosomes. Indeed, incorporation of radioactive precursors showed that the puffs are regions of preferential incorporation in the synthesis of ribonucleic acid and protein (e.g., Pelling, 1959). That a particular puff may be associated with a specific product was shown by Beerman (1961), who found that the presence of a Balbiani ring in "special" salivary gland cells was always accompanied by certain cytoplasmic granules which occur in the secretion.

Our interest has been in the normal development of the gland and in its metabolic and secretory activities. In addition, attention has been focused on the influence of hormones on gland development, secretion, and chromosomal puffing (Laufer, Nakase and Vanderberg, 1963).

The results, summarized briefly, are:

1. At least seven enzymes are present in salivary glands of Chironomus thummi. These enzymes exhibit different degrees of activity in other tissues and thus show tissue specificity. These enzymes may reflect some of the functions of the gland.

2. Of the seven enzymes studied, three (trehalase, hyaluronidase and protease) are present in the salivary gland secretion. They account for a major portion of the protein and indicate possible functional activities of the secretion.

3. Other enzymes of the salivary gland not present in saliva are malate dehydrogenase, esterase, phosphatase and ribonuclease.

4. During normal development of the fourth larval stage and during the formation of the prepupa, the salivary gland enlarges. Final breakdown occurs shortly after pupation. In the course of these developmental changes the activities of trehalase, hyaluronidase, malate dehydrogenase and protease are altered in precise patterns. The total activities per gland may increase as much as 100% in the course of glandular growth while the total activities of the other enzymes (esterase, phosphatase, and ribonuclease) do not change during this period. Thus we have observed two classes of enzymes, those which respond to developmental stimuli and those which remain unaffected even in an environment of rapid growth, and altered hormone concentration. The increases in activity of the enzymes influenced by development closely follow the increase of protein in the gland.

5. Development of the larva can be arrested by positioning a ligature between the first and second thoracic segments, a treatment which separates the salivary gland from its normal source of brain hormone. The enzymes which ordinarily increase in activity during the normal course of development, decrease following thoracic ligature and remain at low levels without subsequent recovery. The larval arrest and depression of enzyme activities after experimental intervention may indicate a dependence of the synthesis of specific proteins (and of development) upon hormones, presumably through the appropriate interactions with brain, prothoracic gland and juvenile hormones.

6. Control ligatures, between the head and the thorax, subject the larvae to trauma without the segregation of the endocrine sources and the salivary gland. This operation retards development by several days, and concurrently decreases the activities of the development-dependent enzymes, i.e. hyaluronidase, trehalase, protease, malate dehydrogenase. The animals appear to recover from the trauma of this operation and proceed through metamorphosis. At the same time, the depressed enzyme activities also recovered at times to near normal levels. The second group of enzymes, i.e. esterase, phosphatase, and ribonuclease, remain unaffected throughout this treatment.

7. Several conditions alter enzyme activities in characteristic ways: a. normal development, b. wounding or trauma, c. starvation, d. interruption of metamorphosis through manipulation of the endocrines. These procedures also alter the puffing activities of the chromosomes (Laufer, Vanderberg, and Nakase, 1963), the effects on the chromosomes being different and characteristic for the various treatments. Two Balbiani rings on the fourth chromosome and the nucleolus are of particular interest since these may be associated with specific gland functions. These chromosomal structures appear to be strikingly susceptible to environmental changes. Reduction in enzyme activities tends to follow the reduction in size and staining intensity of the Balbiani rings and the nucleolus. Concomitant with a depression of chromosomal puffing activity is a loss of activity of several enzymes, including those present in the salivary secretion. Many other

puffed regions remained unaltered after manipulation of hormonal levels by the above treatments. Interestingly, correlated with this observation is the finding of several enzymes whose activities also remain unchanged in the treated larvae. Thus treatments that alter puffing patterns also alter in specific ways the activities of some of the final gene products - enzymes. The differential susceptibility of some enzymes to hormonal control and, indeed their dependence upon it, during both experimental procedures and normal development, strongly support the idea of differential genic activities controlled by hormones.

8. Specific metabolic inhibitors affect the enzyme activities of the salivary gland. Both puromycin and actinomycin D, when introduced into the medium, affect the activities of the developing salivary gland by reducing the development-susceptible enzymes. Actinomycin D also reduces or abolishes the more active chromosomal puffs, as well as reducing nucleolar activity as revealed by size and stainability. The results with puromycin indicate that the increased enzyme activities observed in the course of salivary gland development are dependent on protein synthesis, rather than being accumulated from reserves in other tissues. The experiments with actinomycin D suggest that the synthesis of these enzymes is dependent on the synthesis of RNA. The major salivary gland puffs, following actinomycin D treatment, are reduced in size. The enzyme activities developed by the gland depend upon the dose of actinomycin in the medium. Indeed, all developmental processes appear to depend on the concentration of actinomycin. At low concentration of the antimetabolite (10^{-4}mg/ml) most of the animals remain as larvae for extended periods - as much as 4-5 days longer than controls (controls metamorphose in 1-2 days, depending on the stage at the start of the experiment). At higher concentrations (10^{-2}mg/ml) there is virtually complete inhibition of enzyme activity within 24 hours and the larvae are killed in about 2 days.

Hormones, differential gene function, and development

These experiments indicate that the increases of salivary gland enzyme activities are dependent upon protein synthesis, probably by the cells of the gland. In turn this synthesis appears to be dependent upon the synthesis of specific RNAs (presumably messenger RNAs). The results are consistent with the idea that m-RNAs are made on the chromosomes at the site of particular puffs.

The consistently detected and rapid responses to hormone reduction and following addition of actinomycin D suggest that the half-life of the messenger RNA and these proteins is short; and that this synthesis is rapid in the normal course of development.

It is interesting to note that the manipulation of the endocrine system by means of thoracic ligatures also inhibits puffing at these same chromosomal loci and effects a decrease in activity of the specific salivary gland enzymes. That other manipulations such as trauma can also achieve enzyme reductions and puff reductions is instructive; it suggests that the hormonal effects

may not be direct, but rather may be mediated by other factors,or hormonal effects may be mimicked in some way by certain treatments. It may also be that injury affects hormonal activity. Many of the changes in puffing pattern during development may be necessary for normal developmental processes but they need not be sufficient to bring about or cause normal development to occur. We conclude from our experiments that:

1. The amount of enzyme activity in various tissues differs depending upon the tissue and upon the developmental stage.

2. The activities of enzymes are correlated (and are possibly directly related) to activities at particular chromosome regions. The treatments that affect specific enzymes also alter particular chromosomal puffing events within the same period.

3. Since all genes are not acting at the same time or at the same rate in all tissues, as revealed by enzyme activities, it is suggested that these results are consistent with the idea that insect development can be viewed as a sequence of genic activations and inactivations and that hormones act in some way to regulate genic activities.

4. Hormones affect the tissues and influence enzyme activities. Whether the effects are produced by direct or by indirect means remains to be determined.

This major problem, analysis of the processes by which hormones influence the actions of genes, can be attacked from many aspects. A number of suggestions are to be found in the recent literature. The action of hormones can be at the level of the chromosomes, that is on the DNA template itself, or the control may be more indirect, for example on extrachromosomal regulator substances, or one or more of the various types of RNAs, or on membranes (as those in the nucleus, the mitochondria or the endoplasmic reticulum). Alternatively, hormones may affect key enzymatic reactions and only subsequently force a shift in metabolism in new directions. I hope that we will be able to discuss fruitfully as many of these views as possible.

Insect tissues provide excellent analytical material for examining the problem of genic action and expression in development. One of the major purposes of this symposium then is the bringing together of a group of scientists who have been actively investigating such problems. An expression of experiences in this area and an examination of the current views concerning these investigations will be helpful in bringing about future progress in this field.

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THE CHEMISTRY AND BIOCHEMISTRY OF ECDYSONE. Peter Karlson, Physiologisch-Chemisches Institut der Universität, München, Germany.

Ecdysone is the hormone of the prothoracic gland. It has also been termed "molting hormone", "metamorphosis hormone", "growth and differentiation hormone" (a term of doubtful value), according to its physiological functions in postembryonic development. It was the first insect hormone to be isolated in pure, crystalline form (Butenandt and Karlson 1954).

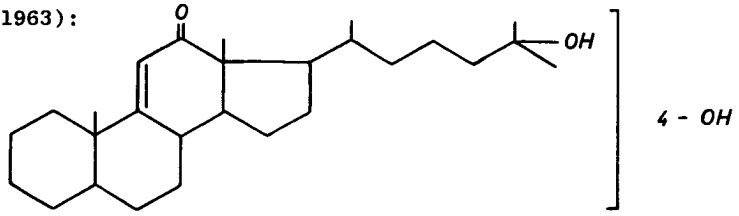
Isolation of Ecdysone. - Though various insect species, e.g. Calliphora or locusts, gave extracts that are active in the bioassay, crystalline ecdysone has been prepared only from Bombyx pupae. The yield is low; in the first large-scale extraction, we obtained 25 mg of crystalline hormone from 500 kg pupae. The method of purification has been published (Karlson 1956).

Owing to the low yield, only very little crystalline ecdysone was available for chemical studies. Two years ago, we made another large-scale extraction of pupae to obtain more ecdysone. We finally got about 200 mg from 2 tons of starting material; this enabled us to do some chemical studies on its structure. Many of the old data could be confirmed. The biological activity was found to be only slightly less - 0,01 µg being equal to one Calliphora unit - and the absorption in the UV-region slightly higher. However, the molecular weight had to be corrected; X-ray-data and mass spectrometry clearly indicated a value of 464. According to the new molelucar weight, the empirical formula must be $C_{27}H_{44}O_6$ instead of the old C_{18}-formula.

Chemical Structure of Ecdysone. - Taking into account that one carbonyl group and one double bond was present in the molecule, one could calculate that four rings should constitute the basic molecule. Actually, the corresponding saturated hydrocarbon should have the composition $C_{27}H_{48}$ which is the formula of cholestane. This implied a relationship to the steroids, and IR and NMR spectra pointed in the same direction. This suspicion was confirmed by dehydrogenation experiments; from 30 mg ecdysone, we obtained a few µg of a mixture of hydrocarbons, among which 3'-methyl-cyclopenteno-phenanthrene was identified: this is a well-known dehydrogenation product of steroids.

Ecdysone is an α, ß-unsaturated ketone. The keto group is probably located in position 12, with the double bond in 9:11.

We assume that the side chain is of the cholesterol type, but with a hydroxy group at C-25. The structure of ecdysone can therefore be represented by the following preliminary formula (Karlson et al. 1963):



The position of the four remaining hydroxy groups cannot be given at present.

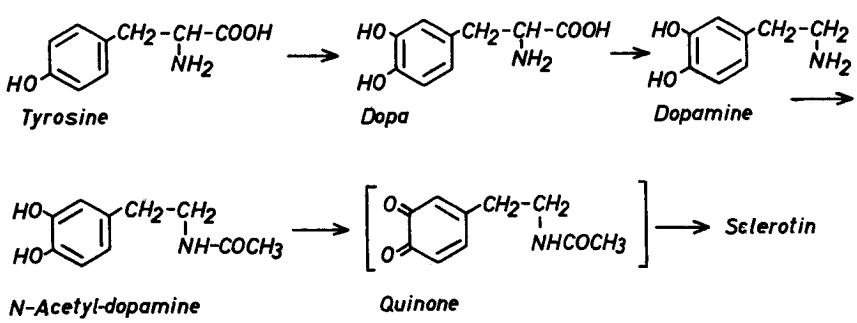
Biogenesis of ecdysone. - It is well known that insects are unable to synthesize steroids, though they require them as essential nutrients. We assumed therefore, that ecdysone would be formed from cholesterol. This conversion of cholesterol to "other substances (hormones?)" had already be postulated by Clark and Bloch (1958). After injection of highly labelled ^{3}H-Cholesterol into Calliphora larvae, we obtained an extract which contained radioactive ecdysone. This could be isolated by the dilution technique and crystallized to constant specific activity (Karlson and Hoffmeister 1963). This result proves a close metabolic relationship of cholesterol and ecdysone.

The physiological actions of ecdysone will be dealt with only briefly. (For review see Gabe, Karlson and Roche 1963). Ecdysone stimulates, in all species studied, the process of molting and all changes preparatory to a molt. In Lepidoptera, a stimulation of pupal molt and imaginal molt, i.e., onset of development after diapause, have been observed. Ecdysone is also responsible for the color change in some species, e.g. Cerura vinula. In several other orders (e.g. Hemiptera, Isoptera,Hymenoptera), either larval molts or imaginal molts are induced, depending on the physiological state of the test animal. In Diptera, puparium formation is controlled by ecdysone; this is the basis of the Calliphora assay, which was used in the purification process. The biochemistry of puparium formation has been studied in our laboratory (see below). Another dipteran species, Chironomus, has been investigated for the puffing pattern in relation to development, and ecdysone has been shown to induce certain puffs. The details of this work will be dealt with by Dr. Clever, and the implications are discussed below.

Biochemical effects of ecdysone. - Mention should be made of the studies on the respiratory enzymes in diapausing and developing H. cecropia pupae. (Williams 1951, Harvey and Williams 1958, Kurland and Schneiderman 1959.) Not only oxygen uptake, but also the amount of some enzymes (cytochrome c) is adjusted to the need of the animal. However, this might be related only indirectly to ecdysone; injury has very similar effects on respiratory metabolism, but does not initiate development.

Our own studies on the biochemical actions of ecdysone have centered around the biochemical interpretations of the Calliphora assay. The visible changes in puparium formation are darkening and hardening of the last larval cuticle, a process known as "sclerotization". This seemed to be a much simpler system than a molt which involves the shedding of the old cuticle. Earlier work by Pryor, Hackmann, Dennell and others had shown that o-diphenols are involved in sclerotization. We found that tyrosine metabolites are incorporated into the cuticle, and that the main sclerotizing agent is N-acetyl-dopamine (Karlson and Sekeris 1962). The experimental evidence is (a) isolation of this compound from mature Calliphora larvae, (b) a sharp decrease of its concentration at puparium formation, (c) a high incorporation of labelled N-acetyl-dopamine into the cuticle, and (d) the fact that the phenoloxidase of Calliphora (which we have purified and crystallized) acts preferably on dopamine and N-acetyl-dopamine, but not on protocatechiuc acid or dihydroxyphenylacetic acid.

Next, the pathway of the biosynthesis of N-acetyldopamine was investigated, especially in relation to its control by ecdysone. The synthesis proceeds through the following pathway:



Tyrosine is first hydroxylated to dopa, which is then decarboxylated to dopamine. The latter is then acetylated by acetyl-coenzyme A. At the onset of puparium formation, N-acetyl-dopamine is presumably oxidized by the phenoloxidase system of the cuticle. This enzyme is at first present in form of a proenzyme which is activated by another enzyme, the "activator enzyme" (Horowitz and Fling 1956, Karlson and Schweiger 1961).

The control of this reaction chain by ecdysone is exerted at two stages: First, the decarboxylating enzyme is induced by ecdysone. We have indications that this is a true enzyme induction, i.e. a de novo synthesis of enzyme protein. Secondly, the activity of the "activator enzyme" is controlled by ecdysone, possibly also through enzyme induction.

Mechanism of action of ecdysone. - We have seen that one important point in the control of sclerotization is the induction of enzyme(s). This is generally interpreted as interaction of the "inducer" with the nucleic acids or related substances, since the production of (enzyme) proteins is controlled by DNA and mediated by RNA.

In this context, the induction of puffs by ecdysone is of special importance. Puffs are known as sites of RNA synthesis, and it is presumed that messenger-RNA is produced here. If through some unknown mechanism ecdysone can produce puffs and initiate synthesis of messenger-RNA, the latter could then be transferred to the ribosomes where it serves a template for protein synthesis (Fig. 1). This would explain the synthesis of dopa decarboxylase in Calliphora.

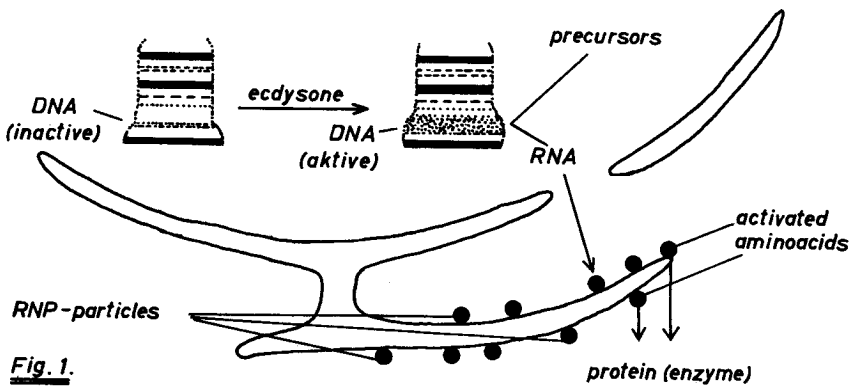


Fig. 1.

We believe that this mechanism of hormone action may be true also for vertebrate hormones. Cortisol, estradiol, testosterone and some other vertebrate hormones have been shown to stimulate protein synthesis and act as inducer for special enzymes.

This concept on the mode of action of hormones has been discussed in detail elsewhere (Karlson 1960, 1963).

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THE CHEMISTRY AND PHYSIOLOGY OF THE BRAIN HORMONE. Masatoshi Kobayashi, Sericultural Experiment Station, Suginami-ku, Tokyo, Japan.

In insects, the endocrinological function of the brain was first described in Lymantria dispar by Kopeć (14). Then neurosecretory cells, i.e. nerve cells which cytologically show signs of secretory activity, were found in the brain of Apis mellifera by Weyer (19). The first evidence of a function of neurosecretory cells was provided by Wigglesworth (20), who showed that in Rhodnius prolixus a factor regulating molting was produced by the pars intercerebralis of the protocerebrum. From that time to the present, many men have studied neurosecretory cells in the brains of insects (cp. Van der Kloot (18)).

Neurosecretory Cells in the Brain

Neurosecretory cells are located in definite parts of the brain in insects. In the silkworm, Bombyx mori (8), neurosecretory cells were located in the brain in four groups in different parts, namely, two medials in the intercerebralis, and two laterals in the protocerebrum. The medial neurosecretory cells were found in the brain through the whole developmental stage, from early larval stage through pupa to moth inclusive, while the lateral cells could be seen only in the period from the 5th larval instar to moth. The neurosecretory cells were distinguished from ordinary nerve cells by their larger size, large nuclei and characteristic staining reactions. The author classified these cells into two types, A- and B-cells depending on their cytological features. A-cell: Elliptic in shape, or tapering off in the axonal region, and measuring 40 μ to 70 μ in major diameter, including a large vacuole in the apical part of the cytoplasm. B-cell: Round in shape, measuring 18 μ to 25 μ in diameter having no vacuoles in the cytoplasm. The medial groups of neurosecretory cells consisted of both A- and B-cells, the lateral groups only B-cells.

The total number of A and B neurosecretory cells in the brain was about 14 in the 5th larval stage, increasing with the age, and about 28 and 34 cells were seen in pupa and moth, respectively. From each of the A-cells, left and right, in medial groups of neurosecretory cells, an axon started, crossing with that of the opposite side in the medulla of the brain, and was incorporated into nervus corporis cardiaci.

A secretion produced by the neurosecretory cells flowed in two pathways: One was from the cells into corpus cardiacum through the nervi corporis cardiaci and from there to the corpus allatum through the nervi corporis allati; the other was from the cells to the blood circulation through the cortex in the brain.

Neurosecretory cells in the brain showed a strong affinity to Sudan black B for staining lipids and to acid haematein for staining phospholipins, suggesting that a large amount of lipids and phospholipins were contained in the cytoplasm of the cells. After treatment with several organic solvents for lipids, neurosecretory cells became less stainable with Sudan black B.

Hormonal System of Growth and Development

Concerning the system of hormonal action in an imaginal differentiation, wonderful experiments were done with Hyalophora cecropia by Williams (22, 23, 24). He found that the pupal diapause of the insect is caused by inactivation of the pupal brain. The brain is activated with low temperature treatment and secretes brain hormone affecting the prothoracic gland. The latter organ secretes prothoracic gland hormone, inducing metamorphosis. This

system of hormonal action has since been confirmed by many workers in several different insects (cp. Schneiderman and Gilbert (17)). But it has not yet been proved that the brain of the silkworm in which diapause does not occur in the pupal stage plays a similar important role in metamorphosis as it does in other insects, e.g. Hyalophora cecropia (24) and Rhodnius prolixus (21), etc.

In the silkworm, a few scientists (3, 15) say that the brain is not necessary for the onset of pupation and for the development of the imaginal characters. For the purpose of answering the question whether, in Bombyx, the brain is responsible for imaginal differentiatiation or not, the author performed an experimental study on the function of the brain during the pupal stage (7, 8).

The Bombyx-pupae of F_1 hybrid between the two races J. 122 and C. 115 were used as materials and were kept at 25°C. throughout the experiments. Two controls received only incisions in the head and were not cerebrectomized, while the brains of the experimental animals were extirpated.

The controls emerged as moths in 11-14 days after pupation. In the pupae whose brains had been extirpated immediately after pupation no imaginal differentiation was observed for more than 40 days after extirpation, although some of these pupae survived for 300 days. Such a pupa was called 'Dauer-pupa', following Wigglesworth's 'Dauer-larva'. When a fresh brain, obtained either from larva, pupa or moth was implanted into the head of 'Dauer-pupa', the latter became an imago in 16-25 days after implantation.

After two larval brains were implanted into the 5th abdominal segments of 5th instar larvae, two different procedures were carried out following pupation of the operated silkworms. In one group of pupae, the brain of each host was extirpated immediately after pupation. In the other group, the pupae were ligated at a level between the thorax and abdomen at 2 hours after pupation. The part anterior to the ligature was cut off. All specimens were kept at 25°C. throughout the experiment, with 2 exceptions, the isolated abdomens of the pupae of the second group showed no imaginal differentiation for 40 days, even though brains had been implanted into each abdomen during the larval stage. Two of the pupae became moths in 34-35 days after ligation, but this result could not be repeated when 10 larval brains were implanted into each of 11 abdomens in another experiment. On the other hand, in the pupae of the first group, in which larval brains were implanted into each abdomen and the native brain was extirpated immediately following pupation, imaginal differentiation was shown (10).

It is concluded from these experiments that in Bombyx, both brain and thoracic segment with prothoracic glands are necessary for the initiation of adult development. The function of prothoracic gland was proved by Fukuda (2). The brain first secretes a factor for the activation of the prothoracic gland, and the prothoracic gland then releases into the blood stream a hormone which initiates imaginal differentiation.

This conclusion can not thoroughly explain all the phenomena of the foregoing experiments, i.e., it can not explain the fact that two isolated abdomens became moths. Subsequently, a similar phenomenon was reported by Ichikawa and Nishiitsutsuji-Uwo (5) in Philosamia cyntia ricini. Therefore, whether the metamorphosis of insects is induced only by brain hormone is an undetermined matter.

In 1961, this matter was made clear by Kobayashi and Burdette (12). Mature larvae of Calliphora erythrocephala were ligated at the seventh segment and were observed for 24 hours at 25°C. Those showing pupal characteristics in the segment anterior to the

ligature were selected for use in the bioassay. Ecdysone was prepared from pupae of Bombyx mori preserved in methanol, and its activity confirmed previous to the experiments. Brain hormone was prepared from pupal brain of Bombyx mori dissected free of corpora cardiaca and corpora allata. Methanol-acetone-ether extract yielded a crude but active fraction. Experience in the bioassay with such preparation has indicated that the tests may be considered positive when 50 per cent. or more of the specimens pupate. A dosage of ecdysone of 29 γ yielded a positive bioassay; a lesser amount did not. Negative results were obtained when brain hormone up to a dose of 150 γ was injected. However, the addition of 75 γ of brain hormone to 7 γ of ecdysone and 150 γ of brain hormone to 15 γ of ecdysone gave positive bioassays, despite negative results would have been obtained from both the amounts of ecdysone and from the brain hormone.

The results obtained by using crude brain hormone and ecdysone suggest that hormonal extract of the brain has two functions. The first is its tropic function on the prothoracic gland and the second is its direct, synergistic action with ecdysone on induction of metamorphosis. This experiment demonstrates that metamorphosis of the insect is not induced by the brain hormone alone. However, whether separate brain hormones are responsible for the two different functions is a matter to be studied.

Physiology of Metamorphosis

Regarding the mode of action of the brain hormone, it is well known that in the pupal stage the respiratory curve of insects makes a typical U-shape, showing that oxygen consumption of the pupae immediately after pupation sustains a high level, then the amount decreases with the lapse of time. Again the amount increases with the progress of imaginal differentiation and reaches the maximum on the day before imagination. Moreover, the glycogen content of the normal pupae decreases with the lapse of pupal time, reaching the minimum just before imagination. In silkworm pupae (8), the rise and fall of both oxygen consumption and glycogen content were the same as in other insects, while in the 'Dauer-pupae', oxygen consumption remained at a low level for long time and glycogen content at a high level. The electrophoretic pattern of blood protein in the 'Dauer-pupae' coincided with that of normal young pupae because of the lack of brain hormone (1).

With this result, it seems that 'Dauer-pupae' sustain the physiological nature of normal young pupae for 3 months after the extirpation of their brains.

Pupae were exposed to gas mixtures of N_2 and O_2 in ratios of 99:1, 95:5, 90:10, 85:15, and 80:20, respectively, for 24 hours immediately after pupation. The brains of the pupae were then extirpated, while control pupae were left intact. All pupae were kept in an atmosphere at 25°C. The results were that the 'Dauer-pupae', those which were still in pupal form 40 days after pupation, occurred among those pupae exposed to the 1 per cent. and 5 per cent. oxygen gas mixtures, but not from pupae exposed to the other three mixtures of higher oxygen content and also from the controls. Similar results were obtained using carbon monoxide instead of nitrogen in the gas mixture. Furthermore, conditions of light and dark during exposure to the gas mixture had an effect on the yield of the 'Dauer-pupae'; a light field gave rise to fewer 'Dauer-pupae' than a darker field. Such an illumination effect had never occurred in the case of the nitrogen-oxygen mixture. These facts suggest that in carbon monoxide the production and release of brain hormone are controled by light. Then the G-Nadi reaction for cytochrome oxidase was histochemically excercised on the pupal brain.

Although the reaction was positive in pupal brain, those kept in the $CO:O_2 = 99:1$ gas mixture for 24 hours and illuminated showed the reaction to almost the same degree as those kept in fresh air for 24 hours, and to far greater degree than those kept in the same gas mixture in darkness for 24 hours or pupal brains immediately after pupation. Therefore, it seems that the brain hormone is produced and released under conditions maintaining a high activity of cytochrome oxidase in the brain. Thus it is concluded that in a low oxygen tension, the action of the brain on hormone secretion reaches a low level, a normal release of hormone from the brain does not take place, and many 'Dauer-pupae' occur among the pupae (11).

Extraction of the Brain Hormone

The extraction of the hormone from insect brains was first reported by Kobayashi and Kirimura (9). Until recently, only a few scientists (4, 6) had carried out extraction of active ingredient from the brain or central nervous system in insects. The author and his collaborators have tried to obtain an effective preparation of the brain hormone as described below.

Brains of 8,500 pupae collected within 24 hours after pupation and stored under refrigeration in methanol were used as the starting material. These brains were homogenized in methanol for 10 min. and centrifuged at 2,500 rpm for 15 min., and this procedure was repeated three times adding 65 ml. of 80 per cent. methanol. The total 200 ml. of supernatant methanol solution was concentrated to 38 ml. and extracted three times with 145 ml. of ethyl ether. The ether was evaporated from the ether layer and about 2 mg. of oily substance, yellowish brown in colour, was obtained as a residue. Throughout the treatments the temperature was never allowed to rise above 38°C.

About 0.1 mg. of the oily substance obtained from the foregoing procedure was injected into the head of each 'Dauer-pupa' about 1.1 g. in fresh weight on the 30th day after pupation. Of five 'Dauer-pupae' injected, one died, and the others survived, among which one on the 25th day, the remaining three on the 40th-47th day, became imagines from the injection, while not one of twenty 'Dauer-pupae' without injection became imagines for 87 days.

In the second experiment 0.1 mg. of the oily substance, dessolved in 0.01 ml. of sesame oil, was injected into each of four 'Dauer-pupae', 64 days old. Among these, two pupae died, the other two survived and became imagines on the 26th and the 43rd day respectively after injection.

In the third experiment each of four 'Dauer-pupae', 51 days old, was injected with 0.06 mg. of the oily substance which was dissolved in 0.0075 ml. of sesame oil. In 22 days, two died and the other two showed no sign of further development. At this time, each of the latter two was again given 0.1 mg. of the oily substance which was dissolved in 0.015 ml. of sesame oil. After 22-25 days from the second injection, they became moths.

Among nineteen 'Dauer-pupae' which were used as a control for the foregoing two experiments and were injected with 0.01 ml. of sesame oil alone, eighteen survived, one of them becoming an imago on the 71st day, the rest remaining in a diapause state for 120 days. It is supposed that in the one pupa, by the time when it was deprived of its brain, a brain hormone already existed above threshold value for imagination.

Concerning the problem of whether the prothoracic gland hormone was contained in the oily substance or not, it seems that this oily substance does not contain the hormone, because only brains were used as the source of this preparation.

Crystallization of the Brain Hormone

Since 1957, about 750,000 brains of pupae, dissected out free of corpora cardiaca and corpora allata, have been used as material in the purification of the hormone.

This work was done by the collaboration of Kobayashi, Kirimura and Saito (13). They succeeded in the isolation and crystallization of the brain hormone through several treatments of a fraction which was separated by the counter-current distribution from a crude but active extract of the brain hormone as mentioned below.

Crude brain hormone extract was prepared from 50,000 brains dissected from Bombyx pupae by the method of solvent treatment using methanol-acetone-ether.

Several active fractions were separated by means of the countercurrent distribution. An automatic counter-current distribution apparatus with fifty 10 ml. tubes was utilized, and the material was carried through 50 transfers using 500 ml. for each phase. The solvent system employed was carbon tetrachloride (88.5 parts), methanol (11.5 parts) and distilled water (0.75 parts) in the lower phase and carbon tetrachloride (46 parts), methanol (54 parts) and distilled water (4.75 parts) in the upper phase. After the distribution, the solvents evaporated off from each tube, and the residue was weighed. Generally, amino acids and peptides are distributed near the first tube (tube number 0), for the transfer of these substances is comparatively difficult by the solvent system. Neutral fats are distributed near the last tube (tube number 49), for the transfer of fats is very easy by the solvent system. Also, conjugated lipids and sterols both are distributed in tubes between proteinous and faty substances. These containers were divided into eleven fractions following the chemical properties. In the results of the bioassay of the brain hormone, about eleven fractions separated out by counter-current distribution method. Most of the activity was in tubes 30-42 and in tubes 0-1, with tubes 36-42 showing the greatest activity. Therefore, the fractions in tubes 30-42, which showed the higher activity, were used as the material for further experiments.

Active fraction was prepared from 220,000 isolated brains by means of both the solvent extraction and the counter-current distribution method mentioned above. Colourless, active solution was separated from the active fractions with petroleum ether (b.p. 40-60°C.). Three kinds of unpurified parts were separated by organic solvents from the precipitate which was obtained from the colourless, active solution in the process of cooling to -18°C.

Using two of the above-mentioned three parts as material, the recrystallization was repeated twice with acetone and ethanol. Finally, 18 mg. of crude crystal was obtained from 3,539 mg. of crude but active extract of the brain hormone. The melting point of this crystal was 142 to 143°C.

Thirty-seven day old 'Dauer-pupae' of Bombyx mori were prepared for use in the bioassay. When 0.02 µg. of the crystal which was dissolved in 0.01 ml. of ethanol was injected into each 'Dauer-pupae', the five injected animals emerged as moths on the 22nd (♀), 24th (♂), 28th (♀), 29th (♀) and 35th (♂) day respectively, following injection. However, no moth emerged from 38 control animals, having been given either no injection or a mock injection of 0.01 ml. of ethanol without the crystal, for 100 days following pupation.

This result shows that the crystal consists of the most important factor of the active principle of the brain hormone.

Chemical Character of the Brain Hormone

This section deals with the progress of research into the chemical character of the brain hormone.

The melting point of the crystal mentioned above remained unchanged by recrystallization and no ultra-violet spectrum of the crystal was found. The infra-red spectrum of the crystal in KBr exhibited that it has a RR'C = CR"'H type band which was characteristic in the region 850 to 750 cm^{-1}, and a hydroxyl band which was shown at 3480 cm^{-1}. Furthermore, the characteristics of the absorption spectrum of the crystal were very similar to those of pure cholesterol in all regions. The crystal was also analyzed by means of gas chromatography on a Baber Colemann model 10 with an argon ionization detector. The result showed that there was one main peak with trace amounts of three other peaks and that the retention time of the main peak coincided favourably with that of pure cholesterol. Therefore, it appears that the crystal was cholesterol, together with trace amounts of three impurities.

Fifteen mg. of crude sterol mixture was collected from several active fractions of brain hormone extract by the counter-current distribution method, and it was then acetylated in pyridine by a common method. After the acetylated sterol mixture had been adsorbed to 5 g. of aluminum oxide column (according to Brockmann) and eluted with n-hexane, the elute was collected carefully. The solvent was evaporated from the elute, and, upon recrystallization with acetone and methanol, white needle-like crystals of acetylated sterol formed. Following further purification by recrystallization, the melting point of the crystal became constant at 115 to 116°C.

After the purified acetylated sterol had been saponified with alcoholic potash solution, 2 mg. of crystal was obtained by recrystallization with acetone and methanol. The crystal appeared as pearly leaflets or plated forms when crystallized with dilute alcohol. The melting point of the crystal was 148°C., and the melting point of a mixture of the crystal with pure cholesterol was determined to be the same as that of the crystal alone.

The chemical characters of purified brain hormone was found to be as follows: The melting point was 148°C.; no ultra-violet spectrum was found; both the infra-red spectrum and the Rf on paper chromatogram were identified with those of cholesterol; the Liebermann-Burchard reaction was positive; a gas chromatogram exhibited only one peak, showing the same retention time as cholesterol; the melting point of acetylated pure hormone was the same as acetylated cholesterol.

From these chemical properties, it was determined that purified brain hormone is identical with pure cholesterol.

Bioassay of Purified Brain Hormone and Its Related Sterols

'Dauer-pupae' of Bombyx mori were used as test-objects for the assay of brain hormone activity. They were observed for about 6 weeks at 25°C. prior to use. Each test animal was injected with 0.01 ml. of sterol dissolved in 9 per cent. ethanol. In the first experiment, each of the 'Dauer-pupae' was injected with 0.02 µg. either of cholesterol isolated from pupal brain or of cholesterol purified from commercial reagents (Merk). In both cases, the animals emerged as moths in 18-27 days following injection at 25°C. (Table 1).

In the second experiment, cholestanol and 7-dehydrocholesterol, two sterols related to cholesterol, were used in the bioassay of brain hormone activity. When 2 µg. of cholestanol or 10 µg. of 7-dehydrocholesterol, was injected into each of three 'Dauer-pupae', the following results were observed. In the case of cholestanol, two out of three 'Dauer-pupae' injected became moths in 16-19 days following injection but the other animal remained in the pupal form without any differentiation for 30 days following injection. In the case of 7-dehydrocholesterol, two out of three 'Dauer-pupae'

Table 1. The Brain Hormone Activity of Pure Cholesterol Isolated from Brain Extract.

Dose of pure brain hormone (μg.)	No. of test-objects	No. of moths emerged*	Days from injection to emergence	No. of individuals died
10	6	4	15-23	0
2	6	5	18-23	0
0.2	6	4	17-23	1
0.02	6	3	18-27	3
0	24	0	-	0
0.02**	6	4	19-22	1

* The results obtained for 30 days following injection at 25°C.
** Colesterol purified from commercial reagent (Merk).

became moths in 19 days, while the other animal remained in the pupal form for 30 days. From this experiment it seems that these two chemicals also have brain hormone activity, but to a lesser degree than cholesterol.

In the third experiment, three groups, of six 'Dauer-pupae' each was injected with 100 μg. of β-sitosterol, stigmasterol and ergosterol, respectively. In each group, two of the six animals emerged as moths in 14-29 days following injection. A total of six individuals in this experiment died, but no other animals emerged for 30 days. However, no moth was obtained from twenty control animals for 30 days following non-injection or mock injection of 0.01 ml. of 9 per cent. ethanol alone. From these results, it appears that these three chemicals do display brain hormone activity, even though the results of this bioassay were inconclusive.

From the foregoing experiments, it is concluded that cholesterol is one of the active principles of the brain hormone, and its related compounds, such as cholestanol and 7-dehydrocholesterol, also have a certain degree of hormone activity.

Generally speaking, cholesterol is the main sterol of most insects and phytosterols derived from diet are also found in phytophagous insects. Most insects, however, can not synthesize cholesterol from substances which are known to be cholesterol precursors in both mammals and microorganisms. According to Saito, Yamazaki and Kobayashi (16), in the silkworm, Bombyx mori, incorporation of C^{14}-acetate into digitonide was very weak, if at all present, in the larval stage, while its incorporation was conspicuous in both prepupal and pupal stages. Therefore, it was belived that C^{14}-acetate is a sterol precursor in silkworm pupae as in mammals. The 'Dauer-pupa', 30 days old, however, could not synthesize sterol from C^{14}-acetate, or if the system was present it was very weak. This fact suggests that the brain takes a part in the sterol metabolism in the silkworm. Although the hormone activity of sterols in insects was unknown until 1961, it has been determined that sterol, like hormone, is one of the biologically vital active substances in insect development.

The Other Active Brain Hormone

Furthermore, the chemical properties of the active fraction other than cholesterol, obtained by the counter-current distribution method in tubes 0-1 as described above, was similar to those of Ichikawa and Ishizaki's extract (6). This suggests that the brain hormone consists of sterol and other chemicals. The separation and purification of the active crude substances which have a positive protein reaction are now being carried out.

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THE JUVENILE HORMONE AND AGENTS WHICH MIMIC ITS ACTION.

Howard A. Schneiderman, Developmental Biology Center, Western Reserve University, Cleveland 6, Ohio.

The discovery by P. Schmialek (1961) that the terpene alcohol farnesol copied the action of juvenile hormone in a number of test systems led us to examine a variety of other polyisoprenoid compounds. Among the active compounds uncovered were solanesol, phytol, geranyl-linalool, farnesyl acetate, farnesenic acid, nerolidol and linalool. Beta sitosterol also had slight juvenile hormone activity.

Other classes of compounds were also examined and several active compounds were uncovered notably 1-heptanol and certain substituted carbolines. The significance of the juvenile hormone activity of these diverse compounds will be discussed and their relation to the natural juvenile hormone examined. Possible chemical and physiological relationships among the juvenile hormone, brain hormone and ecdysone will be considered.

THE ENDOCRINE BASIS OF THE INSECT DEVELOPMENT.

Vladimír J.A.Novák, Dept. of Insect Physiology, Entomological Institute of the Čs.Academy of Sciences, Praha, Czechoslovakia.

The aim of this communication is to show the possibility of a comprehensive theory of insect development based on the present stage of knowledge of the metamorphosis hormones ; to ask to what degree the author's earlier gradient-factor theory is able to fulfill the purpose of such a theory ; and to summarize the contributions of our department to the problem.

The most characteristic feature of postembryonic morphogenesis in insects, as in all other arthropods, is its cyclic character due to the necessity of a periodic removing of the unexpansible chitinous cuticle. The postembryonic development is thus subdivided into a number of instars separated by periods of more or less complete reduction of most of the physiological activities. This is of great advantage for the analysis of various interrelations in growth and development. A further advantage is the interaction of a system of the s.c. metamorphosis hormones, by means of which deep experimental interferences may be effected without seriously disturbing the other physiological processes.

Thus far three principal metamorphosis hormones are known of which the secretion of the corpora allata, the juvenile hormone or neotenin /JH/ discovered by Wigglesworth /1935/, is of greatest importance for the study of morphogenesis. Its morphogenetical activity is a favouring of the larval type of growth and a prevention of metamorphosis. A close analysis of its mode of action in the lygaeid bug Oncopeltus fasciatus has shown /Novák, 1951/ that it produces its effect by activating the growth of the larval parts of the body, while it does not affect the growth of the imaginal parts directly. The larval parts, those which do not grow and are eventually histolyzed during metamorphosis, are unable to grow in the absence of JH since the late embryonic period while the imaginal parts continue to grow. There is no other general difference between these two types of tissue, neither in their morphology nor in their physiology.

A hypothesis has been formulated of a special factor responsible for the independence of the imaginal parts from JH. The term gradient-factor /GF/ has been suggested for it, as it is supposed to induce the gradients of imaginal growth. It is supposed to be contained in all parts of the body originally ; its inactivation in the late embryonic period, according to a species specific pattern, thus gives rise to the origin of larval tissues, their growth being activated by the JH until the beginning of metamorphosis.

No indications of the chemical character of the hypothetical GF are available so far. As it can be completely repla-

ced by the JH and as it is completely independent from the histological structure and physiological activity, however we may assume that it is an individual chemical substance.From its distribution and its mode of activity may be concluded that it is a tissue bound factor which may eventually spread into the neighbouring structures /tissues, cells, parts of the cells/ under specific conditions such as regeneration. As a secondary hypothesis the possibility was suggested that the GF is identical with the JH except for its tissue bound /desmohormone/ character. /Novák, 1951, 1956/. An other recent explanation seems, however, to be more likely - that of its corresponding to a link of the chain between DNA, RNA and the corresponding proteins /Novák, 1956/, perhaps to the messenger RNA /Unger, 1962/.

The GF hypothesis, together with the author's findings on the non specificity of the JH /Novák, 1949/, has made possible a new general theory of insect metamorphosis, unifying the earlier morphological /histological/ data with recent physiological evidence, especially that dealing the metamorphosis hormones. The gradient-factor theory of insect metamorphosis has not only amalgamated the existing notions on the three metamorphosis hormones, but has enabled a consequent explanation of various experimental findings on each of these hormones which were not fully understood before. The ever increasing amount of knowledge on this subject has more than doubled since the theory was first proposed, but none of the new findings has contradicted it, whereas many of them have uncovered new important evidence in its favor. Let us consider the present stage of knowledge of each of the metamorphosis hormones and of their effect on insect morphogenesis from the point of view of the theory.

The activation hormone /AH/, being a product of the neurosecretory cells of the pars intercerebralis of the protocerebrum, derived its name from its effect on the prothoracic glands. A series of futher principal physiological processes has recently been shown to depend on the AH. It activates the midgut proteases /Thomsen, E. and Möller, 1960, 1961, Strangways-Dixon, 1961/. It is perhaps the most important internal mechanism for regulating the water balance /Altmann, 1956, Nayar, 1958 etc., rev. Raabe, 1959/. As shown by Sláma /1963/, neither growth nor the development of eggs in ovaries occurs in absence of the AH due to the extirpation of the corpora cardiaca in the bug Pyrrhocoris apterus. The oxygen consumption is reduced to a very low and constant level corresponding to that in the natural imaginal diapause /being itself a syndrome of AH deficiency/, and this state is reversed after a cardiacum-allatum complex implantation.

New insight was gained into a mechanism of reaching the site of action of the AH. As shown by Wigglesworth /1956/ in Rhodnius prolixus, the function of the AH is inhibited by blocking the haemocytes with an injection of Chinese ink, trypane

blue or iron saccharate, which do not interfere with the hormone activity. From this it may be concluded that the role of the haemocytes consists of phagocytizing the neurosecretory granules reaching the blood stream through the axons of the neurosecretory brain cells via the corpora cardiaca and making free the AH while digesting the carrying substance. This seems to be a general mechanism, at least in insects.

The previously supposed close chemical relation between the AH and the various pterine substances occuring in close connection with neurosecretion /L'Hélias, 1956 etc./ has been disproved by recent findings /Gersch, Unger and coauct.,1962/. Similarly, the suggested identity between the AH and cholesterol has not been corroborated by further experiments /Gersch, Sláma independently, personal communications/.

The recent findings of Gersch and coworkers /1952/ suggest that the neurosecretory brain cells are the not only source of AH in the body. Being identical with neurohormone D_1 it also occurs in most of the ganglia of the ventral nerve cord, in subthreshold concentration for prothoracic glands activation. This is in full agreement with the very early phylogenetical origin of the AH, which may be supposed to be in the annelid stage already. Here a serial homology between the brain and ventral ganglia is out of the question according to the strobilation theory. A hypothesis has been put forth, proposing phylogenetical relations between the carrier substance of the neurosecretory granules, the various secretory products of the glial cells /gliosomata, trabeculae, the neural lamella etc./ and the secretory activity of the original epidermis /ectoderm/ cells. In connection with this it may be understood also that the endocuticle and the chitinous intima show the Gomori and paraldehyd-fuchsine positive reaction /Novák and Gutmann, 1962/.

The production of AH in the form of neurosecretory granules in the neurosecretory brain cells and its release into the blood stream by the corpora cardiaca or the walls of the aorta seem to be processes of cyclic character regulated by both environmental and internal medium stimuli. A temporary inhibition of either production or discharge /or both/ of the neurosecretory products containing the AH by the species specific environmental stimuli is the primary internal cause of the diapause.

The reaching of an effective concentration of the AH in the haemolymph of an insect thus constitutes the primary indispensable precondition for the start of growth in each instar, both by conditioning the food-proteins utilization and by activating the prothoracic and other glands. It does not, however, interfere with the principle of morphogenesis itself.

The moulting-hormone /MH/, or ecdysone, produced by the prothoracic glands and related structures /ventral glands, peritracheal and pericardial glands, ring glands/, used to be

called growth and differentiation hormone or metamorphosis hormone. As a matter of fact, it is quite indispensable for growth and especially for any morphogenesis. This does not mean, however, that it would affect the processes in other than an indirect way. As supposed originally by Wigglesworth /1934/, its most important effect is the initiation of secretory activity in the epidermis cells and related structures. Their secretion, the moulting fluid, the most important component of which is a chitinase /Williams, 1956/, produces a chitinolytic activity, first dissolving the internal surface of the endocuticle and thus loosening the epidermis cells for morphogenetical activity. Later it conditions the chitinogenous activity of multiplied epidermis cells depositing the new cuticle.

Little is known of the effect of the MH on other than ectodermis structures. There is irrefutable evidence against its direct inducing growth except perhaps that of the epidermis cells. First, the growth of specific tissues such as gonads continues in adult insects, where no MH is present. And secondly, there occurs moulting without growth; e.g. starving cloth-moths larvae /Tineolla/ may moult even 40-times without reaching metamorphosis. The recent findings of various authors show that the MH is able to induce moulting without growth experimentally also /cf. e.g. Halbwachs, 1957 in Locusta migratoria, Karlson and Lüscher, 1958 in termites/. And as more pure ecdysone is applied, less growth occurs. A high dosage of subcutaneously applied ecdysone causes a local area with moulted cuticle /Karlson, 1956/.

Also the newly shown effects of ecdysone on the puffing patterns in giant chromosomes of salivary gland cells in Drosophila and Chironomus /Beermann, 1959, Kraeger, 1960 etc./, irrespective of their importance, do not bring significant evidence in favour of the "growth and differentiation activity" of the MH. The same is true of the earlier findings on the influence of MH on enzyme activity, e.g., that of cytochromoxydase /Williams, 1949, 1954, Shappirio and Williams, 1952, 1953/. However interesting and important these findings may be in the study of enzyme activity -showing among other things the importance of insect hormones as a method in the investigation of any question of insect physiology - they cannot be viewed as evidence in favour of the direct participation of the MH in the growth and differentiation processes in general.

Thus the MH is nothing more but one of the preconditions of growth and differentiation in specific parts of the insect body, while it does not interfere with these processes in general.

The juvenile hormone /JH/ or neotenine, on the other hand, being a true growth hormone, affects the very principle of differentiation. Also the earlier conclusions on its metabolism /oxygen-consumption/ favoring character /cf.Thomsen, 1949, Sägesser, 1961/ have not been corroborated by recent experimental results. As

shown by Novák, Sláma, Wenig /1959, 1961/, Novák and Sláma /1962/, and Sláma /1963/, there is full reason to suppose that the observed increase in oxygen consumption following active corpus allatum implantation may be most reasonably interpreted as an increase in the amount of metabolizing tissues due to the activation of the larval parts of the body, which would not participate in the oxygen consumption otherwise. This is true both in the last larval instar and in the adult female. In the latter the ovarian folicles are activated, and through them the ripening eggs, causing distinct cycles of oxygen consumption in connection with the corresponding ovarian cycles, whereas no cycles appear after the allatectomy /Sláma, 1963, in Pyrrhocoris apterus/.

Another question which has been often discussed in recent years is that of there being more than one c.allatum hormone. In contradiction of this idea is the fact that imaginal c.allata are active in favoring larval growth while the larval c.allata are completely equivalent with the imaginal ones in activating the development of eggs in ovaries. Similarly, the physiological findings show that only one type of secretory cells exists in the corpora allata. As shown by Sláma and Hrubešová /1963/, no qualitative differences can be seen between the effect of the implanted corpora allata from IVth instar larvae and those from adults on the oxygen consumption of allatectomized female adults. The same cycles in connection with the egg ripening periods appear after a larval c.allatum implantation as after on imaginal one. Moreover, the experimental results interpreted as favouring the two-or-more hormones hypothesis /cf.Sägesser, 1961/ may be at least equally well explained by supposing only one c.allatum hormone. Finally, there is no principal difference between the activation of the larval parts of the body and that of the ovarian follicles according to the gradient-factor theory /cf.Novák, 1956, 1960/.

Even the slightest deviations from the general trend in the postembryonic development of insects may be understood on the basis of the above mentioned GF-theory. For example, the relative increase of the imaginal discs during the individual larval instars /cf.Novák, 1951/, the changes in larval coloration with each subsequent instar as in saturniid catterpillars, the positive effects of the c.allata on imaginal differentiation as shown by Wigglesworth /1948/, the partial reversal in the metamorphosis of the epidermis cells /cf.Novák, 1960/, etc. There are, however, experimental results which seem to contradict the theory and were interpreted in this way by their authors. Out of the several cases discussed in earlier contributions /cf.Novák, 1960/ Bodenstein's /1953/ findings on the negative effect of implanted corpora allata on the survival of the prothoracic glands in Periplaneta americana adults are the most interesting. A very complicated interaction among the three metamorphosis hormones, as well as the supposed secretion of the c.cardiaca, is suggested by Bodenstein to explain these fin-

dings. However, the much simpler following explanation, consistent with the GF-theory, is possible : The failure of the prothoracic glands to degenerate is not due to a special hormonal medium but to the stimulatory effect of the extensive regeneration caused by the operation injury. The precondition for this is the presence of an active concentration of the AH; this may be prevented by extirpation of the c.cardiaca. When, however, active c. allata are present, the extensive development of the ovaries removes the necessary nutrition from the haemolymph, so that regeneration is reduced and its stimulatory effect is insufficiant for the prolongation of the functioning of the glands. This effect of regeneration on prothoracic glands survival may be limited to Blattoptera and some other phylogenetically low Hemimetabola. Here prothoracic glands degeneration occurs in the beginning of the adult period as a relatively new achievement of evolution and it is not yet completely fixed. It is also the most probable explanation of the observed occurence of two moultings /the preadultoid and the adultoid/, following allatectomy in that order /B.Scharrer, 1946, in Leucophaea/,in place of the normal one moulting in most other insects, which has been previously explained as a "tissue competence".

The analysis of the various abnormities produced by experimental deviations in JH activity has shown that the earlier distinguishing of only prothetely and metathetely cases is not satisfactory, and it has unabled a new classification of this type of heterochronies /1951/. A precocious adult stage, the prothetely, may be reached in two different ways : By limiting ~~the~~ the growth of the larval parts, as is observed following allatectomy in earlier larval instars, the total amount of growth is notably lower during the given time and the moulting is delayed in comparison with normal specimens - this is a case of the regressive prothetely. When, on the other hand, the moulting is accelerated so that the increase of the larval parts is lessened without any delay of the imaginal parts, as following prothoracic glands implantation, it is a case of the progressive prothetely. The metathetely is a case of prolonged larval stage. This may be reached by delayed adult differentiation and moulting, as in the incomplete adults which are sometimes found in overpopulated or starving cultures. We speak then of a regressive metathetely. Or it may be caused by a surplus growth of larval parts together with a speeding of the moulting process, as following an active c.allatum implantation in last instar larvae. Then we speak of a progresive metathetely.

A refined analysis of the resulting morphogenetical changes on the basis of this ~~xxx~~ classification has enabled a distinction to be made between the effects of the induction of the true JH by c.allata implantation and the similar effects of the extractions of various lipoids from insects and other animal and plant tissues, as discovered by Williams /1956 etc./ and by Schneidermann and Gilbert /1958 etc./. As shown by Sláma /1961,

1962/, the implantation or injection of various fatty acids, both unsaturated and saturated, of higher alcohols /farnesol, cf. Wigglesworth, 1961/ of colchicin and of some other substances results in a distinct, and sometimes very extensive, local or general regressive metathetely effect. The same is true in the case of the above-mentioned lipoid extractions from various animals. The action of the true JH, on the other hand, results in a progressive metathetely. The possible slight differences seem to be due to in the presence of some amount of the AH or AH-like substances, which cause a progressive prothetely through the acceleration of the moulting process. Thus practically identical results /especially in the less JH-sensitive pupae/ may be obtained, on the one hand, by suppressing the imaginal growth without interfering with moulting /the pseudojuvenilizing effects/ by means of the extractions and chemicals mentioned, and, on the other hand, by activating the growth of the larval parts by means of the JH.

To what has already been said about the hypothetical gradient-factor /GF/, several further notes on its character must be added. Only the larval and imaginal parts of the body have been mentioned so far in relation to the GF. There are, however, structures which do not fit into either of these groups; For example, the special colour patterns and cuticular structures in the saturniid moth do not appear until the later larval instars. It is therefore supposed that the GF of the corresponding epidermis cells remains active until this period; it is, however, inactivated afterwards, before the beginning of metamorphosis, so that these late larval structures perish at the same time as the other larval structures. Other structures occur in insects with regressive metamorphosis, such as the pupal wings in female Orthoptera brumaria, which develop as true imaginal structures until the pupal instar and then perish together with the larval structures before the imaginal moulting.Another type of structure are the epidermis cells of Hemimetabola, which function in both the larval and imaginal periods undergoing only a partial change in their structure and in the type of cuticle deposited. Similarly, the shape and structure of the corpora allata undergo a partial change, while the gland continues its secretory activity in the adults. There are also the cells of the ovarian follicles in the females /of most species/, which first reach their full development in the adult insects, but are unable to function in the absence of the JH. The various parts of the body may thus be divided into a series of structures subsequently losing their GF and thus becoming dependent from the JH.

The series of tissues successively losing their GF may be followed deeper into embryogenesis in the case of such structures as the embryonic appendages of the polypod stage, and even later, into the adult period,when the sex-cells in contradiction to the entire imaginal body, continue their growth as a new individual. From this a new, more general definition of the GF and

metamorphosis may be derived : The function of the GF is produced by some of the components essential of growth of the living body; it is originally contained through the entire body and its inactivation in form of a species specific pattern conditions the morphogenesis. From this, all of morphogenesis may be understood as a series of ~~processes~~ successive processes of gradient growth, each of which functions by stopping the growth of one part of the body /in a species specific pattern/, while the other parts continue to grow. This sequence is only temporarily broken by the larval proportionate /harmonic/ growth period due to the action of the JH. Metamorphosis is thus nothing but the last portion of the embryonic morphogenesis, which has been shifted by the JH activity to the last period of postembryonic development /Novák, 1960 etc./. It is identical with all other morphogenetical processes in principle, differing only in its extent and time of occurence.

The gradient-factor theory of morphogenesis. There is no principal differences in the morphogenesis of insects from that of other animals. Here also its principle is the subsequent gradient growth processes. It thus seems very reasonable to suppose an identical cause for each of these processes also, that is, the inactivation of a general gradient-factor - the gf. Several reasons seem to justify such a broad generalisation : It is a well known fact that the most important biologically active substances are of very common occurence in organisms. For example, the nucleic acids seem to be quite indispensable for all animals, plants and microorganisms. Some of the enzymes of tissue respiration are common to most animals and the same is true of chlorophylls and auxins in plants. The supposition of gf as a material principle common to all animals cannot thus be viewed as improbable, a priori. Tumor growth or blastomogenesis may be interpreted as a failing of the mechanism of morphogenesis. Recent findings show the occurence of tumor growth in all the different groups of animals. The fact that the same phenomenon results in all cases from the failing of the mechanism of morphogenesis suggests the existence of a common principle of morphogenesis in all animals.

The gradient-factor theory of blastomogenesis. The gradient-like character of the mentioned hypothetical substance /or of the atomic configuration common to a group of substances such as the various messenger - RNS's/ thus depends on nothing but its inclination to be inactivated in a lawful way by various other substances and by the physical factors of both external and internal environment. On this basis the principle of action of the various morphogenetically active substances known from experimental morphology and developmental physiology, such as organisers and inductors, may be simply understood as due to a specific inactivation of the gf and to the influence of this upon further morphogenesis ~~may be understood~~ /cf.Novák, 1960/. On the same principle, blastomogenesis may be understood as a change in the gf, which prevents its further inactivation by the

physiological inactivators /organizers, etc./,perhaps by blocking the corresponding reactive atomic group of its molecule, while its growth activity remains untouched.

The conclusions from the findings on morphogenesis hormones thus enable us to establish a common theoretical form into which all the morphogenetical facts may be arranged. The resulting theory may serve as a tool of thought, means for the orientation of further experimental work, and a help in classifying the results obtained, irrespective of the definitive confirmation of the primary hypothesis of the existence of a specific gradient-factor.

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FUNCTIONS OF THE CORPORA ALLATA IN THE DEVELOPMENT OF TERMITES.

Martin Lüscher, Div. of Animal Physiology, Dept. of Zoology, University of Bern, Switzerland.

As in other insects, development and metamorphosis of termites are controlled by hormones. But development in termites can lead to the normal winged adult as well as to different polymorphic forms; i.e., the castes of soldiers, workers and replacement reproductives. Caste differentiation is also regulated by hormones and the endocrine glands seem to be controlled by factors of the social environment of the individual termite. The study of the endocrine mechanisms in such a complex system may well disclose new aspects of the functions of hormones and of the mechanisms controlling the endocrine glands.

The author studied particularly the functions of the corpora allata in the development and caste differentiation of the European dry-wood termite Kalotermes flavicollis (Fabr.). In this primitive species there are only three fixed castes: alates or winged imagos (which later develop into primary reproductives), secondary or replacement reproductives, and soldiers. The functions of the workers are carried out by the fullygrown larvae which are therefore termed pseudoworkers or pseudergates (Grassé and Noirot 1947). The developmental possibilities (Fig. 1) have been elucidated by marking single individuals in laboratory colonies. The pseudergates and the nymphs* have at each moult 4 or 5 possibilities in regard to differentiation. They can develop in the adult direction or undergo stationary moults, but they can also differentiate into replacement reproductives or pre-soldiers, and nymphs may undergo regressive moults and become pseudergates. The functions of the corpora allata have been studied in view of these possibilities in three ways: by volume measurements, by transplantation and extract feeding experiments, and by observation of the development of colonies which were collected at various seasons.

Progressive and regressive development will first be considered. Fig. 2 shows the seasonal changes in volume of the corpora allata of pseudergates and first-stage nymphs which had shown no signs of an approaching or just completed moult. The termites were collected and fixed immediately at the indicated times. During winter the corpora allata gradually decrease in size until they reach a minimum in March. No moults occur during this period. Then the corpora allata increase in size and reach a maximum in May. They then go through a second minimum in July and a second maximum in September. In laboratory colonies which were set up at 26°C, and composed of termites collected in the field at various periods, progressive moults occurred in appreciable numbers only in colonies collected in January and March, i.e., when the corpora allata activity, to judge from the volume, is low. The number of regressive moults showed two peaks in the colonies collected in May and September. These peaks correspond to the maxima of the corpora allata-volume curve. It therefore seems reasonable to postulate

* The term "nymph" is used to denote forms with wing pads, and the term "larva" to denote forms without wing pads.

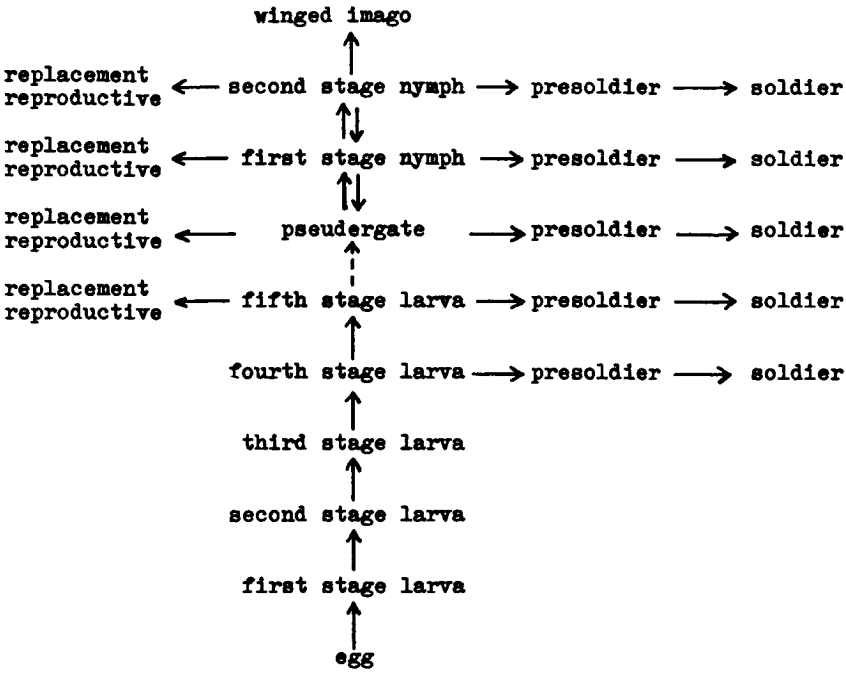

Fig. 1. Course of development in Kalotermes flavicollis. Each arrows indicates one moult; the broken line the occurrence of several moults.

that the relative activity of the corpora allata before each moult is responsible for progressive or regressive development.

This conclusion is supported by results of an experiment in which second stage nymphs were fed a derivative of farnesol, for which a strong juvenile hormone action had previously been found in the roach Leucophaea. A number of the treated nymphs developed into intermediate forms between the winged adult and nymph. As in other insects the juvenile hormone seems to inhibit or prevent imaginal development. It can be assumed that (a), in nymphs of the first stage a high juvenile hormone titer will induce regressive development, (b) an intermediate titer may lead to stationary moults and that (c), a low juvenile hormone titer allows progressive development. In field colonies a pseudergate or a first stage nymph undergoes only two moults per year, generally a progressive moult in late March or in April and a stationary or a regressive moult in August or September. The second stage nymphs which are produced in spring, moult again only in August or September of the following year to give rise in the majority of cases to winged imagos.

The development of replacement reproductives is also dependent on the corpora allata. It has been shown that their differentia-

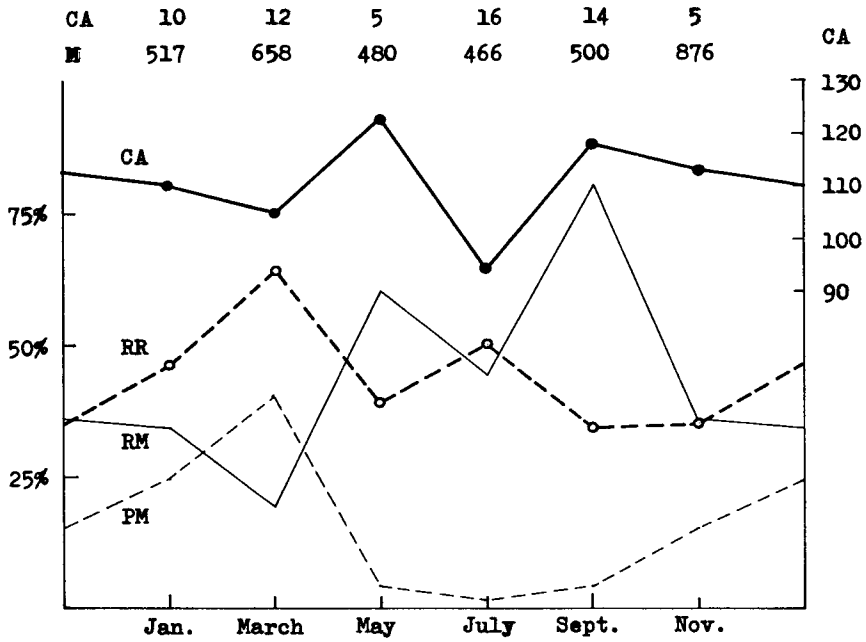

Fig. 2. Mean volume of the corpora allata of pseudergates and first stage nymphs during the seasons, and percentage of different moults which occurred in orphaned groups of termites collected at different seasons.
CA Volume of the corpora allata in units of 1000 cubic microns.
RR Percentage of individuals changing into replacement reproductives.
RM Regressive moults as percentage of all larval moults.
PM Progressive moults as percentage of the larval moults.
The figures above the curves represent the number of individuals used for the calculation of the points (CA for the corpora allata volume; M for the percentage of moults).

tion is prevented by a pair of functional reproductives which give off inhibiting pheromones with their excrements, and that not all larvae and nymphs are always competent to change into replacement reproductives (Lüscher 1961). In laboratory colonies which are kept at constant temperature competence is dependent on the age within the moulting interval and is highest soon after each moult (Fig. 3). The volume of the corpora allata shows a marked decrease just before and just after the moult, and following this a slight increase with a first peak about 10 days after the moult (Fig. 4). The volume increases steadily after the 30th day until the next moult is induced. If a replacement reproductive moult is induced in a competent pseudergate by removing the sexual pair from the colony, the corpora allata suddenly grow very rapidly and reach a volume which is about 4 times larger than that of the corpora allata of the

pseudergates. It was therefore at one time assumed that the first maximum of the corpora allata volume within the intermoult period corresponds to an activity phase of these glands and that a high juvenile hormone titer at this stage would be responsible for competence (Lüscher 1960). It has now been proved that this assumption was wrong and that the juvenile hormone actually decreases competence.

An extract of adult males of Platysamia cecropia (kindly supplied by Prof. C.M. Williams) and an extract of Bombyx mori cater-

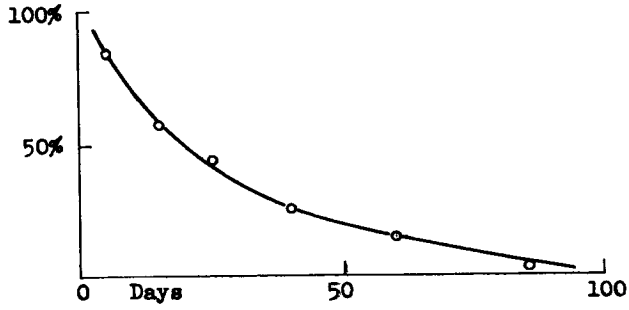

Fig. 3. Competence of pseudergates for replacement reproductive development in relation to the age within the moulting interval.

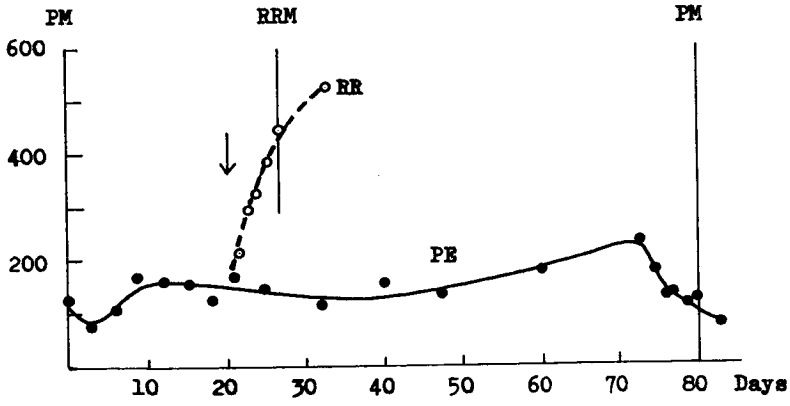

Fig. 4. The volume of the corpora allata of pseudergates (PE) between two pseudergate moults (PM) and of replacement reproductives before and after their moult (RRM) in units of 1000 cubic microns. Each point represents the mean of the volume of both glands of 3-6 individuals. The arrow indicates the time when replacement reproductive development was induced.

pillars (kindly supplied by Prof. P. Karlson) both had a significant inhibitory effect on replacement reproductive development when fed to orphaned colonies. Also synthetic substances with juvenile hormone action, like farnesol and less toxic derivatives of farnesol, had the same effect. On the other hand the honey-bee queen substance (kindly supplied by Dr. C.G. Butler), which has been shown to be a corpora allata inhibitor in the honey-bee (Lüscher and Walker 1963), when fed to termites significantly increases the number which change into replacement reproductives in orphaned groups. It could also be shown that the honey-bee queen substance, when fed to orphaned groups of termites, significantly inhibited the slight increase in volume of the corpora allata which normally takes place in all termites of a colony after removal of the sexual pair.

The observations on the development of orphaned groups of termites collected in the field at different seasons, also support the assumption that competence is dependent on low corpora allata activity. As can be seen in Fig. 2, the proportion of termites changing into replacement reproductives shows a negative correlation with the volume of the corpora allata. Competence is high when the corpora allata volume is small and vice versa.

As it has been shown that juvenile hormone as well as farnesol and its derivative have an inhibitory effect upon replacement reproductive differentiation, it seems not impossible that the active principle of the pheromones produced by the reproductives might be the juvenile hormone or derivatives of this hormone, and that the inhibiting action is brought about by reducing the competence of the pseudergates and nymphs to zero. The fact that products with juvenile hormone action can be extracted from insect excrements (Schmialek 1961), and the finding that the corpora allata of the pseudergates are inhibited by the pheromones, just as they are in hosts which receive implants of corpora allata, support this idea; but it is not easy then to explain the sex specificity of the pheromones (Lüscher 1961).

The sharp increase in volume of the corpora allata in developing replacement reproductives (Fig. 4) occurs only after this development has been induced. It is probably correlated to the production of a "gonadotropic" hormone, but it is as marked in males as in females and it is conceivable that a different hormone is being produced at this time.

If corpora allata of primary or secondary reproductives are implanted into pseudergates, most of the pseudergates develop into presoldiers and later into soldiers (Lüscher and Springhetti 1960). Soldier development is therefore induced by an activation of the corpora allata, but the hormone which is involved in this differentiation is probably not identical with the juvenile or the gonadotropic hormone.

Wigglesworth (1963) has shown that in Rhodnius farnesol and its derivatives have a juvenile as well as a gonadotropic effect, but that it needs much higher doses to demonstrate the latter effect. We have confirmed these results in Leucophaea, but here both effects are brought about by equivalent doses in relation to the

body weight. As these two effects are caused by the same substance it is reasonable to assume that the juvenile hormone and the gonadotropic hormone are (as Wigglesworth has claimed) identical. However, Sägesser (1960) has demonstrated that the hormone which increases respiratory metabolism cannot be identical with the juvenile hormone. His results and those of Engelmann (1957) show that the corpora allata volume of adult females of Leucophaea reaches a peak when the rate of growth of the oocytes is highest, but that the highest oxygen consumption occurs much earlier. Therefore the metabolism stimulating hormone is probably different from the gonadotropic hormone, and this is probably the reason why it could never be demonstrated in insect larvae. Also Leuthold, working in the author's laboratory, was able to show that the locomotory activity in Leucophaea can be stimulated by implanting the corpora allata of adult females, but not by injections of a farnesol derivative that had juvenile and gonadotropic actions.

Implanting the corpora allata of pseudergates or of last stage nymphs, and also the injection of the farnesol derivative mentioned above, do not induce soldier development in termites. Soldier development can however easily be induced by implanting corpora allata taken from last stage nymphs shortly before the adult moult. At this time the corpora allata have probably neither juvenile nor gonadotropic action. These results suggest that soldier differentiation is not brought about by the juvenile hormone but by a different hormone which is only produced by the corpora allata of adult, or nearly adult, insects. It cannot be a specific hormone of termites, because implanted corpora allata of adult females of Leucophaea can also initiate soldier differentiation. The hormone may be identical with the hormone which stimulates respiratory metabolism.

The evidence presented here shows that the corpora allata play a crucial part in development and caste differentiation in termites. The juvenile hormone titer is responsible for competence of the replacement reproductives as well as for progressive and regressive development and metamorphosis. The juvenile hormone probably also brings about egg development in the different reproductives and it may be contained in the pheromones which are produced by the reproductives. Another hormone, which is only secreted by the corpora allata of soldiers, reproductives, winged imagos, and nymphs shortly before metamorphosis, induces soldier differentiation. It is also conceivable that this hormone might be produced at certain times by certain castes as a pheromone. There is not a single differentiation step in termites in which the corpora allata are not involved. It therefore seems to the author that the corpora allata hormones are true differentiation hormones rather than ecdyson, the hormone of the prothoracic glands.

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CELLULAR MECHANISMS REGULATING THE ACTIVITY OF GENES IN INSECT DEVELOPMENT. Heinrich Kroeger, Eidgenössische Technische Hochschule, Zoologisches Institut, Universitätsstrasse 2, Zurich, Switzerland.

The Cell in Metamorphosis. It is the same cuticle cell that produces skin of a larva, of a pupa and of an adult in successive stages of postembryonic metamorphosis. We can imagine that the cell changes its performance by reading, at different times, different parts of the instructions coded in its genome. However, the information it needs at any given time is distributed all over the genome in a random fashion. There must be a mechanism that can selectively incite the right loci to yield their information at the right time, and that coordinates the selection of information to yield an integrated performance of cell and body.

Metamorphosis is under the direction of two hormones: ecdysone causes molting, juvenile hormone modifies the action of ecdysone to result in larval, pupal or adult structure according to its titer. By a change in the hormonal balance a cell can be forced into any performance that is within the limits of its determination, even into a reversal of the stages of metamorphosis: it seems to react blindly to orders of the hormones.

The manner in which the hormones exert their control is at present completely obscure: if the hormonal milieu induces the cells to read different chapters in their "book of heritage" then every tissue must understand this message in its own way: the cuticle cell draws information on pupal cuticle while the Malpighian cell draws information on the functioning of Malpighian vessels in a pupa.

The process of obtaining information from a part of the genome can actually be made visible in cells with giant chromosomes. Those regions which are actually "read" by a cell appear in a "puffed" state. Having a grasp on the two ends of the chain of action - hormonal milieu and puffs - the problem is now to uncover the manner in which the intermediate members function. It is these intermediate steps that must be responsible for the differential effect of hormones on the tissues and for the coordination of the effects in cell and body.

Hormones and their Imitators. CLEVER and KARLSON[1] have shown that 30 min. after injection of ecdysone into larvae of Chironomus tentans certain loci in the salivary gland chromosomes have reacted by forming puffs, the pattern of which corresponds to a more advanced stage of development. In Chironomus thummi, I have found that this effect can be copied by some heavy metals and by many narcotics. 30 min. after an injection of sufficient $ZnCl_2$ to bring the concentration in the hemolymph to $2,3 \times 10^{-2}$ molar (standard), the same effect ensues as with ecdysone. The experiment can be successfully repeated with glands explanted into oil with a droplet of hemolymph. The reaction of the puffing pattern to ecdysone shall be called "acceleration of development".

A larva responds only at a certain phase of development shortly before entering the prepupal stage. In this stage I call the larva competent. Occasionally whole cultures are encountered which even when at this phase of development do not respond to the stimulus. Non-responding larvae are called incompetent.

The effect of juvenile hormone consists of blocking the action of ecdysone: if active corpora allata are transplanted into a larva competent to respond to ecdysone imitators it is rendered incompetent within 5 days. This blocking effect of juvenile hormone, in turn, can be copied by inducing a "wounding metabolism" in a cell. A cell is forced into wounding metabolism by mechanical injury, explantation, X-rays, or oxygen poisoning. The wounding metabolism copies the effect of juvenile hormone in most cases in a more drastic way than is attainable by implantation of corpora allata. Blockage in most

cases does not occur at the stage the gland was in at the beginning of the experiment: the nuclei reverse their course of development, return to a puffing pattern characteristic of a younger stage and become blocked to the action of ecdysone in this state. This process shall be called "rejuvenation". BECKER was the first one to describe rejuvenation in Drosophila. In the rejuvenated pattern the puffs are oversized as compared to those puffs that appear in normal development.

In the rejuvenated state the puffs are biochemically highly active: as judged by autoradiography they synthesize RNA at a high rate (LEZZI).[2]

Rejuvenation occurs in glands of all ages except after pupation when the glands are in the process of dissolving, and in glands from very young larvae. Whatever the age at the start of the process, rejuvenation proceeds to the same juvenile stage. A cell can be prevented from developing the wounding metabolism by the administration of ecdysone imitators immediately upon giving the wound stimulus: once established in a cell the blockage of the ecdysone effect is complete: no titer of heavy metals or narcotics will override it nor any physiological titer of ecdysone.

Heavy metals and narcotics do not imitate ecdysone by increasing the sensitivity of the cell to the action of the hormone: they exert their effect independently of the titer of ecdysone present when the imitator is added. This is demonstrated when the two glands from a competent larva are explanted into hemolymph differing in ecdysone content: no difference in reaction is observed when Zn^{++}-ions are added in standard concentration.

The antagonism between juvenile hormone and ecdysone functions as a balance: if the titer of $ZnCl_2$ is successively decreased below a concentration of $2,8 \times 10^{-3}$ M, more and more nuclei exhibit rejuvenated puffing patterns as a result of the wounding metabolism in the explanted gland. They may lie side by side with nuclei accelerated in their development forming a mosaic gland. Few nuclei are neither rejuvenated nor accelerated. They have probably been wounded only very slightly and developed a wounding metabolism not strong enough to effect acceleration of development, but strong enough to halt rejuvenation. After transplantation of corpora allata into a competent larva the same situation is created: the titer of juvenile hormone is slightly risen so as to block acceleration but it remains insufficient to rejuvenate the puffing pattern. With Zn^{++}-ions no concentration has been found which in a whole gland blocks acceleration without causing rejuvenation.

It is known (SCHNEIDERMANN and WILLIAMS)[3] that wounded insect cells exhibit an increased O_2-consumption, as well as other biochemical changes. It is also known (PIEPHO and HEIMS)[4] that "regenerating tissue" undergoing a molt, behaves as if it were more sensitive to juvenile hormone than the rest of the body. Apparently the observation of rejuvenation of the puffing pattern reflects this reaction to wounding on the cytological level.

Since the existence of two independent systems that control gene activities in a similar fashion can be dismissed as highly unlikely and since the imitators closely copy their respective hormone each imitator seems to exert its effect via the same system as the hormone it copies. In addition the antagonistic relationship between the hormones makes it likely that both act by way of a common system, which in turn controls and coordinates the activity of genetic loci.

The Control System. The target on which the hormones act does not reside in the hemolymph. Glands explanted into oil without hemolymph rejuvente like glands surrounded by hemolymph: strong treatment of hemolymph with chloroform, evaporation to dryness and replacement of water yields a medium that neither accelerates nor keeps a gland from rejuvenation.

Nor is it in the cytoplasm: nuclei stripped of their cytoplasm except for a thin layer covering the nuclear membrane and kept alive in Drosophila egg cytoplasm rejuvenate at normal pace (LEZZI).[5] At present an influence of the attached layer of cytoplasm on the process of rejuvenation cannot be excluded: however, the absence of any quantitative changes in the course of rejuvenation after removal of the greater part of the cytoplasm makes it more probable that rejuvenation is a process triggered from the cytoplasm but which proceeds inside the nucleus.

If the control system resides in the nucleus it may involve an interplay of parts of the genome or may be an independent response of single loci (or groups of closely linked loci) to a specific situation in the nuclear sap. If gene activities are regulated by other parts of the genome (JACOB and MONOD,[6] McCLINTOCK)[7] removal of certain parts of the chromosome set should interrupt this function and should allow for the localisation of such parts of the genome that are indispensable for the function of others.

A newly developed technique for the production of highly deficient karyotypes makes it possible to perform such an experiment. By a process of elimination it was demonstrated that for the appearance of two puffs and for the enlargement of a third one during rejuvenation no other part of the genome is necessary. Only short streches of chromosome material neighboring on the respective loci on either side were not tested for dispensability because they cannot be removed by this technique.

It has so far been impossible to repeat these experiments under conditions leading to acceleration of development because the excessive wounding metabolism induced by the microsurgical procedure blocks acceleration. However, the results gained by testing rejuvenation alone establish that in being activated a locus responds directly to a conditions prevailing in the nuclear sap. It cannot be excluded that closely linked loci interact in a similar way as an "operon" in bacteria. However, the activation of the whole functional unit cannot depend on parts farther removed in the chromosome set.

The operation of the control system can be depicted as follows: Ecdysone and juvenile hormone act in an antagonistic fashion on some parameter in the nuclear sap varying its level. The genetic loci in turn, respond to various levels of this parameter by being activated or remaining dormant according to their individual sensitivity. Since, in the process of metamorphosis it is the level of the controlling parameter which changes, not the sensitivity of the loci, only specific combinations of activities can appear in response to a given hormonal situation.

The addition of ecdysone causes, in competent larvae, only limited acceleration in the development of its puffing pattern. No titer of ecdysone imitator promotes acceleration all the way to its final stage characteristic of early pupae. This could mean that other parts of the sequence of puffing patterns are operated by other factors than the hormones. However, the occurence of rejuvenation at all stages shows the same control system to be operative along the whole developmental sequence. Since in addition rejuvenation can be halted at all stages by ecdysone imitators and since the artificially introduced blocking action of juvenile hormone and its imitators acts in exactly the same manner as naturally occuring incompetence, it seems that all progress in puffing patterns is effected by ecdysone. It is, however, blocked over long stretches of development by juvenile hormone being uncovered only step by step as the titer of juvenile hormone falls.

Progression from one stage of metamorphosis to the next requires two events: the juvenile hormone titer must fall and the ecdysone titer must rise. However, the juvenile hormone titer may fall without ecdysone being secreted immediately. This is presumably the case in a competent larva: addition of

exogenous ecdysone or of imitators results in a sudden acceleration of development. An incompetent larva has maintained a high juvenile hormone titer, such that additional amounts of ecdysone are ineffective. Suboptimal breeding conditions (such as overpopulation) seem to further the development of the "hormone gap" characteristic of competent larvae.

What is the nature of the control system in the nuclear sap? Our knowledge on this is very limited. Heavy metal ions as well as narcotics in the concentrations used have a mildly denaturing action on proteins. They seem, therefore, to poison a particularly sensitive enzyme or group of enzymes. For narcotics the primary targets are the middle members of the respiratory chain, viz flavoprotein and/or cytochrome b. The depressant action on these targets seems to effect a redirection of metabolic pathways which is not yet fully understood. In the nuclear sap this redirection of metabolism to a state typical of narcosis seems to proceed further and further with an increasing titer of ecdysone: at every level reached in this process certain genes respond by being activated. In view of the independence of the juvenile hormone effect from the titer of ecdysone imitators it seems unlikely that juvenile hormone renders the primary targets insensitive to the poisonous action of heavy metal ions and narcotics: it seems more likely that it can open up pathways that bypass the affected reactions and thereby prevents the development of the metabolism typical for narcosis.

That the loci respond to a redirection of metabolism rather than to a mere depression of oxidative processes is shown by the fact that KCN does not accelerate the puffing pattern in a competent larva: in contrast in glands subjected to KCN puffs disappear altogether. Also ecdysone itself is known to raise respiration (ZWICKY and WIGGLESWORTH)[8] and to cause synthesis of cytochromes (SHAPPIRO and WILLIAMS),[9] findings not concomitant with the assumption of a depressant action of ecdysone on respiration.

Metamorphosis and Differentiation. One parameter is changed: in response to this a number of genes changes its activity, - some being activated, some becoming dormant. Evidently specificity and coordination are not so much a product of the hormones but a result of the specific response of the genetic loci. The hormonal situation undergoes rather simple, cyclic changes during metamorphosis: it is because they have specific sensitivities that single genes out of the whole complement are excited by the stimulus for activation. A larva hatching from the egg seems to carry, therefore, a large part of the course of metamorphosis programmed in the sensitivities of its genetic loci.

How does a gene acquire its sensitivity? The easiest assumption would be that a gene carries encoded in its DNA not only the information for a specific protein but also, as part of the common heritage, an individual sensitivity. However, in the same hormonal situation the tissues show different puffs (BEERMANN).[10] Evidently the same gene has one sensitivity in one tissue, another sensitivity in another tissue. The process by which they acquired different sensitivities must have occured during the life of the individual. It must have been concomitant with the diversification of the progeny of the zygote into different cell types, which we call determination and differentiation. It may, in fact be the cause of this diversification.

These are very different phases in the life of an individual: Determination, the process which imprints individual reactivities on the parts of the genome and predetermines to a large extent the course of events in development and metamorphosis: and differentiation, the call upon these potencies by the hormones which transforms the program into actual events. It is only within the limits of reactivity set by determination that a cell can respond to the stimulus of the hormone: it is without respect to the needs of the organism that a cell will also follow the call of abnormal hormonal situations set by the experimenter.

The physical basis of different sensitivities of genetic loci is entirely unknown. It may consist of an association with different proteins, it may consist of different types of coiling. Even more of an enigma is the selective action of whatever force imprints the "epigenetic" changes onto the genome. The problem of selection and coordination has been pinpointed to a sensitivity of the gene: what confers it onto the gene remains unknown.

It seems possible that the mechanism of redirecting gene activities is not restricted to developmental processes. Rejuvenation follows wounding and may be a reasonable response aimed at coping with the abnormal situation and furthering the cause of healing. The same system may be operative in other physiological or pathological reactions of a cell.

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GENE ACTIVITIES AND GENE ACTIVATIONS IN THE HORMONAL CONTROL OF MOLTING IN INSECTS. Ulrich Clever, Max-Planck-Institut für Biologie, Abt. Beermann, Tübingen, Germany.

The post-embryonic development of insects is characterized by a series of moltings. The last ones differ markedly in character from the earlier ones. However, all these moltings are induced by the same hormone, the ecdysone, which is secreted by the prothoracic glands. The character of each molt is, as is now generally accepted, determined by the presence or absence of a second hormone, the juvenile hormone of the corpora allata. Target organs of both hormones are the individual cells of the different tissues. What are the intracellular regulatory mechanisms of their molting behaviour?

Studies on the mechanism of action of ecdysone by several authors during the last few years indicated that one of the early effects of this hormone is an increase in the synthesis of proteins (see review by GILBERT and SCHNEIDERMAN, 1961). In some cases it has been demonstrated that the proteins which are newly synthesized or enhanced are enzymes (e.g. LAUFER, 196o, KARLSON, 1962). According to the modern concepts of biochemical genetics, the primary products of genes are the enzyme proteins, the synthesis of which is immediately directed by messenger RNA at the ribosomes. Indeed, changes in RNA metabolism are the second group of early effects of ecdysone observed in past years (WIGGLESWORTH, 1957, WYATT, 1959, LINZEN, 1962). These findings made it tempting to speculate that the hormone might fulfill its function by regulating the synthetic activity of genes (SCHNEIDERMAN and GILBERT, 1959, LAUFER, 1961, WILLIAMS, 1961, WIGGLESWORTH, 1962). A first direct approach to this problem came, however, from studies on the pattern of puffing in giant chromosomes of Diptera.

Puffs are local and reversible modifications in the structure of giant chromosomes. Each puff takes its origin in a single band. Studies of several authors in the past few years confirmed the hypothesis of BEERMANN (1952) that puffing of a chromosomal locus is related to synthetic activity of genetic material located there (review see BEERMANN, 1962). The patterns of puffing loci are specific for each tissue. However, even in the loci which form puffs in a given tissue, phases of puffing alternate with phases of non-puffing. Furthermore, puffing is not an all-or-none effect; the size of a puff may vary within a rather large range. The durations of the puffing phases and the size of each puff at any moment are directed by external factors, influencing each individual locus specifically The duration of puffing of a given locus - and, therefore, the frequency of this puff within a sample of animals to be studied- and the average size of the puff are parameters of the activity of this locus. In puff sizes and puff frequencies of giant chromosomes we have, therefore, simple measures of gene activities. We used them to study the behaviour of gene activities in salivary glands during the course of the third and fourth larval instar of Chironomus tentans, including one larval and the pupal molt. Our first question was whether there are any temporal relationships between puffing of chromosomal loci and the changes in hormonal situation regulating larval development.

With respect to this two main types of puffs must be distinguished (CLEVER, 1962b). The first group contains loci which form puffs, regardless of what period of a molt or intermolt the larva just is in. To this group belong most of the puffs in the salivary gland chromosomes of Chironomus tentans. Clearly, the puffing phases of these loci are not related to any process of larval development or changes in the hormonal situation. It is concluded that genes whose activities are represented by these puffs take part in processes of basal or functional metabolism of the gland cells. - The second group of puffs is comprised of those loci which form puffs only during certain phases of moltings. Genes whose activities are represented by these puffs take part in metabolic processes which are specific for certain phases of development. The kinds of these processes may vary from puff to puff and are in the most cases still unknown.

In last instar larvae which have not yet begun the pupal molt, the pattern of puffs is formed by a larger or smaller number of puffs belonging to the first group. Alterations of this pattern start very soon after the induction of the pupal molt. The extent of the alterations seem to vary in different species, e.g. in Drosophila many more loci seem to puff only during moltings than in Chironomus (see BECKER, 1959). These differences are, however, of no principal meaning for our considerations. We will describe the alterations as they appear after an experimental induction of the molt by injection of ecdysone into last instar larvae of Chironomus tentans (CLEVER and KARLSON, 196o, CLEVER, 1961a, 1961b).

Within the first hour after the injection of ecdysone into young last instar larvae two puffs appear in sections I-18-C and IV-2-B. The appearance of puff I-18-C is recognizable after 15 to 3o minutes, that of puff IV-2-B after 3o to 6o minutes. The induction of these molting specific gene activities is the first effect of ecdysone so far known.

During the next hours a larger number of loci react. These are alike in that their puffing is not specific for molting periods. The large sizes, however, which these puffs always reach during the pupal molt, are only occasionally achieved during intermolts. These results indicate that certain parts of the non-molting-specific metabolism also are regularly enhanced in the course of the molting process as an effect of ecdysone, and that this is accompanied by an enhanced activity of related genes.

As cited above, several authors found increases in RNA and in various proteins closely related to development and thus to the action of ecdysone. Many of these changes, however, could be induced without ecdysone, merely by injuring the insects (for review see GILBERT and SCHNEIDERMAN, 1961). The molt itself could not be provoked in this way. It follows, therefore, that although these processes are necessary for development, they are not sufficient to induce it. Moreover, they are not specific for it. These results correspond on the level of cytoplasmic metabolism with the behaviour of the puffs of the last group which led us to comparable conclusions.

Later on a third group of loci begins to puff. All the puffs formed are specific for older prepupae. The beginning of puffing of these loci is strongly correlated to certain stages of the molting process. One may assume that some puffs of this group have something to do with the production of enzymes responsible for the breakdown of the gland cells. In any case, their appearance indicates the action of metabolic processes specific for pupation and the participation of gene activities in the processes. At the level of protein metabolism the increase of some enzymes responsible for sclerotization of the cuticle in Calliphora (KARLSON, 1962) may belong to this group.

So far, we have only reported enlargements of puffs, indicating an increase of gene activity as a consequence of the induction of molting. Moreover, a larger number of those loci which had been puffing before molting begun decrease in activity. The frequency of these puffs becomes lower and lower beginning approximately at the middle of the prepupal phase. It seems reasonable to assume that this decrease of activity of large parts of gene metabolism is a consequence of the beginning breakdown of the gland cells.

Our findings demonstrate very clearly that alterations of gene activities take place during the sequence of events induced by ecdysone. Moreover, it can be shown that all these alterations of puffing are in some way under control of the hormone: if the hormone production ceases by a relapse of prepupae into a phase of dormancy, all molting specific puffs disappear (CLEVER, 1962a). The same conclusion may be drawn from studies of PANITZ (196o) in Acricotopus and of BECKER (1962) in Drosophila. However, the lapse of time between hormone injection and the reactions of most of the loci as well as the non-specificity of puffing of some them indicate that this dependency at least in most cases is an indirect one. So far, only a few cases are known in which puffing of chromosomal loci seems to depend on ecdysone in a more direct way. These cases are the puffing of loci I-18-C and IV-2-B in Chironomus. As stated above, these puffs appear very shortly after an injection of ecdysone. In normal development they are, at least in larger sizes, specific for molting phases (CLEVER, 1962b). We will now describe experiments which demonstrate the dependency of puffing of loci I-18-C and IV-2-B on the hormone concentration (CLEVER, 1963).

The duration of puffing of both loci depends on the amount of ecdysone injected: Two hours after injection of a dosage of 2×10^{-5} µg both puffs are present, but they have disappeared after eight hours. However, eight hours after injection of 2×10^{-3} µg small puffs are still present which have disappeared after 24 hours. After injections of higher doses, the induced puffs are still present even 24 or 48 hours later. Both puffs even reappear if ecdysone is injected once again immediately after their disappearance. Therefore, the puffs had disappeared because of an elimination of the ecdysone injected. Both puffs gradually decrease in size. The hormone also will be eliminated gradually; the duration of its elimination is the longer the

more hormone was injected. From these results we have concluded that activity of loci I-18-C and IV-2-B as indicated by puffing is under control of the titer of ecdysone.

In other experiments we have injected various amounts of ecdysone and compared the sizes of the induced puffs after three hours of incubation. The reaction threshold of locus I-18-C lies at a concentration of $10^{-7}\mu g$, that of locus IV-2-B at a concentration of $10^{-6}\mu g$ of ecdysone per milligram larval weight. Both puffs increase gradually with increasing hormone doses. The maximal sizes are reached at concentrations of $10^{-2}\mu g$ (I-18-C) and $10^{-4}\mu g$ (IV-2-B) ecdysone per milligram larval weight. Thus, the size of puff I-18-C depends on the hormone concentration in a range of about 5, that of puff IV-2-B in a range of about 2 orders of magnitude.

The finding of different reactivities of loci I-18-C and IV-2-B against different hormone concentrations makes it possible to understand the behaviour of both puffs in normal development. It differs in some degree from that after injection of ecdysone: In normal pupal molt, puff IV-2-B appears one to two days later than puff I-18-C, although locus IV-2-B reacts earlier than all the other loci. The lapse of time between the appearance of the two puffs in the experiment is about 30 minutes. Furthermore, in the experiment both puffs have reached their maximal sizes about two hours after the injection. In normal development, however, both puffs increase gradually. Puff I-18-C reaches its maximal size in old prepupae, puff IV-2-B in prepupae of middle age. The results just described led to the following conclusions: During normal development the concentration of ecdysone rises gradually and the activity pattern of these loci is adjusted in dependence on the altering hormone concentration. Thus, the two gene activities seem to be included in the reading of the information which lies in the alteration of the hormone concentration during the course of metamorphosis. From the sizes of the puffs in normal prepupae the actual concentrations of ecdysone may be estimated. We found they rise from about $10^{-7}\mu g$ in young to about $10^{-2}\mu g$ of ecdysone per milligram larval weight in old prepupae. The rise of concentration of ecdysone during metamorphosis could be proved directly in new experiments.

From our results the following conclusions may be drawn about the mechanism of inducing metamorphosis by ecdysone: The hormone itsself regulates quantitatively only one or a few specific metabolic processes within the cell. Gene activations are included in these early processes and it may be that regulation of these gene activities is the primary action of ecdysone. As secondary effects , many alterations in the synthetic machinery of the cells are set in motion. The exact way in which ecdysone influences the activity of these processes of genic and cytoplasmic metabolism is not yet known. It seems probable, however, that it does so by means of the reactions of the early induced chromosomal loci.

So far, we have only described the alterations of gene activities which occur if ecdysone has induced metamorphosis. If, however, larger amounts of juvenile hormone are present, ecdysone does not induce metamorphosis, but a larval molt. To learn something about the interactions of the two hormones in regulating insect development, we have studied puffing during a larval molt and compared it with the puffing during metamorphosis. Our results may be summarized as follows (CLEVER, 1963b): (1) The pattern of puffs in third instar larvae before beginning of the molt seems not to differ from that in last instar larvae. Even the frequency of puffing of the different loci is more or less indentical during both larval stages. (2)The first alteration in the puffing pattern after the onset of the larval molt is the appearance of puff I-18-C. The puff reaches its maximal size at about the middle of the molting phase. It remains very large for some time, then it becomes smaller and has completely disappeared one to two days after the molting is finished. Puff IV-2-B appears later than I-18-C but it reaches maximal size earlier than that one. Puff IV-2-B completely regresses before the molt has finished.
(3) No alterations in the activity pattern of those puffs which are not specific for molting have been observed: neither is the frequency of puffing of these loci during the molt lower than before, nor is there an increase in size of some of them.
(4) Most of the molting specific puffs which appear later on during metamorphosis do not appear during larval molt, but some of them do. Furthermore, there may be a very few puffs which appear only during the larval molt or which, at least, become larger then than at any other stage. Puffing of all loci of this group begins only toward the end of the molt.

The results show that in contrast to the many alterations of gene activities which take place during metamorphosis, the pattern of gene activities remains nearly unchanged from at least young third instar larvae - and probably from the beginning of larval life - until the end of the last larval stage. The confrontation very clearly demonstrates that most of the effects of ecdysone described for the pupal molt are characteristic not for molting but for metamorphosis. The gland cells break down during metamorphosis. Consequently, the functioning of the gland is finished and the cytoplasmic and genic metabolism becomes more and more restricted. Only those metabolic processes which are included in cell autolysis may remain active for some time. It follows that the effect of juvenile hormone in the salivary gland cells of Chironomus is the prevention of ecdysone from setting in motion such part of metabolism which lead to a break down of the gland. Only if no ecdysone is present or if the effect of ecdysone is altered by the juvenile hormone, are the gland cells able to live and to fulfill their secretory functions.

In species other than Chironomus tentans , the salivary glands, before breaking down at the end of metamorphosis, seem to exert a secretory function somewhat different from their former one (e.g. Acricotopus after MECHELKE, 1953). It is probable that in these speciessome of the observed alterations of puffing are related to the synthesis of new secretory products. The behaviour of the glands in these species leads to that of tissues

which are not broken down in metamorphosis, but whose function is changed as a result of it. E.g., the synthetic machinery of every individual epidermal cell must be somewhat different during the different types of moltings. These tissue specific differences in detail are, however, of no importance for our considerations: in all cases under discussion in the absence of juvenile hormone other sequences of metabolic changes are induced by ecdysone than in its presence.

This statement is, however, not pertinent to every individual step in the sequences. In the salivary glands of Chironomus tentans in the larval as well as in the pupal molt, locus I-18-C is activated first, followed by locus IV-2-B. Furthermore, the temporal relationships between the enlargements of the two puffs in the larval molt also correspond to those during metamorphosis. As mentioned above, the activity of these loci depends very specifically on the concentration of ecdysone. We followed that they are included in the primary action of the hormone. From the behaviour of these loci in larval molt, therefore, is to be concluded that the primary effect of ecdysone in the salivary gland cells is identical in the larval and in the pupal molt. Generalizing this result, it may now be stated that the primary effect of ecdysone is independent from any action of the juvenile hormone. As we have described previously (CLEVER, 1961b), the primary effects of the ecdysone seem also to be identical in different tissues. Probably, therefore, the only action of ecdysone is to induce the beginning of the molting processes within the cells, and this in the same way in any sort of cell. It is only later that the specific developmental behaviour of a cell is determined - according to the tissue and according to the developmental stage of the larva. The mechanism of the tissue specificity as well as the stage specificity of the of the cellular reactions seems to be independent of the primary actions of ecdysone.

Let us now return to the question of hormonal control of gene activity patterns. In the larval molt three kinds of puffing reactions can be distinguished in this respect:
(1) The juvenile hormone is responsible for maintaining in action all non-molting-specific puffs during larval molt in contrast to pupal molt. This, however, does not mean that all these loci stand under the direct control of juvenile hormone itself. This is clearly demonstrated by the details of their puffing behaviour during molts and intermolts. The function of juvenile hormone is to prevent the induction of cellular breakdown by ecdysone, and the maintenance of the general metabolism is only a consequence of this action.
(2) A few molting-specific puffs appear at the end of the molting process. The alterations of none of these puffs seem to be correlated to changes in the concentration of either of the hormones. There is no doubt, however, that these loci take part in the regulatory mechanisms of molting behaviour of the gland cells.
(3) As in the pupal molt, two specific puffs appear at the beginning of the larval molt and their sizes change in a very characteristic manner depending upon the changes in the concentration of ecdysone.

Thus, our results demonstrate that alterations of gene activities are included in the molting processes of insects. Furthermore, they demonstrate that the pattern of chromosomal loci active at a given molt is controlled by the hormonal situation. In most cases, however, this hormonal influence on genic activity is only a secondary one, mediated by other intracellular processes controlled by the hormones in a more direct manner. The alterations of gene activities in this connection demonstrate that genes take part in many of the metabolic processes within a cell which are associated with its basal, functional or developmental functions. Therefore, these results disprove the view of some authors which believe the activity of genes to be restricted to some special points of development. The results also demonstrate that the occurence of alterations of gene activities related to molts is not in itself an argument for a direct action of hormones on the genes - it may be directly observed by alterations of puffing in giant chromosomes or it may be deduced from alterations of RNA or protein synthesis. It cannot yet be decided definitely whether the target point of the hormones is at the chromosomal or at the cytoplasmic level. The close relationship which exists between the titer of ecdysone and the activity of loci I-18-C and IV-2-B led us, however, to the suggestion that the primary effect of at least this hormone might be to regulate directly the activity of specific genes. The mode of action of ecdysone in this case might be comparable to that of an effector in the regulator-operator systems in bacteria proposed by JACOB and MONOD (1961). The results of some recent experiments from our laboratory to test this hypothesis do not disagree with it.

With regard to the juvenile hormone, we have no indication of a direct action on chromosomes as yet. To study this problem we used several preparations which had shown juvenile hormone activity in insects other than Chironomus (Extracts had been kindly supplied by Dr. P. Karlson and Dr. H.A. Schneiderman, samples of farnesol and some of its derivatives by Dr. P. Schmialek). We injected these substances into larvae of Chironomus tentans of different stages of development. Alterations in the sequence of puffing normally occuring during the last stage and the pupal molt could not be induced by these injections. However, the experiments are not very conclusive as yet, because there was no indication of any effectiveness of the preparations in Chironomus larvae at all. There are some puffs which appear for the first time during larval life soon after the beginning of the last larval instar. One might assume this to be a consequence of the decreasing concentration of juvenile hormone, controlling some metabolic processes these loci take part in. It seems to us more probable, therefore, that the character of a molt is determined by the metabolic situation interferring with the primary action of ecdysone than by a later alteration of the induced events by the juvenile hormone directly. The mechanism by which the juvenile hormone brings forth this alteration of metabolic processes and the target point of its action remain, however, still to be elucidated.

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BIOCHEMICAL AND MORPHOGENETIC ACTIVITIES OF THE CORPUS ALLATUM HORMONE ON THE SUBCELLULAR LEVEL

D.Stegwee, Laboratory for Entomology, Agricultural University, Wageningen, The Netherlands

The regulation of cell metabolism is brought about by different mechanisms. The primary regulatory mechanisms mostly involve intricated biochemical feedback systems. Superimposed upon these primary mechanisms are the regulatory actions of hormones and the nervous system. Hence, the study of the biochemical mode of action of a given hormone involves the search for the primary key process or processes upon which the hormone might act.

In the course of our investigations on the mode of action of the corpus allatum hormone in insects (1, 2) we have obtained evidence that this hormone acts by influencing the total energy supply of the cell. This holds true for the process of oxidative phosphorylation, whereby chemical energy is generated in the form of ATP, but assumedly also for anaerobic processes involving phosphorylation reactions leading to energy conservation. The latter assumption is based upon the observation of a peculiar phenomenon in diapausing Colorado Potato Beetles, viz. the reversible degeneration of the flight muscles (3, 4). In the course of this degeneration the whole respiratory system contained in the sarcosomes is lost. Accordingly, in what is left of the thoracic tissues an increased rate of anaerobic metabolism is found. Under the influence of the corpus allatum hormone already during diapause development regeneration, i.e., morphogenesis of sarcosomes and muscle fibrils, starts. A complete regeneration of the respiratory system, however, has to await the termination of diapause. There are strong indications that this involves de novo synthesis of respiratory enzymes in the sarcosomes.

It seems obvious that for the regeneration to take place the energy supply has to be increased considerably. It is known that for mitochondrial enzyme synthesis oxidative phosphorylation is required. Presumably, the corpus allatum hormone stimulates the energy supply of, first, anaerobic and, later on, aerobic processes. But not only the restoration of the normal pattern of metabolism, also its maintenance seems to require the presence of the corpus allatum hormone. The observed effects of the hormone upon oxidative phosphorylation in isolated flight muscle sarcosomes of Locusta migratoria bear upon such a relation. It would seem that the degree of coupling between respiration and phosphorylation is dependent on the level of corpus allatum hormone.

Undoubtedly, for a complete unraveling of the whole complex problem a good deal of work has still to be done, in close collaboration with the endocrinologist, the physiologist, and the histologist.

Part of the results, discussed in the present paper, have been submitted for publication (3, 4).

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CHROMOSOME STRUCTURE AND FUNCTION

A symposium organized for the
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STRUCTURAL AND PHYSIOLOGICAL HETEROGENEITY OF MAMMALIAN CHROMOSOMES

T. C. HSU and CAROLYN E. SOMERS

Section of Cytology, Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Inst., Houston, Texas

Cytologists have long recognized that chromosomes are not morphologically homogeneous. The centromere and the nucleolus organizer are definitely differentiated regions which can easily be observed in mitosis. From breakage and reunion data, it has been inferred that the tips of a chromosome, telomeres, are at least functionally different from the rest of the body.

It has been found previously that the thymidine analog, 5-bromodeoxyuridine (BUdR), damages mammalian chromosomes at specific regions. One of the regions responding to BUdR preferentially is the telomeres. In the Chinese hamster, it is found that chromosome No. 1 contains an interstitial region (Region 7) which is extremely susceptible to BUdR toxicity. These regions are interpreted as areas rich in adenine-thymine (A-T) pairs.

To test this hypothesis further, hydroxylamine, a mutagen which allegedly deaminates cytosine, was employed to treat the Chinese hamster cells. Breakages are found on all chromosomes following the hydroxylamine treatment, but those regions that are particularly susceptible to BUdR are not significantly more damaged than other areas. Region 7 of chromosome 1, for example, shows relatively low frequency of breaks. However, hydroxylamine produces its own "hot spots". The centromeric region of all chromosomes appears especially vulnerable to the action of this agent. On chromosome 1, region 5 shows a high frequency of chromatid breaks. It is deduced, therefore, that the centromeric regions and other hydroxylamine hot spots may be rich in guanine-cytosine (G-C) pairs.

When the cells were X-irradiated and harvested 4 hours thereafter, the distribution of chromosome damage was at random. However, when the cells were sensitized with BUdR at a dose and duration not enough to produce visible damage, X-irradiation, also at a low dose, induced a nonrandom distribution of chromatid breaks at 4 hours postirradiation. Region 7 of chromosome 1 sustained significantly higher damage than other regions.

Autoradiographic studies of the Chinese hamster cell lines showed that mitoses found 4 hours postirradiation (250 rads) were principally cells in the G_2 phase when radiation was applied. The random distribution of chromosome breakages from this sample indicated the randomness of radiation damage induced. However, if samples are taken 8 or 12 hours postirradiation (S or G_1 cells

respectively), the distribution is found to be nonrandom, with region 7 showing a distinct peak (approximately 25%). The nonrandom distribution is thus regarded as a result of nonrandom restitution. Since region 7 is considered as A-T rich, a break in this region would probably retain sufficient A-T pairs to serve as a new telomere. Interstitial A-T regions, therefore, may be regarded as interstitial telomeres.

To test this hypothesis further, it is anticipated that region 7 of chromosome 1, after induced breakage by BUdR, does not necessarily require rejoining. Thus in the ensuing cell generations, a number of cells would contain, in replacement of a chromosome 1, a new subtelocentric element whose long arm should be equivalent to the short arm of chromosome 1 and the short arm equivalent to the length between region 7 and the centromere. This was found to be the case. The experimental results support the hypothesis that some chromosomes may bear interstitial telomeres.

The effect of BUdR on chromosomes must follow the incorporation of this agent into cellular DNA. Cells already completed DNA replication (G_2) are not damaged until they enter the next synthetic cycle. Likewise, the damaging effect of 5-fluorodeoxyuridine (FUdR), an inhibitor of thymidylate synthetase, appeared to manifest only on cells synthesizing DNA. In the Chinese hamster, chromosomes replicate themselves following a definite time sequence. The sex chromosomes are among the last to replicate. Using this as a guide, FUdR damage on chromosomes is found to be proportional to the activity of DNA synthesis. For example, the damage on the sex chromosomes is higher when samples are taken 3 hours following FUdR treatment than those taken 4 or 6 hours. The mechanism of FUdR damage, as suggested by Mennigmann and Szybalski, may be the production of nicks in the DNA molecule when the thymine moiety is absent.

When the Chinese hamster cells are treated with FUdR for 12 hours or longer, mitosis virtually stops. If the cultures are now supplied with thymidine, DNA synthesis resumes and a wave of mitosis occurs several hours thereafter. However, severe chromosome damage is observed during this period. Cells with completely shattered or pulverized chromosomes are frequent. This phenomenon is not observed among cells from a diploid human strain or L strain similarly treated. The difference is not explained, but it may be related to the mode of DNA synthesis in response to FUdR inhibition.

Autoradiographic Studies of the Structure and Reproduction of the Chromosomes of Phages and Bacteria

J. Cairns

Cold Spring Harbor, Long Island, N.Y.

Thanks to the formidable armoury of biophysical and biochemical techniques developed in the last ten years, the detailed structure and the manner of replication of DNA are now very precisely known. Many models have been proposed to describe how such DNA might be arranged in chromosomes, but the very profusion of these models shows how great the gap is between the study of DNA in solution and the genetic and histological studies of DNA in chromosomes. Recently, however, special techniques have been devised for determining what might be called the molecular structure of chromosomes. These techniques owe their origin to the demonstration by orthodox biophysical procedures that the "chromosome" of certain bacterial viruses comprises a single molecule, with a molecular weight of about 10^8, and that molecules of this size are exceedingly fragile in solution. Since conceivably the DNA of larger chromosomes, such as the bacterial chromosome, might similarly constitute a single molecule, special methods were developed for determining the size of DNA molecules without subjecting them to any stress. These measure the size of DNA molecules by looking at them rather than by measuring their movement in solution.

So far, two methods of visualizing DNA molecules have been used. One of these, namely direct electron microscopy, is discussed elsewhere in this issue. The autoradiography of tritium-labeled DNA is reviewed here. The results of the two methods show most satisfactory agreement wherever they overlap, and between them they promise ultimately to scan the entire subject of chromosome structure, from the smallest viruses to the chromosomes of higher organisms.

Autoradiography of Bacteriophage DNA.

Once tritiated thymine of sufficiently high specific activity (10 mC/mM) came on the market, it became practicable to label DNA sufficiently heavily to get an autoradiographic image from individual molecules after an exposure under film of about two months. The DNA of bacteriophage T2 was the natural choice

for testing the procedure since its molecular weight was known fairly precisely and the methods for extracting it intact had been worked out in great detail (1) (2).

Autoradiographs of T2 DNA, labeled by growth in the presence of H^3-thymine and extracted from the phage in the presence of a great excess of unlabeled phage, showed that those labeled molecules that had been caught in the extended state were about 50μ long (3). As this value for the length agrees with the estimated molecular weight (and, incidentally, has recently been confirmed by direct electron microscopy (4)), it seems that here we have a " chromosome" which is nothing more or less than a single molecule of two-stranded DNA.

Molecules of this size are not, however, the most suitable objects for autoradiography. For they are not inordinately longer than the resolution of the method (± 1μ), so that autoradiographic measurement of their length gives a very imprecise measure of their molecular weight. Further, they are now amenable to measurement by electron microscopy. Nevertheless these early efforts did show that the method worked and should therefore be applied to the DNA of more complex chromosomes for which, at present, neither electron microscopy nor ordinary biophysical procedures are particularly suitable.

Autoradiography of Bacterial DNA.

There were several reasons for thinking that the bacterial chromosome might also prove to be a single molecule of two-stranded DNA: genetically, it comprises a single linkage group which may be exchanged entire at the time of mating (5); physically, it is partitioned at cell division as if it contained two subunits that invariably separate at the time of duplication but usually are not further subdivided thereafter (6) (7). One other important fact demanded that bacterial DNA should be investigated: unlike the cells of higher organisms, a bacterium such as E.coli is engaged in DNA synthesis during the whole of its division cycle (8) (9); in other words, if its chromosome could be isolated intact and visualized by autoradiography, one should be seeing a molecule engaged in self-duplication.

In devising a procedure for the isolation of bacterial DNA, the molecules were assumed to be potentially so fragile that they could not be transferred from one container to another without being broken. Labeled bacteria were placed in a narrow chamber, bordered on one side by a Millipore filter, and were lysed either by lysozyme or by dialysis against Duponol. After a prolonged period of dialysis the chambers were drained slowly

and dried, and the filters autoradiographed. In this way it was possible to look at DNA which had not been subjected to any unnecessary stress. The results (10)(11) may be summarized as follows:-

(1). The chromosome of E.coli is indeed a single molecule whose length (1.0-1.5 mm) agrees reasonably with various estimates of its DNA content.

(2). The molecule is in the form of a circle.

(3). The circle is duplicated by a process that starts at a relatively fixed point on the circumference and proceeds in one direction from there.

(4). In the region of duplication, the two strands of the parent molecule separate and a new strand is laid alongside each; thus both daughter molecules are being made in the one region of DNA replication.

(5). During this process of duplication, the ends of both daughter molecules remain attached to each other and to the far end of the parent molecule; in other words, the circularity of the molecule is preserved during the period of its duplication.

In most respects these results are hardly unexpected. It has long been known that bacterial DNA replicates semi-conservatively (12), in the manner proposed by Watson and Crick (13). More recently, it has been found, both genetically (14) and physically (15), that the entire DNA is duplicated by a single process that moves in one direction only. And yet, finding that the molecule is in fact circular provokes the following rather unexpected argument:-

The molecule cannot be a double helix covalently linked into a circle; such a circle could not unwind and so could never be replicated. Whatever unites the ends of the molecule to form a circle must allow the two ends to spin relative to each other and so must have the properties of a swivel. Granted this, it is natural to consider whether this swivel might be the seat of some mechanism that actively spins the molecule and thereby drives the process of replication; since the chromosome is a circle, every revolution of such a spinner would inevitably unwind the parent molecule at the point of duplication and thereby provide single-stranded DNA as primer for the polymerase.

Certain pieces of evidence can be listed in support of this rather fanciful hypothesis. Immediately before the onset of

each round of DNA duplication, but not at any stage later, there is a short period of essential protein (and/or RNA) synthesis without which there can be no DNA synthesis (16); this would correspond to the stage of duplication of the spinner that, according to our hypothesis, must come between one round of duplication and the next. If the polymerase is prevented from replicating the DNA by depriving it of thymidine triphosphate, "thymineless death" ensues (17); one might think of this as the result of duplicating the spinner without first completing the duplication of the DNA; appropriately, such death does not occur if protein synthesis is inhibited at the same time, for example by chloramphenicol (18). There certainly must be some feature that distinguishes the resident DNA of a cell from any DNA acquired, for example, as a result of mating, since the latter generally can be replicated only if it is first integrated into the resident genome. Lastly, it is not by any means certain that the energy available from the action of the polymerase is great enough to unwind a molecule of 1 mm length at sufficient speed (10,000 rpm) to complete the act of duplication in each generation time (20-30 minutes). At the moment, however, there seems unfortunately to be no simple way of testing the spinner hypothesis directly.

Autoradiography of Animal Cell DNA.

Like the DNA of bacteria, the DNA in each chromosome of higher organisms may, on occasion, be partitioned at the time of duplication as if it too comprised a single molecule (19). Each chromosome in a human cell, for example, contains about ten times as much DNA as the bacterial chromosome (i.e., about 1 cm of DNA, or more). If this is all in a single molecule, the technical difficulties of isolating it intact promise to be formidable. So far, applying the method used for bacterial DNA to labeled HeLa cells has only yielded molecules about 1 mm long. However this value may merely indicate the limits of this particular method, rather than the real length of the DNA. And until some better method is devised, the molecular structure of the chromosomes of higher organisms must remain obscure.

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ELECTRON MICROSCOPIC STUDIES OF DNA AND CHROMOSOMES.

A. K. Kleinschmidt†, D. Lang and R. K. Zahn, University of Frankfurt am Main, Germany.

Deoxyribonucleic acid (DNA) seems to be suitable for a molecular morphology by electron microscopy. The diameter of the three-dimensional double helix model, after WATSON and CRICK usually given as about 20 Ångström units, is greater than the limiting resolution of commercially available instruments. The relation of length to width, however, in every kind of DNA is extremely high; DNA has all properties of linear macromolecules. The image of these filaments arises by differential contrast to the support film while the preparation is exposed to the electron beam in the vacuum. Constitutive phosphorus atoms or artificially synthesized nucleic acid bases with halogens like Br or F, and other components of organic material with higher contrast do not give enough scattering of electrons at present for a visible image of DNA lying as single threads on the background as seen in the photographic plate or screen.

The contrast usually has to be enhanced by apposition of heavy metal or metal salts. The electron staining consists in a more or less strong adsorption from the solution, often combined by chemically changed solubility. Shadowing uses the contrast by deposition of evaporated metal (or oxide) around particles distributed on the support film. Both methods give a positive contrast of the object itself and thicken the contour of the structure; they have been used in dried preparations or in thin sections.

Negative staining means the embedding of particles in phosphortungstate (PTA) which has not yet proven good for demonstration of single filaments of nucleic acids. The reasons for this unexpected event are not fully understandable since nucleoproteins as ribosomes or small viruses are easily shown by their negative contrast. It seems necessary not only to develop very thin sheets of PTA of similar thickness to the DNA strand itself but also to prevent a breakdown of the unprotected macromolecules during the drying process.

In the present state of knowledge on electron microscopy of DNA and its complex structures in cells there are several aims of visualization. Cytologists look for proof of chemically defined intracellular structures in their natural configuration; for example DNA in chromosomes which regularly are surrounded by ribonucleic acid (RNA) combined with proteins, or amino acids. Biochemists tend to isolate and to work with these cell constituents on the pathways of protein biosynthesis. RNA occurs in three different forms: The most abundant components are ribosomal particles of fairly uniform size easily broken down to smaller elements; more recent results show the messenger RNA in combination with ribosomes to polyribosomes with the aspect of pearl-like structures of different lengths; in contrast to the

†Present address: Virus Laboratory, Univ. of California, Berkeley

particulate matter the third component, transfer or soluble RNA an activated product for amino acid attachment to polypeptide chains, seems to be an unsuitable object for electron microscopy, because of its small size. Besides the investigation of natural nucleic acids, denatured and renatured DNA or hybridization of single strands with similar RNA, and synthesized copolymers of pairs of mononucleotides, are appropriate objects for molecular morphology. These natural or artificial components can be isolated in the test tube by subtle methods, and one of the essential difficulties in getting exact results using the microscope lies in sensitivities to physical influences or chemical breakdown of the original macromolecules during preparation. Often the so-called usual methods for isolation of cellular DNA result in a shortening of the intracellular length by shearing forces or by activated nucleases. The same seems to be valid for chains of RNA with higher sedimentation coefficients.

Another technique for the demonstration of individual DNA molecules uses the decay of labelled nucleic acid bases especially H^3-thymidine or thymine to produce autoradiograph effect using thin sections or dried preparations. The limit of this elegant method depends upon the maximum length in which decays can be distinguished from each other. This can be reduced now to a fraction of one micron.

Electron microscopy of isolated particles then will be a helpful proof for the biochemical investigation. Such proof also needs data of other physical chemical methods, as centrifugation, viscosity measurements, optical absorption in the ultraviolet light or light scattering. The visualization of single macromolecules gives new results since the sensitivity of bulk methods in the test tube is limited to the investigation of some millions, or more, of molecules. An example is the most recent experience with branched DNA molecules synthesized by in vitro polymerization with the polymerase-primer system of KORNBERG. These in vitro results are concerned with more or less diluted complex systems of DNA to investigate molecular lengths, configuration and cell reactions without cells. Cytology and virology use the electron microscope for the demonstration of topologically defined structures and DNA configuration in cells or in elementary organelles. Viruses as intracellular parasites are one of the typical examples. The particulate structure shows a central core with the nucleic acid, either DNA or RNA, and a surrounding shell, in simple cases as a capsid with subunits, the capsomers. The latter are the main objects of morphological investigation, since in many viruses a crystal-like order has been found by negative staining. On the other side the packing of nucleic acid in the core cannot be derived directly by electron microscopy. With DNA containing viruses as some bacteriophages of E. coli now it is possible to open the head by osmotic shock or detergent extraction; the DNA filament is released from its primary tight formation and shows, under optimal conditions, the length.

How viruses arise by intracellular growth, after infection by the nucleic acid, often is supported by a stepwise morphological study of the intracytoplasmic or intranuclear behaviour in thin sections of virus-infected cells. In bacteriophages (T-even of E. coli) the polymerized phage-DNA is condensed from loose pools while the bacterial DNA is broken down and transported from its central position to the peripheral cytoplasm. Other phages as λ use the cell during vegetative growth without attack of the cellular DNA.

More recent observations point to the probability of DNA content (but in apparently small amounts) of other intracytoplasmic organelles as chloroplasts and mitochondria.

The behaviour of superstructures of extremely long DNA strands packed to chromosomes is exhibited during mitosis and meiosis. Here, the electron microscope fully supports the findings of the light microscope, mostly done by thin sections. Extremely long and extended DNA molecules, however, cannot be measured in number and length in these tightly packed gel systems. The methods of autoradiography and the more or less direct conclusions from the growth cycle using pulse labelling open one approach to a better understanding of DNA configuration in growing cells. Another possibility is digestion by deoxyribonucleases in thin sections to show total DNA configuration in the protein environments at different stages of cell division.

The interest in the occurrence, length or molecular weight, and configuration of nucleic acids in the cell nucleus and/or in specialized structures of the cytoplasm arises mainly from the knowledge that they carry genetic information. DNA and the stable messenger RNA e.g., RNA containing viruses, contain the genetic information for the progeny. But only in a few examples the investigations by molecular morphology elucidate the mechanism derived from chemical investigations. Further work will give more information on the multiplicity of structural arrangements during growth or resting stages of cells, and their constituents.

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Structure and Function of Lampbrush Chromosomes

J. G. Gall, Department of Zoology, University of Minnesota, and
H. G. Callan, Department of Natural History, University of St. Andrews, Scotland.

The lampbrush chromosomes of amphibian oocytes each consist of two homologues in a modified diplotene stage of meiosis. The structural changes which accompany oocyte development seem to be related primarily to increased synthesis of ribonucleoproteins by the chromosomes. Each homologue displays several hundred pairs of laterally projecting loops, which represent regions where the two sister chromatids are not in close proximity. On these loops ribonucleoprotein is synthesized. It accumulates in various granules and masses whose morphology is often sufficiently distinct to permit identification of particular regions of the chromosome.

Callan and Macgregor (1) demonstrated that the loops contain DNA even though they are negative in the conventional Feulgen cytochemical test. This they did by isolating unfixed chromosomes into a DNase solution, and noting that the loops were extensively fragmented by the enzyme. RNase and several proteolytic enzymes remove the ribonucleoprotein matrix of the loops, (which then collapse into the chromomere from which they project), but these enzymes do not cause breaks in the linear continuity of the strands. DNase also causes breaks in the interchromomeric regions of the chromosome axis between successive loop pairs; this latter observation indicates that DNA is present in parts of the chromosome which are not synthesizing RNA.

A kinetic analysis of the fragmentation by DNase has been made by counting the number of breaks as a function of time (2). If we assume that the chromosome consists of $\underline{n}$ longitudinal subunits which are attacked independently and with equal probability by the enzyme, then we may predict that the number of visible breaks, $\underline{b}$, will be given by the expression, $b = kt^n$

in which $\underline{k}$ is a proportionality constant and $\underline{t}$ is time. The value of $\underline{n}$ was determined in separate experiments for the loops and for the interchromomeric regions. The values are 2.6 ± 0.2 for the loops and 4.8 ± 0.4 for the interchromomeric regions. These values are interpreted to mean that there are two subunits in a single chromatid (loop) and four subunits in a pair of chromatids (interchromomeric regions). The possible existence of an odd number of subunits cannot, however, be ruled out on the present data. Since it is known that the two polynucleotide strands of the DNA molecule are attacked independently by DNase, it seems possible that the two experimentally defined subunits of the chromatid correspond to the two polynucleotide strands of the molecule. If this is true, linear continuity of the

lampbrush loop is maintained by only one double helix of DNA.

The rapid incorporation of RNA precursors suggests that the loops of the lampbrush chromosomes are involved in RNA synthesis (3). Radioactive amino acids are also actively incorporated into protein in these chromosomes. The great majority of lampbrush loops label uniformly along their length after a single administration of H^3-uridine. A few loops, however, show a different pattern of incorporation. In these the isotope is incorporated in a restricted region near one of the two insertion points of the loop. As time passes, radioactivity spreads along the loop, until some days later the entire loop is radioactive. We interpret this experiment as evidence that the loop is spinning out of its parent chromomere at one of its insertion points, and is reeling in at the other, somewhat like a conveyor belt. It is believed that RNA synthesis proceeds for a relatively short time after a segment has unwound. If this interpretation is correct, it implies that the loop is a polarized structure in the sense that RNA synthesis begins at one end and proceeds sequentially to the other end. Although the majority of loops do not show sequential labeling, we believe that these, also, are moving in conveyor belt fashion (see discussion in 3). It is probable that all the loops are moving, but that this movement is demonstrable in only those few which in addition have a restricted region of RNA synthesis.

As shown recently by Izawa, Allfrey, and Mirsky (4), RNA synthesis in the lampbrush chromosomes is inhibited by actinomycin D. The loss of synthetic activity is accompanied by the loss of the ribonucleoprotein matrix of the loops. Izawa, et al. suggest that the loops are not only involved in RNA synthesis, but require synthesis for maintenance of their morphological structure. We have also carried out actinomycin experiments and have confirmed the loss of synthetic activity and collapse of the loop structure.

It is tempting to regard the RNA synthesized in the lampbrush loops as "messenger" RNA. The large size of the oocyte nucleus permits the manual separation of chromosomes, nucleoli, and nuclear sap. The RNA in these fractions has been analyzed for base ratios by the microphoresis technique of Edström (5). The pooled RNA of the chromosomes has the GC content of the DNA, although the values for individual bases are not the same in DNA and chromosomal RNA. The RNA of the nucleoli is very different from that of the chromosomes, but closely approximates the RNA of the cytoplasm (ribosomal RNA). A similarity between ribosomal and nucleolar RNA has been noted by Edström in several different organisms, and suggests that the nucleolus may be the major source of ribosomes. The nuclear sap RNA has shown significant, but as yet unexplained variability from sample to sample.

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STRUCTURE AND FUNCTION OF THE Y HETEROCHROMATIN IN DROSOPHILA

Wolfgang BEERMANN, Oswald HESS, and Günther F. MEYER

(Max Planck-Institut für Biologie, Tübingen, Germany)

In spermatocyte nuclei of Drosophila melanogaster at the beginning of the meiotic prophase strongly osmiophilic structures are formed around the chromosomal fibrils.These structures consist, as seen in the electron microscope, of tubuli with a diameter of 300-400 A. Besides the tubuli reticular elements and strongly osmiophilic granules of 400-700 A diameter always occur. Tubuli and reticular elements are limited to the first spermatocyte stage. Both types of structures are decomposed during diakinesis when they separate from the chromosomes.

In studies on other Drosophila species it became evident that in spermatocyte nuclei of all these species intranuclear formations occur, but their morphological appearance varies greatly and is species specific. Although organized structures (such as tubuli, for instance) may be absent in some species,the spermatocyte nuclei always contain some striking formations which are not found in other nuclei of the organism.The spermatocyte nuclei of D. busckii, D. miranda, D. pseudoobscura, D. persimilis, D. virilis, and D. funebris contain only aggregated granules of different sizes. The most characteristic component of the spermatocyte nuclei of nearly all species so far examined is a special type of granules with a diameter of about 300 A.

In D. willistoni we found ribbons of tubuli and many globular masses of nucleolus-like material. The spermatocyte nuclei of D.simulans contain bands of 300 A-granules, reticular elements, and near the nucleolus two short and highly refractive threads. The nucleolus is surrounded by a dense network of strongly osmiophilic material in the form of a ring or a hollow sphere. More highly organized functional structures have been observed in D.repleta. A large part of the dumbbell-shaped nuclear space is occupied by an elongated area of granules of different sizes. In the neighbourhood of the nucleolus there are found tubuli and a complex of tightly coiled thread-like material.

So far the most complicated and differentiated intranuclear formations have been found in D. hydei. Most striking is a pair of compact and strongly refractive threads which arise in the neighbourhood of the nucleolus and become diffuse distally. The diffuse sections are continuous with each other, so that the two compact threads are connected in a single loop. The threads are surrounded also in their compact sections by diffuse material which appears in the electron microscope to be formed of tubuli. Second are found a pair of club-shaped bodies with a reticular ground substance to which are attached large, strongly basophilic and mostly hollow granules. The third structure was called "pseudonucleolus". It consists of a loose spongy ground substance which is penetrated by canals. These are filled with less electron dense filamentous and reticular material. On the two opposite sides can be recognized by phase contrast two dark protrusions.In many cases the pseudonucleolus consists of two separate parts which then have one protrusion each. Fourth, tubuli are found in the nuclei. They are formed into ribbons and the ribbons are wound in knotes which lie in the

central region of the nucleus.

In four other species of the D. hydei-subgroup, namely D. neohydei, D. bifurca, D. eohydei, and D. nigrohydei, principally similar formed intranuclear structures are found. The different loop-like formations may be classified on the basis of their morphological appearance into compact threads, diffuse threads, clubs, pseudonucleoli, and bands of tubuli. Not all of these types are found in every species, and there is a characteristic topographical arrangement for each particular species. Therefore, the five species of the D. hydei-subgroup can be more easily defined on the basis of the appearance of their spermatocyte nuclei than by the normal morphological criteria.

The intranuclear formations of spermatocytes of Drosophila are considered to be phase specific chromosomal functional structures. Direct proof for this hypothesis such as the incorporation of RNA precursors as shown by autoradiography is not yet available. However, in one experiment we have already obtained a selective labelling of the "clubs" in D. hydei after injection of H3-uridine. Recent experiments with Actinomycin C, on the other hand, strongly support our views. Actinomycin is known to react with DNA and then to block the synthesis of messenger-RNA. If Actinomycin C is injected into larvae or imagines of D. hydei, changes in the intranuclear structures are found as early as 4-5 hours after injection. The matrix of the clubs is dissolved, the threads disappear, of the pseudonucleolus there only remains the apparently empty coat and the two uneffected protrusions, and in the tubuli definite alterations can be observed in the electron microscope. Two days after the injections all the functional structures are returning again to normal. Similar results have been obtained in the puffs of polytene chromosomes (CLEVER, personal communication) and in the loops of lampbrush chromosomes (GALL, personal communication), so that this seems to be a general reaction of the functional structures in chromosomes.

In attempting to explain the question of the physiological significance of the chromosomal functional structures it appeared necessary to establish which chromosome is involved in their formation and to localize the relevant sites on the chromosome. In view of its well known indispensibility for normal sperm functioning the Y chromosome can be considered as the most likely member of the genome to have an influence on spermatocyte metabolism. Therefore, we examined the spermatocyte nuclei of X/O males and other sterile males which lack a part of the Y chromosome. In fact, the spermatocyte nuclei of X/O males of D. melanogaster contain no tubuli and no reticular material. The Y chromosome must, therefore, play a decisive role in their formation. The examination of males possessing only a part of the Y chromosome shows that at least the tubuli are not dependant on the activity of a single locus on the Y chromosome. This follows from the fact that both the X/Y^L and X/Y^S spermatocytes contain tubuli and the other formations. Moreover, a quantitative correlation is found. The X/Y^L spermatocyte nuclei possess tubuli more regularly and in larger quantities than the X/Y^S spermatocytes. The wide variation from cell to cell which in contrast to the X/Y and X/O testes characterizes X/Y^L and especially X/Y^S nuclei, appears peculiar. It is certainly the rule in the X/Y^S testes that the spermatocyte nuclei are "X/O-type", but

there are also a few nuclei with tubuli. The X/Y^L spermatocytes behave in a corresponding way: most of them have the appearance of normal X/Y cells, but one finds some with only a few or even no tubuli. This type of variegation has, however, no parallel with the functional effectiveness of the sperm because the spermatozoa of X/Y^L and X/Y^S males are all, as far as we know, non-motile. Perhaps in the spermatocytes the realization of an intrinsic tendency to structural modifications is more or less hindered in a mode similar to that of a heterochromatic genetic variegation effect.

In X/O spermatocyte nuclei of D. melanogaster are found long needle-shaped protein crystals which occur at the same time as do the tubuli in X/Y spermatocytes. The crystals always appear when the formation of the tubuli is lacking or seriously inhibited. The conclusion can probably be drawn that the crystals are composed of material which in the normal X/Y spermatocyte is used for the construction of tubuli and the other functional structures and which in the absence of the Y chromosome crystallizes in a different form. However, it could also be the case that the crystals are intermediate products in a chain of syntheses, and are normally used up by the activity of the Y chromosome, i.e. by the tubuli.

From the investigations on D.melanogaster it could not be decided whether the functional structures in the spermatocyte nuclei were formed by the Y chromosome itself, or whether they are formed by the X chromosome or by the autosomes in which case the Y chromosome would act only as inducer. An opportunity to clarify this arose when we studied other species of the genus Drosophila. In D. hydei, experimentally induced changes of the paired loop-like formations in the spermatocyte nuclei can easily be analyzed because of their size and their distinctive individual appearance. In D. melanogaster the intranuclear formations depend upon the presence of a complete Y chromosome. This relationship holds for D. hydei, too, as was shown by the investigation of X/O spermatocytes. The paired loops (threads, clubs, and pseudonucleolus, as well as the tubular ribbons) which are so striking in normal spermatocyte nuclei are lacking. On the other hand, the crystal needles which were found in X/O spermatocytes of D. melanogaster do not occur in D. hydei. Another difference between X/O males of these two species is that spermatogenesis in D. hydei is arrested in early growth stages of the spermatocytes. Testes of X/O males do not contain any spermatids or spermatozoa.

A conclusion as to the specific mode in which the Y chromosome may be involved in the development of these structures may be expected from an investigation of males with two Y chromosomes. The following situations might be encountered in spermatocyte nuclei of double Y males: 1) if the intranuclear structures were formed by the Y chromosome itself, males with two Y chromosomes should possess two pairs of each loop instead of the normal set of one; 2) if the structures were formed by homologous elements in both the X and Y chromosome, XYY males should possess each of the intranuclear formations in triplicate; 3) if the loops were formed by the X chromosome alone, or by an autosome, under the inductive action of the Y chromosome, no numerical increase should be observed at all, although an increase in the material composing each loop might be found in

XYY spermatocytes. In spermatocyte nuclei of males with two Y chromosomes a complete duplicate set of paired loop formations was found. This shows that the paired structures of the spermatocyte nuclei are structural modifications of parts of the Y chromosome. The possibility that the loops are formed by the X chromosome or by an autosome under the specific inductive influence of the Y chromosome is definitely excluded.

The question of whether the Y chromosome itself is also responsible for the species specific appearance of the loop-like formations was investigated by studying the spermatocytes of hybrid males from species crosses between D.hydei and D.neohydei. The general result of these crosses is that in the spermatocytes of hybrids only the intranuclear formations of that type are formed which are characteristic for the species from which the Y chromosome was introduced into the cross. This means that the aspect of species specificity of the paired structures depends entirely on the constitution of the Y chromosome. It seems possible that the species specificity of the loop-like formations is based on the synthesis of specific proteins the polypetide sequence of which might be coded on the Y chromosome itself.In this case the Y chromosome would be directly responsible for species specificity. One might assume, on the other hand, that the information for the synthesis of proteins which are needed for the development of the intranuclear loops, might be localized elsewhere. In this case one must consider the possibility that hybrids are synthesizing the specific proteins of both species, and that the Y chromosome then selects the "suitable" protein for the development of the structures. In this case the X chromosome would, however, have to be excluded as carrier of this information. Repeated backcrosses to either of the parental species always ended up in complete sterility of the cultures after 2-3 generations, so that the question could not be decided yet.

In order to get some information about the extent of the Y chromosomal sections involved in the formation of the paired loops and the sequential arrangement of the loops on the Y chromosome we studied X ray-induced autosome-Y translocations in D. hydei. In one of the translocation stocks there is a break in the long arm of the Y chromosome and a second break immediately next to the kinetochore of one of the rod-shaped autosomes. One of the translocation chromosomes consists of the distal part of the long arm of the Y chromosome (approximately two thirds of the overall length) and a very short piece of the autosome which is presumably completely heterochromatic ($A_1.Y^{L2}$).The other translocation chromosome consists of the short arm and the proximal one third of the long Y arm and of the distal section of the autosome, presumably including the whole euchromatic part of that chromosome ($A_2.Y^{S.L1}$). In this translocation stock the following classes occur: 1) normal spermatocyte nuclei ($X/A_1.Y^{L2}$; $A_2.Y^{S.L1}/A$); 2) pseudonucleolus and threads missing ($X/-$; $A_2.Y^{S.L1}/A$); 3) clubs and tubular ribbons missing($X/A_1.Y^{L2}$; A/A); 4) a normal set of intranuclear formations,with addition of a second pair of threads and pseudonucleolus ($X/A_1.Y^{L2}/A_1.Y^{L2}$; $A_2.Y^{S.L1}/A$); 5) a normal set with addition of a second pair of clubs,duplication of the tubular ribbons is pre-

sumed but is very difficult to demonstrate ($X/A_1.Y^{L2}; A_2.Y^{S.L1}/A_2.Y^{S.L1}$); 6) complete duplication ($X/A_1.Y^{L2}/A_1.Y^{L2}; A_2.Y^{S.L1}/A_2.Y^{S.L1}$). From this it may be concluded that the sites of formation of the threads and the pseudonucleolus are on the Y^{L2} and those of the clubs and tubular ribbons are on the $Y^{S.L1}$ fragment. In further radiation experiments more Y-autosome translocations were found but these have not yet been analysed completely. It is highly probable that the site of formation of the threads will be found to lie distal to the pseudonucleolus, because several animals have been found lacking only the threads but never animals lacking only the pseudonucleolus.

With the help of our translocation stocks the question of the physiological significance of the functional structures during spermiogenesis can be tackled. In contrast to X/O males where spermatogenesis is completely blocked at the stage of the first spermatocyte we found that in testes with deficiencies for clubs and tubuli spermiogenesis proceeds further, at least to the formation of elongated spermatids. In cases in which threads and pseudonucleolus are lacking, spermiogenesis proceeds even to the formation of non-motile sperm. Moreover, there exists a correlation between chromosomal functional structures and sperm length: spermatozoa of normal X/Y males are 6.6 mm, of X/Y/Y males 13-14 mm (!) long. Non-motile sperm of males without threads and pseudonucleolus are shorter than normal; males with partial duplication of the set of loop-like formations have sperm of a length lying between that of normal and double Y males.

Corresponding correlations have been found in D. melanogaster, too. We have established the sperm of this species to have the following varietions of lengths: XO-♂♂ about 1.1 mm, X/Y^S-♂♂ about 1.3 mm. X/Y^L-♂♂ about 1.5 mm, XY-♂♂ about 1.8 mm, and XYY-males more than 1.8 mm (with considerable varietions).

Such a correlation exists not only between chromosomal functional structures and sperm length within the same species but also between the degree of differentiation and the quantity of intranuclear structures and the sperm length of the particular species. For instance, the species specific sperm length within the D. hydei-subgroup varies from 1.2 mm for D. nigrohydei which has only one single loop formation in the form of a double loop of diffuse material to about 6.6 mm for D. hydei which has at least four different loop forms. We also found corresponding correlations in members of other species groups of Drosophila.

It is not only the length of the sperm which is influenced by deficiencies in the functional structures but there are also structural alterations. In all the species of Drosophila which we have so far examined occur in the tail of the sperm beside the flagellum inside extraordinarily elongated mitochondria remarkable rod-shaped "intramitochondrial bodies" which stretch from the rear of the head almost to the tip of the tail. They are composed of material with periodic cross striations. On the basis of their fine structure as seen by negative staining of total mounts we presume that this is paramyosin or a related protein.

In the tail of the sperm of D. melanogaster two elongated mitochondria of which only one contains an intramitochondrial body are found beside the flagellum. In the testes of X/O-♂♂ besides

the apparantly normal sperm are found some in which the arrangement of the organelles is disturbed and the mode of crystallization of the intramitochondrial bodies is altered. In D. hydei each of the two mitochondria contains one body, but the two bodies have different diameters from each other and a different periodicity of striation. In individuals without threads and pseudonucleolus no alterations of these bodies in the sperm have been found.By way of contrast when the clubs and tubular ribbons are missing in no case so far examined have the intramitochandrial bodies been found. In several cases the flagellum was also missing.

The structures in the spermatocyte nuclei of D.hydei and related species are paired and polarized (asymmetrical) formations. Very similar paired and polarized formations are realized in the loops of lampbrush chromosomes which are found in oocytes of Amphibia and other animals. It is, therefore, very obvious to homologize the intranuclear formations of Drosophila with the loops of lampbrush chromosomes. However, some additional assumptions have to be made. In those cases, where no closed paired loops are seen, one has to suppose that unvisible submicroscopic connections to the (also unvisible) chromomere are existing. In some of the intranuclear formations, for example the compact threads of D. hydei, axial breakages similar to those obtained experimentally in Amphibia occur regularly. In addition, there are some characteristic differences between the Amphibian lampbrush chromosomes and the chromosomal functional structures in Drosophila spermatocytes. In Drosophila no chromosomal axis is detactable by means of the available technics.While in the lampbrush chromosomes of Amphibia very many loci make up loops, in Drosophila only single loops are formed. Perhaps the most important point is that the functional structures of Drosophila are made up only by one chromosome of the genome, namely the Y chromosome, and that this chromosome is completely composed of heterochromatin. In other words, the Y chromosome in spermatocytes of Drosophila may be considered as a solitary (univalent) lampbrush chromosome with only a few but very large loops.

The small number and the enormous size of the Drosophila loops leads one to think that we are perhaps dealing with multiples of identical information units (for example, for a structural protein which is needed in especially large amounts for the formation of giant Drosophila sperm) or with genes which belong to one particular functional unit. What is particularly difficult to explain is the fact that our experiments have rarely enabled us to decide between qualitative alternatives but on the other hand have commonly demonstrated the existance of quantitative relationships. This can presumably be attributed to the nature of heterochromatin. No concrete assertions can be made about these problems until more is discovered about the function of the heterochromatin.

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THE DEEP SCATTERING LAYER

A symposium organized for the
XVI International Congress of Zoology

by

S. R. Galler
Biology Branch
Office of Naval Research
Department of the Navy
Washington

THE BEHAVIOUR OF ORGANISMS CONSTITUTING THE DEEP SCATTERING LAYER. Hilary B. Moore, Institute of Marine Science, University of Miami, Florida.

Various types of animal may, under different conditions, be responsible for the deep scattering layer. A preliminary study of four plankton groups showed a consistent behaviour pattern, suggesting that a study of copepods would yield information of general application. A mathematical model has been proposed which assumes that the depth occupied by an animal results from a balance between stimuli of temperature, illumination and pressure. For each factor there is a nul value and a relative sensitivity to departure from this nul. For each species there is a diurnal rhythm in both the nuls and the sensitivities, and also a seasonal rhythm possibly related to differences between successive broods. Within a species there is also a wide variation between different individuals, resulting in vertical spread of the population.

A comparison of the characteristics of ten species of copepods is in progress. These span a wide range of characteristic depths and of extent of diurnal migration. A general pattern is apparent, and it is hoped that it may be possible to relate this to the geographic distribution of the species.

AN INSHORE SONIC-SCATTERING LAYER. Brian P. Boden and Elizabeth M. Kampa, Scripps Institution of Oceanography, University of California, La Jolla, California.

At the invitation of Dr. Brian Bary of the Oceanographic Department of the University of British Columbia we undertook a short investigation of the sonic-scattering layers encountered in the waters of Saanich Inlet, Straits of Georgia, British Columbia. The purpose of the enquiry was to determine the nature of the ambient light at the depth of the layers and to perform electrophysiological experiments on animals collected at these depths.

Measurements at sea:

Saanich Inlet is rather shoal and the R. V. "Whitethroat" was at anchor throughout the operation. For observation of the scattering layers the following echo-sounders were used: two Furunos, F 701 and F 705 operating at 50 and 200 kc/sec respectively; an EDO operating at 12.5 kc/sec and a Minneapolis Honeywell PDR operating at 12 kc/sec. We have described the basic design of the irradiance meter used for determination of light conditions elsewhere. It embodies a selected 931-A multiplier phototube and a series of eight narrow-band interference filters in the 400-600 mμ range which are placed in front of the photocathode by means of a filter changer operated from the deck. Desaturation of the narrow spectral bands because of large angles of incidence is avoided by placing a collimating tube between the filter and an irradiance plate. This limits the angle of acceptance of the filter to its useful 5°. The whole system is calibrated by means of an Eppley thermopile against a U. S. Bureau of Standards light source. The depth-sensing circuit of the meter was not used since the ship was stationary, and the weight of the instrument and weakness of current resulted in a zero wire-angle. Reflection from the instrument appeared on the fathometer trace and simultaneous records of depths of instrument and layers were obtained.

The experimental procedure was as follows. The instrument was lowered to the depth of the top of the layer and the irradiance value recorded through an arbitrarily selected 502 mμ-peak reference filter was noted. The instrument was raised or lowered to maintain it at this irradiance value at λ 502 mμ throughout a 36-hour period. The filters were cycled periodically, and the fathometer record was ignored by personnel operating the photometer after the initial matching of depths of instrument and scattering layer.

Behavior of scattering layers: During the day the layer was well-defined at a rather shoal depth of about 90-100 m. During our observations the layer was usually split into upper and lower components (Slide 1). The lower component maintained a constant depth throughout the diurnal cycle. The upper component appeared very sensitive to fluctuations in light intensity and on the PDR record it would merge with the lower part, settle just above it (10-15m.) or show a tendency to diffuse according to the variable

state of the sky. The upper component migrated toward or from the surface at twilight (Slide 2). Fish traces were also apparent, associated with the layer. It is Bary's opinion that most of the scattering in the migratory component is caused by the crustacean Euphausia pacifica. The dominant plankton organisms in the lower layer appear to be amphipods. Bary (pers. comm.) tells us that this has been confirmed by television observations and that quantities of fish appear to be sandwiched between the layers (Slide 1). Our hauls with a 1 m. closing net through the upper layer yielded E. pacifica which were taken, alive, to Scripps for experimentation.

Light conditions: During the daytime the two components of the layer frequently merged at a depth (85-100m.) at which the transmitted sun-and sky-light is blue-green with a peak between 490-500 mμ. At this depth the intensity of irradiance at λ 494 mμ was about 1.2×10^{-1} μW/cm^2 (Slide 3). The spectral distribution and intensity of the natural light from the time it forms at dawn throughout its downward morning migration are very similar to conditions that pertain at midday (Slide 4). During the sunset migration, while the irradiance intensity at reference λ 502 mμ is the same, the peak intensity is reduced and the peak has shifted toward the longer wavelengths (Slide 5). Total irradiance (the areas under the curves) does not vary very significantly in the three situations. Planimeter readings are as follows: Slide 3 = 4.83 in.^{2}, Slide 4 = 5.14 in.^{2}, Slide 5 = 4.94 in.^{2}. We have reported this shift toward longer wavelengths at twilight previously in California. At sunset the diffuse, upwardly migrating component overtook the λ 502 mμ isolume (Slide 6). We have seen this occur in the Mediterranean too. The position of the peak at λ 495-500 mμ is at a considerably shorter wavelength than that demonstrated by Utterback in the nearby Straits of Juan de Fuca (λ 530 mμ). John Tyler, Vis. Lab. Univ. Calif., (pers. comm.) considers this could be attributed to Utterback's use of broad-band filters to which "effective wavelengths were arbitrarily assigned." Differences in the clarity of the water possibly contributed to the discrepancy.

The intensity of the light at scattering layer depths is about two orders of magnitude higher than that we have reported in other localities. This suggests a hitherto undetected seasonal or geographical effect.

Bioluminescence: Very little bioluminescence was encountered although a few very bright flashes - up to $1\text{-}2.5 \times 10^{-1}$ μW/cm^2 - were observed.

Measurements in the laboratory:

Electroretinograms were obtained from a number of specimens of E. pacifica collected from the upper layer in Saanich Inlet and flown to Scripps Institution.

The experimental animal was placed in a narrow groove in a lucite plate and confined with rubber bands in such a way that it could not swim, but that its breathing was not impaired. The plate was placed in a sea-water bath at 9°C. Recordings were made

with micropipettes (tip diameter 0.5 to 1 μ) filled with 3 M KCl. The pipette was inserted into the eye and connected by way of a Ag-AgCl electrode to the G_1 grid of a Grass P-6 preamplifier. A strip of Ag-AgCl gauze in the sea-water bath served as the indifferent electrode. It was connected to the G_2 grid of the preamplifier. The G_2 grid was grounded, and recording was single-sided. Responses to flashes of light were displayed simultaneously on a Tektronix 502 oscilloscope and a Sanborn 320 recorder.

The stimulating light source was a tungsten lamp (GE, 110-v, 500-w) housed in a 35-mm slide projector and operated at constant voltage. The light beam was collimated and entered the experimental block box, which housed the preparation, through a camera shutter. Narrow-band interference filters (half-peak band width, 7-12 mμ) were used to select various wave lengths and neutral-density filters modified intensity. The light passed through a diaphragm and two lenses inside the box to a mirror mounted at 45° directly over the experimental preparation. The positions of the lenses and the aperture of the diaphragm were adjusted to provide a uniform light field at the level of the preparation.

Light intensities through the various interference filters were measured at the level of the preparation with a thermopile and galvanometer.

The preparation was left in the dark to adjust to the experimental temperature. Test flashes were given at intervals during adaptation. When the amplitude of the response became constant the experiment was begun.

The sensitivity spectrum of E. pacifica from Saanich Inlet, like that of E. pacifica from the coastal waters off San Diego, is trimodal. In the Canadian form there is a primary peak at 495 mμ and two secondary peaks, one in the blue near 460 mμ, and another in the green near 515 mμ. We attribute the peak at 460 mμ to the presence of euphausiopsin, and we consider that euphausiopsin is the visual pigment. The ratio of sensitivity at 515 mμ to that at 460 mμ is much greater in the Canadian specimens (Slide 7) than that in the Californian specimens. This difference between members of the same species can probably be accounted for by a greater concentration of a screening pigment, astaxanthin, in the eyes of the Northern specimens. There is an implication here that this difference in spectral sensitivity represents an adaptation to environment, for the peak (490 mμ) of the spectrum of transmitted sun-and sky-light at the depth of the euphausiid layer in Saanich Inlet is some 30 mμ toward the red from that (460 mμ) in the waters off San Diego.

It has been known for nearly a century that representatives of a single class, particularly the class Pisces, may have quite dissimilar spectral sensitivities because the responsible visual pigments, rhodopsin and porphyropsin, are constructed from different forms of vitamin A. Recently Munz has shown that certain fishes produce, from a given vitamin A, a visual pigment with an absorption spectrum different from those previously associated with that vitamin. At the same time, but with another technique,

Denton and Warren related a pigment, chrysopsin, with similar absorption characteristics in similar fishes to the environment of these animals.

Apparently, a similar situation exists among arthropods: other workers have shown that lobsters have a rhodopsin-like pigment and spectral sensitivity. A hermit crab that was studied resembles the lobster (Homarus) in spectral sensitivity and habitat; an American freshwater crayfish has quite a distinctive sensitivity with a peak near 575 mμ. No biochemical experiments or *in situ* measurements of environmental light have been reported for this animal.

With euphausiids we appear to have a further refinement, in that, within one pelagic species, by differential deposition of a non-visual pigment, subtle adaptations to photic environment seem to have been achieved.

AN UNUSUAL SOUND-SCATTERING LAYER IN THE SLOPE WATER OFF THE NORTHEASTERN UNITED STATES, Richard H. Backus, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

Widespread exploration with a 12 kcps echo-sounder during the last decade in the western North Atlantic has uncovered an apparently unique sound-scattering layer which is confined to the slope water off the northeastern United States. The shoreward limit of the area in which this feature has been observed is about at the 200-fathom curve; the seaward limit is the warm front of the Gulf Stream. Between these limits the feature has been observed from off Cape Hatteras northeastward to the meridian of 65° W. This layer has the general properties of ordinary deep scattering layers save in the structure of the echo sequences on the echo-sounder record. Successive trains of echoes, instead of forming the usual even stripe resulting from the receipt of sound scattered by numerous targets of low reflectivity, form crescentic echo sequences resulting from the receipt of sound scattered by a few targets of high reflectivity. Such crescentic echo sequences are familiar features of echo-sounder records made in shallow water, especially over known fishing grounds, but are of infrequent occurrence at depth in the deep ocean.

These crescentic echo sequences are arranged to form a layer whose midday depth is about 180 fathoms. The layer migrates to or near to the surface in the evening and away from the surface at dawn. These vertical migrations are slower and begin sooner than those of the ordinary deep scattering layers seen in the area with the 12 kcps echo-sounder.

In 1959 this peculiar layer was virtually continuously distributed over thousands of square miles of the slope water area from Cape Hatteras to the

meridian of 71°W. In 1960 and 1961 the layer was very patchily distributed in this area.

An examination of the broad-band sound-scattering properties of this layer have been made during an evening vertical migration. At midday depth or near it the layer scatters sound which is sharply peaked near 12 kcps. This explains the very high intensity of the echoes recorded by the 12 kcps echo-sounder. During the evening ascent the layer best scatters sound at lower and lower frequencies. Near the top of the ascent at 15 fathoms the sound-scattering peaks between 2 and 3 kcps. Such a considerable change in peak scattering frequency indicates a gas-bubble scatterer, doubtless the swimbladder of a fish. Consideration of the depth-frequency relationship during the migration suggests that the fish was heavy at depth and simply let the swimbladder expand with lessening pressure to bring the fish to neutral buoyancy when near the surface. The surface swimbladder volume of the scatterer has been computed using the frequency-depth relationship and indicates a fish whose total displacement volume is about 40 cc.

Fishing with a large mid-water trawl has not yet assisted in the identification of this sound-scatterer nor have observations with an echo-sounder - camera apparatus been successful. One fish under consideration is the little-known but apparently abundant gempylid, Nealotus tripes Johnson. This bathypelagic fish has been observed in great number at the sea-surface at night shortly after the evening ascent of the layer in question. The displacement volume measured for a number of specimens collected at such a time is smaller by a factor of two or three than that computed from acoustical data.

THE DEEP SCATTERING LAYER AS OBSERVED FROM THE BATHYSCAPH TRIESTE

by
Eric G. Barham
U. S. Navy Electronics Laboratory
San Diego 52, California

Deep scattering layers are broad zones of diffuse acoustic reverberation recorded on echo sounders at mid-depths throughout the temperate oceans of the World. Diurnal vertical migrations in response to changing light intensity clearly indicate a biological causation. However, the applications of this phenomenon to an understanding of the ecology of marine organisms has been limited pending positive identification of the causative animals. Evidence garnered from several types of studies have tended to implicate diminutive fishes with gas-filled swim bladders as the organisms most likely responsible. However, net hauls taken through layers at their daytime depth have shown that there is a closer correspondence with euphausiids and sergestid prawns, but such hauls have not demonstrated large enough populations of these crustaceans to prove acoustic responsibility. Recent evidence gained by direct observation from the TRIESTE is therefore of interest.

During 1962, six dives in the bathyscaph TRIESTE were made in and adjacent to the San Diego Trough, the region of DSL discovery and site of intensive studies. Organisms thought capable of scattering 10-20 kc sound were identified and counted as the TRIESTE descended through the DSL depths. By computing the angle of view from the window of the TRIESTE'S sphere, and the maximum range at which the various types of organisms could be recognized, the volume of water within which they were contained was estimated, and counts converted to numbers per $1000M^3$. Comparisons could then be made with net-hauling results. On three dives adequate acoustic data were obtained by surface ships, and the depth of the bathyscaph was accurately established allowing critical comparison between these data and the observed population densities.

These studies have shown that the best and most consistent spatial relationship between organisms and the deep scattering layers has -- contrary to information gained by net hauling -- been with physonectid siphonophores. Generally the DSL displays an upper and lower component. Euphausiids are usually

common in the upper layer, while a prawn, Sergestes similis Hansen, is consistently present in the lower component. The majority of mid-water diminutive fishes, Myctophids and Cyclothone spp., are located well below recorded scattering layers. Immature Pacific hake, Merluccius productus (Ayres), were found to be associated with a shallow "hard" scattering layer centered at 200 M. above the DSL. Schools of 10-70 Loligo-like squid were observed above, in and below the layers.

In view of the generally accepted concept of the faunal make-up of scattering populations, two aspects of these observations were most surprising. (1) The scarcity of fishes at the depths of the DSL. For example, during Dive 88 (mid-morning 14 February 1963) 92 fishes were counted above and below the depths of the DSL and only 2 fish observed at depths corresponding to the DSL. (2) The large populations of physonectid siphonophores, probably Nanomia bijuga (Delle Chiaje), consistently observed in conjunction with the DSL. Because of the fragile nature of these colonies net hauling has failed to provide this information, but these observations have led us to a consideration of such siphonophores as the major cause of the layers. For example, measurements of their pneumatophores indicate the gas bubbles contained within are close to the resonant size for sound frequencies used in echo sounders. Further, such organisms are widely distributed throughout the temperate seas, but relatively rare in polar waters. This matches the range of the DSL quite well. In addition, observations of their swimming actions in conjunction with speculations based on their anatomy indicate they are capable of undergoing vertical migrations of a magnitude and rate demonstrated by scattering layers. Venting of expanding gas due to pressure release at time of upward migration or as a prelude to descending could also create secondary scattering sources.

On the basis of these considerations it is tempting to conclude that siphonophores are the primary cause of the DSL off Southern California -- and probably throughout the oceans of the World. Rigorous proof, however, requires two more pieces of evidence. Ideally we should have target strength measurements of these organisms in situ, and observations of such populations migrating with the layer. Further observations in other geographical areas would be highly desirable. These observations also corroborate evidence gained by net hauling that euphausiids and sergestids are physically associated with the layers, but the

question of their importance as actual scatterers remains unsettled. It would seem, however, that they probably contribute to overall scattering levels. In EDO sounders, a deep layer below the DSL is rarely present during daylight hours, but is usually recorded, beginning at about 200 meters, as it "follows" the main layers towards the surface. Diminutive, bathypelagic fishes might well be the cause of this layer. In collaboration with others we intend to attempt to gain information on these questions during the next series of dives of the TRIESTE.

Regardless of the outcome of future work, however, it is clear that these findings have a direct bearing on our understanding of ecological relationships within the volume of the sea. Heretofore, our concepts of physonectid siphonophores have been based on fragments taken in nets, or on rare occasions when young colonies are carried into shallow seas or carefully dipped from surface waters. It is now obvious that these organisms, if shown to be the primary cause of the DSL, form a zone of mid-water predators -- a living net -- stretched across the far reaches of the World's oceans. Clearly they must play a key role in the transfer of energy between the euphotic zone and the abyssal depths. Some speculations are suggested here. For example, a possible predator-prey relationship may exist between siphonophores and euphausiids which may help to explain the numerical predominance of the latter organisms in the higher latitudes where such siphonophores are rare or lacking. Or, since physonectids are capable of establishing perfect buoyancy with their density parameters, perhaps we have populations of organisms of high acoustic reflectivity that will mark density anomolies for us. Sensitive to temperature and salinity, such organisms may represent hydrographic tags, for if different species dominate characteristic water masses, and if we can learn to distinguish their acoustic signatures, then we can know a great deal about the ocean in a very short time. And what of their larvae? In some species of physonectids, a gas sac develops by the seventh day. Perhaps diffuse scattering at shallow depths is frequently caused by siphonophore spawn.

At the present at least, we can firmly conclude that the phenomenon of the Deep Scattering Layer will continue to serve a coordinating function -- as it has in the past -- for the many disciplines which we must apply to the study and understanding of marine ecology.

SOME NEGLECTED METHODS OF STUDYING THE DEEP SCATTERING LAYERS. J. B. Hersey, Department of Geophysics, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

The deep scattering layers are studied acoustically almost solely by means of the echo sounder. This habit has retarded progress in this field, especially since other acoustical observations have provided the significant new insights of the past fifteen years. The usefulness of the latter is far from exhausted. Over ten years ago a sparker was used successfully to prove that the impedance of individual scatterers is less than that of sea water. It seems that this technique could well be extended to observe the swimming habits of individuals and to measure population densities. The spectrum of scattering revealed by using explosives as sound sources has provided clues, as yet not fully understood, about the behavior of the scatterers during vertical migration. Research of the past five years includes observations of broad spectrum scattering over large areas of the North Atlantic Ocean and adjacent seas from which zoogeographic patterns can be deduced. The information rate of these observations is limited by safety requirements for explosives or by the design of the explosive train, and by the requirement for stopping the ship for best results. Nearly two years ago an echo sounder employing a spark sound source was shown capable of recording deep scattering layers in the frequency band from 3 to 20 kc from a ship underway at 10 knots. It would seem well worthwhile for students of the deep scattering layers to take advantage of the intensive development of sparkers for reflection seismology and make these broad spectrum observations continuously while underway. Echo ranging from an instrument suspended at the depth of a scattering layer should yield quick return in monitoring horizontal motion of individuals, but there seems to be little, if any use of this well-known method.

New extensions of these basic techniques can probably be designed in the future greatly to the benefit of this field of research. The most profitable technical advances of this sort will come only from the proper partnership of biologists and physicists.

SCATTERING IN AN OXYGEN STRATIFIED COASTAL WATERWAY. Brian McK. Bary, Dept. of Zoology and Institute of Oceanography, University of British Columbia, Vancouver, B.C., Canada.

Probably the chief problem involved in scattering-layer studies is relating cause and effect. This arises because of the frequent need to work in the open sea, on layers which are deep, where it can be most difficult to sample an adequate spectrum of the organisms present and be sure that they are the effective scatterers. Moreover, the scattering may not be readily or continuously available for study; and finally, there is no certainty that the species' composition of the scatterers will remain the same from one time to the next.

Saanich Inlet, a coastal fjord, is 24 km long, from one-half to 6 km wide, with a mouth 3 km wide. A sill across the mouth is about 75 m deep, but a 16 km reach within the inlet approximates 200 m deep.

The inlet is only five hours by ship from Vancouver, B.C. Conditions are ideal - calm, little current, and night and day sampling can be carried out. Buoys to which the ship can be moored are located in the middle of the scattering area, in 200 m of water.

The area has been visited at approximately two-monthly intervals over the past two years. Stratified horizontal plankton sampling has been carried out by day and night using a closing, high-speed sampler and flights of Clarke-Bumpus samplers. Both samplers meter the flow. Echo-sounder surveys of the whole inlet, and day-night series of observations whilst the ship is moored to the buoys have been made.

The echo-sounder observations were "standardised" at first on the 12 kc EDO plus Precision Depth Recorder (PDR). More recently the Gifft Precision Sonar Tranceiver (PST) plus PDR, or Precision Graphic Recorder (PGR), have been available. As well, 50 kc and 200 kc Furuno, portable sounders, and a 30 kc Kelvin-Hughes sounder (of the Pacific Biological Station) have been used. The several sounders are operated simultaneously when the ship is moored to the buoys; only the 12 and 50 kc are operated on surveys of the inlet.

As far as possible joint cruises have been made by the Institute of Oceanography, University of British Columbia and the Pacific Biological Station, Nanaimo (of the Fisheries Research Board of Canada). The Institute of Oceanography has studied primarily the zooplankton; the Biological Station has studied the fish since its vessels are equipped to handle large mid-water trawls.

Hydrography

Saanich Inlet is unusual hydrographically. Below sill depth, the water is relatively stagnant and an oxygen deficit is common. Concentrations approach zero for much of the year; hydrogen sulphide may be present. There is an oxycline between the low concentrations (1 ml/l or less) in the deeper water and the high

concentrations (5 to 6 ml/l) in the upper waters. The oxycline usually has a steep gradient extending over 20 to 40 m of depth at around 100 m, or it may be less steep and extend over depths of 80 or 100 m or more.

Temperature and salinity of the deeper water approximates 8 to 9°C and 31‰. During the summer, temperatures may rise to 16°C at the surface, and 10° to 11°C at about 50 m. Surface salinity is affected by inflow of fresh water from outside the inlet. Typical values are around 27‰, but may be much less; at 10 m the salinity is about 29‰.

Water from outside the inlet has been observed to intrude into the deep basin. Oxygenated water is introduced and the oxycline then has the shallow gradient. This condition begins in late spring or early summer and continues until about August; the strongly demarcated oxycline re-establishes gradually over this period.

Neither the salinity nor the temperature is considered important in relation to the scattering in Saanich Inlet (except in the upper 10 m or so). The oxycline, however, appears to delimit the day-depth of scattering and the distribution of organisms within the layer.

General features of the scattering.

There are several outstanding features to the scattering in Saanich. First: it is permanent; second: it is associated with fish and plankton; third: there are only a few species of "potential scatterers" and the species' composition is relatively unchanging; and fourth: there is differential vertical migration of species.

The layer has been observed for most months over the past two to three years, and there is reason to believe it was present prior to that. Most often there is a diffuse, layer-like scattering combined with group scattering, but occasionally one or other of these is almost absent. Diffuse scattering is usually centred at 100 to 110 m and is about 40 m thick. Group scattering varies in amount and in its location with respect to the diffuse layer. During the daylight it may be very dense in one part of the inlet, and in another sparse; it may span the diffuse layer, or lie above, within or below it. Although the relationship between the two types of scattering differs from cruise to cruise, it is usually maintained for the duration of a cruise, i.e. two weeks, which indicates some slow-changing factor may be affecting the distribution of the group scatterers. These scatterers largely disperse with onset of darkness but regroup with daylight. Locations of groups within the inlet can be constant over 2-week periods. That group scattering is probably from large fish, either singly or in shoals is suggested from trawling results. Hake (40 cm), whiting (30 cm), Sebastodes (40 cm), dog-fish (75 cm) have been the chief fish collected from it; small fish such as herring (10 to 20 cm) the smelt Leuroglossus stilbius (up to 10 cm) and occasional myctophids have also been collected.

Plankton sampling at the depth of the scattering indicates

that plankton may be associated with diffuse scattering; trawling and other observations indicate the presence in this scattering of young herring, hake or other small fish. Common potential scatterers among planktonic organisms are Euphausia pacifica, and the amphipods Orchomonella pingvis, Cyphocaris challengeri and Parathemisto gracilipes. Other organisms include various copepods, occasional shrimps, decapod larvae and small squid.

The species' composition, and the proportions of the several species vary relatively little during the year. The chief changes lie in the life history stages collected. An influx of other species, or a change in relative numbers of some species may accompany the early summer inflow of outside water, e.g. small squid and Leuroglossis are collected and P. gracilipes is more numerous at this time.

Only part of the layer migrates towards the surface from the day level; part remains at the day depth. Organisms migrating are E.pacifica, C.challengeri and P.gracilipes, copepods, and small fish. Those not migrating - or migrating in low numbers - are Orchomonella, some copepods and shrimps. Some small fish apparently remain deep during the night, or there is interchange between shallow and deep levels. Descent begins at dawn (beginning of twilight) and the ascent ends at dusk (end of twilight). Descent and ascent can be divided into three phases each with characteristic rates of movements. The fastest approximates 1 m/min.

Evidence towards interpreting causes of scattering has been derived from plankton sampling, trawling for fish, multiple sounder traces, underwater TV, a few U-W photographs and effects of lights.

Series of 10-minute, horizontal tows at speeds of 5 to 8 knots, from the surface to below the layer, have been made with closing, and more recently opening-closing, high-speed samplers. Flow is metered after filtering. Biomass (drained wet weight/m^3 of water filtered) ~~are~~ is determined for the common amphipods, euphausiids, and residue; some breakdown to species has been made as well.

The plankton is broadly stratified in the layer - probably a response of species with differing oxygen requirements to the range of concentrations in the oxycline. This is being investigated.

The stratification, broadly is:-

Chaetognaths + copepods —— above and clear of the layer.

C.challengeri)
P.gracilipes) + copepods —— upper edge of layer.

Euphausiids)
Euphausiids + Orchomonella) —— middle of layer.

Orchomonella and shrimp + copepods —— lower edge of layer

The total biomass and the biomass of the euphausiids, amphipods and residue (mostly copepods) collected from the layer are very high. Euphausiids frequently occur in quantities of 1 to 4 or 5 g/m^3 and may amount to 7 or 8 g/m^3. Amphipods rarely are above 1 g/m^3. Translated to numbers the maximal quantities represent 250 euphausiids and 40 to 50 amphipods/m^3. Additional to these are very high numbers of copepods and other organisms.

The distribution of biomass has been compared with the location of the scattering shown by the 12 kc sounder. For several cruises wherever there was high biomass there was diffuse scattering and it began to appear as though plankton was an effective scatterer. Of the chief potential scatterers, E.pacifica undergoes vertical migration in highest numbers and the 50 kc and 200 kc sounders have recorded faint diffuse scattering (as well as scattering from small fish) during the migration. This probably is to be attributed to plankton (chiefly E.pacifica).

However, on several occasions, very high biomass of euphausiids could not be associated with scattering. This has caused doubt as to whether the diffuse scattering is to be attributed to plankton, more especially euphausiids, at all times.

The possibility is present as to whether the diffuse scattering may be caused by small fish. Euphausiids are one of the principal plankton foods in Saanich. Herring, young hake and smelts may aggregate on the plankton, but since the diffuse scattering and euphausiids may not coincide, these fish would not always appear to be aggregating on euphausiids, their main food organism. The small fish often occur at the surface in large numbers at night; they descend at dawn. During descent individual traces are distinguishable to about 60 m, particularly on the 50 kc sounder, but these gradually merge into a diffuse scattering. The 12 kc sounder, more especially when used with the Gifft Transceiver, has recorded individual traces (other than group scattering) from the diffuse scattering. Frequently small fish have been trawled, sometimes in large numbers, when the layer has been fished. But as the trawl is shot and retrieved open it is not certain that these fish were actually collected in the layer.

More direct evidence of the presence of small fish in the diffuse scattering has been obtained with under-water TV and from observing the effects of the TV lights on the behaviour of the layer (using the 12 kc sounder to record effects). The camera was held for 15 to 20 minutes at each of several levels, from the surface downwards, on one night and one day lowering.

Herring, young hake and Leuroglossis (smelt) were observed with the TV camera and were associated with the diffuse scattering, by day and night. When the camera, with the light on, was being lowered, the diffuse scattering moved downward ahead of the light, but this did not happen when the light was off. When the light was turned on in the diffuse layer, the scatterers responded immediately, moving upward and/or downward away from the light. Depth changes were as much as 25 m and could be completed in a half minute, or over a longer time, e.g. 1½ minutes. The reactions were typically those previously observed with a sounder and lights

on shoals of herring at the surface. The rate of movement was such that it is unlikely planktonic organisms were responsible.

It is of interest to note that at levels where fish were being observed, larger plankton was not observed (copepods or other small organisms were difficult to see and may have been present). It seemed that the larger organisms occurred above and below concentrations of fish. This needs confirming. A few underwater photos (EGG camera Model 200 and strobe-light) have shown fish, probably herring.

Only preliminary comparisons of the records from sounders of several frequencies have been made. There are differences in the records. Some of these probably result from the inherent characteristics of the sounders, but some result from the use of the different frequencies.

It is clear that even in the good conditions in Saanich Inlet the difficulties of identifying the effective scatterers persist. Nevertheless it appears possible that the roles of fish and plankton can be determined. The permanency of the scattering, the relatively few species involved, and the fact that a variety of observational techniques can be used, will assist.

THE BIOLOGY OF ABYSSAL ANIMALS

A symposium organized for the
XVI International Congress of Zoology

by

N. B. Marshall
British Museum (Natural History)
London

DEEP SEA BENTHIC INVERTEBRATE COMMUNITIES. Robert H. Parker, Systematics-Ecology Program, Marine Biological Laboratory, Woods Hole, Massachusetts, U.S.A.

Four deep sea benthic communities were studied in subtropical eastern Pacific waters, and a fifth is known from work of Danish and Russian expeditions. The shallowest community (from 200 to 1500 meters) occurs in the northern Gulf of California, in deep isothermal basins. Although maximum depths are abyssal, bottom temperatures are 12° C. None of the living animals have been cited previously from abyssal regions. Many common species found between 300 and 800 meters are either known from neighboring shelf depths, or from shelf waters off California to Alaska.

The middle continental slope from 700 to 1800 meters is characterized by few species and individuals, several which also occur in abyssal depths. Although deposit feeders predominate, many suspension feeders live on falling detritus, resulting from intense upwelling and consequent high surface primary production. Low bottom oxygen concentrations distinguish this environment.

Abyssal borderland basins and outer continental slopes off Middle America are characterized by distinct and prolific faunas. The abundant animals are *Limopsis*, *Dentalium*, pogonophorans and holothurians. Over 110 species of invertebrates have been identified from depths of 1800 to 4122 meters, the majority previously found in abyssal depths.

The California borderland basins, from 1640 to 2360 meters, contain a different fauna from the previously mentioned environment. Most species have been reported as either endemic to this region, or have been found in shelf and upper slope waters from Oregon to Siberia.

The hadal community, found below 7,000 meters, is characterized by small endemic populations, each to its own trench. This and other observations permit speculation that most species have arrived at these depths since Cretaceous times. Most of the world's trenches are considered to be less than 70,000 years old.

Feeding type composition of the communities is presented together with factors which are important in limiting the communities discussed.

REPRODUCTION IN DEEP-SEA FISHES. Giles W. Mead (Museum of Comparative Zoology, Harvard University, Cambridge 38, Massachusetts), E. Bertelson (Marinbiologiste Laboratorium, Charlottenlund Slot, Denmark) and Daniel M. Cohen (Ichthyological Laboratory, Fish and Wildlife Service, U. S. National Museum, Washington).

Information on sexuality, fecundity, fertilization, mode of development and habitat of eggs and larvae among deep-sea (ie. below 2000 meters) fishes remains fragmentary and largely speculative. The present paper is a review of the available data for the major groups of such benthonic and bathypelagic fishes. Published data on certain orders such as the Heteromi and the deep-sea Ogcocephalidae are wholly lacking; the reproductive habits of others such as the deep-living elasmobranchs appear to be little different from those of their inshore relatives, while other groups show reproductive specializations of considerable interest. Among shallow-water marine fishes and those from fresh water, one finds reproductive and larval specializations including protandry, proterogeny and simultaneous hermaphroditism with and without self fertilization, parthenogenesis and gynogenesis; oviparity, ovoviviparity and viviparity; superfoetation; parental care of young; direct and indirect development; demersal and pelagic eggs; and fertilization with and without a spawning migration. Now known or suspected among the deep-sea fishes are examples of spawning migrations, viviparity, hermaphroditism, indirect development, demersal and pelagic eggs and young, and parasitism of males on females (in some ceratioids, a situation unique among vertebrates). Those sexual peculiarities known among shallow water forms but not yet from the deep are those, such as parental care, most difficult if not impossible to determine from deep-sea catches at present.

Reproductive adaptation to deep-sea life is discussed, and a distinction is made between those fishes "fully adapted", in which the young live in the deep-sea habitat of the adult; and those "incompletely adapted," in which the young do not.

SOME OBSERVATIONS ON THE BENTHONIC FAUNA OF THE DEEP-SEA. Howard L. Sanders, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

A continuing quantitative and qualitative benthonic investigation is being carried out along a transect between southern New England and Bermuda at bathyal and abyssal depths. The analysis of 25 samples indicates that each region supports a characteristic number of animals: the outer Continental Shelf, 6,000; the Continental Slope, 1,500-22,000; Abyss north of the Gulf Stream, 250; the Sargasso Sea, 25-100; and the Bermuda Slope, 100-1,000/m^2.

A few of these samples have been analyzed in detail. They revealed a pronounced diversity in the fauna and a lack of dominance by any one species. We believe that this pattern of community structure to be characteristic of the deep-sea benthonic fauna and is the result of both the past history and the physical stability of the environment.

The study of the lamellibranch component of our samples demonstrated that no species shows a ubiquitous distribution and, in fact, each species was confined to a specific portion of the transect. These data suggest that in a single ocean basin, despite the apparent uniformity of the environmental conditions, lamellibranch species are not found throughout the basin but, instead, have restricted and circumscribed distribution patterns. Preliminary distributional data for other deep-sea benthonic groups agree with these findings for the Pelecypoda.

REMARKS ON THE DIVERSITY AND DISTRIBUTION OF DEEP LIVING COPEPODA.

George D. Grice, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

A highly diversified copepod fauna consisting of twelve exclusively or predominately deep-living families, numerous genera, and probably more than 500 species occur in the bathypelagic zooplankton. The distribution and vertical occurrence of the species in oceanic areas and in certain adjacent seas is discussed. There is an increase in species diversity from the surface downwards with maximum numbers occurring in the neighborhood of 1000-2000m in tropical-temperature latitudes of the open ocean. In semi-enclosed areas with shallow oceanic connections the bathypelagic element is reduced and maximum diversity occurs at shallower depths. The small diversity and high percentage of endemic species in the central Arctic basin and in Hadal depths is mentioned and a plea made for a more systematic sampling of the abyssal and hadopelagic plankton by use of closing nets.

ASPECTS OF THE FOOD AND FEEDING HABITS OF ABYSSAL BENTHONTS.

R. J. Menzies, Zoology Dept. and Oceanographic Program, Duke University Marine Laboratory, Beaufort, North Carolina.

Various methods of providing nutrient matter to invertebrate organisms living in the sea at depths in excess of 2000 meters are explored from quantitative considerations. These include "rain of dead plankton", "living vertical migrations", "submarine turbidity currents", "floating terrestrial plant debris", and "floating benthic marine plant debris".

The feeding mechanisms of marine isopoda in the abyss suggest selective and non-selective feeding on sedimentary detritus. Records of plant remains in abyssal invertebrate life are discussed. Quantitative considerations of the crudest sort suggest that hadal life exists independent of living vertical (ladder) migrations as a food source. The radular apparatus of abyssal limpets, chitons, and monoplacophorans appears to have undergone marked reduction in musculature strength and volume suggesting a change in living habits compatible with the ooze substrate of the abyss.

BIOLOGICAL PRODUCTIVITY AT THE HETEROTROPHIC LEVELS

Sponsored by the Commission on Ecology of the International Union for the Conservation of Nature and by the Ecological Society of America

A symposium organized for the XVI International Congress of Zoology

by

Eugene P. Odum
Department of Zoology
University of Georgia
Athens, Georgia

Heterotrophic Productivity in the grazing food chain of terrestrial vegetation. D. A. Crossley, Jr., Radiation Ecology Section, Health Physics Division, Oak Ridge National Laboratory.

Some aspects of productivity in the food chain based on green foliage have been under study for centuries. In such disciplines as animal nutrition and vertebrate metabolism, for example, research has reached a level of refinement exceeding the needs of the field ecologist. The term "grazing food chain" is used here to denote the entire food chain based on green vegetation, and includes insects as well as cattle. Studies of invertebrates have proceeded less rapidly than those of vertebrates, and now constitute a limiting factor in the development of information on heterotrophic productivity in the environment.

Productivity cannot be interpreted separately from other phenomena of the food chain. Standing crops, rates of feeding, total energy intake, and energy utilization for maintenance are all variables which ultimately affect productivity. Few ecological studies have managed to measure most of the necessary variables, although many have measured some of them. Enough information has accumulated on caloric contents of organisms that reasonable assumptions can be made concerning energy content of various types and stages of organisms. By contrast, so little data are available on insect biomasses in ecosystems that magnitudes of present estimates require verification. The development of new techniques for measuring variables associated with productivity, and new applications for older techniques, are essential for such verification.

Radioactive tracers are among the newer tools which are being used to measure phenomena associated with food chains. When animals feed on plants containing certain radioactive tracers, those herbivores reach an equilibrium concentration. At that concentration the daily intake of radioisotope is balanced by daily elimination. By measuring the elimination rate the intake rate, a function of the feeding rate, can be estimated. Predatory mortality in herbivore populations can be estimated from feeding rates for predators. Results obtained with this radioactive tracer technique are similar to those estimated by the more conventional respirometry--calorimetry procedures.

HETEROTROPHIC PRODUCTIVITY IN THE DETRITUS FOOD CHAIN IN SOIL

by
Amyan Macfadyen, Zoology Dept., University College of Swansea.

Even to modern urban man soil is one of the most familiar of biological systems: but it is also to the biologist one of the least understood. Because soil is opaque the life within it is difficult to observe without disturbance, and when soil is disturbed many of its properties are at once radically altered. This difficulty has been partly overcome by techniques in which soil is frozen, impregnated and sectioned to reveal animals and microorganisms in their normal relations to the environment.[3,10] In this way the essential qualitative biological facts for food web construction can be amassed, but such methods, although essential, are very laborious and so far have not proved to be of outstanding value for quantitative work.

The study of soil biology has gone forward in three mutually dependent stages. Firstly, we have had to find out what organisms are present; secondly, how numerous they are and thirdly, what they are doing in the soil. We, in temperate Europe, are fortunate in having a century of taxonomic labour on which to base the first of these stages. It is widely argued, particularly in the U.S.A. that this taxonomic stage can be omitted from the study of soil biology but, whilst in certain particular kinds of work it may be permissible to substitute code numbers for taxa, I believe that this is generally an unwise proceeding. The more we learn about the biology of soil animals the more frequently do we detect pairs of species, hardly distinguishable morphologically but showing extreme ecological divergence. Sweeping generalizations about the biological roles of "the oribatids", "the springtails" or the "sugar fungi" nearly always conceal numerous exceptions and if time and money are not available for the creation of a sound taxonomic basis for such work it seems far better to transfer attention to taxonomic groups in which the basic taxonomy has been worked out.

The problem of quantifying the life in the soil is a particularly intractable one which can only be defined in relation to specific objectives. The ecologist may want to quantify his data for many reasons which are not necessarily related to our present topic of heterotrophic production. He may, for instance, be interested in distribution patterns on a geographical, a topographical or a micro scale and frequently he can be satisfied with relative indices of abundance or frequency. But a metabolic study of soil as a whole demands the exact determination of biological activity rates on the basis of soil surface or soil volume. In the case of bacteria, fungi and other microflora the problem of quantitative sampling remains unsolved and perhaps unsolvable. Fungi of course cannot be numbered in any meaningful sense and in some soils they are responsible for up to 90% of total metabolism. Even were it possible to enumerate microbes accurately the variability of their metabolic activity would greatly reduce the

value of such work. For some purposes it follows that our efforts should be directed towards measurement of different aspects of microbial metabolism in soil, by regarding the microflora as a complex system of living catalysts whose significance in the soil is best measured by biochemical means. This approach does not seem to have been extensively applied by the microbiologists except in the case of total metabolism which I shall discuss below. In general, as one studies progressively larger organisms, so one is confronted with greater diversity of behaviour and habits. It follows that the biological differences between animal species and between their life stages can no longer be ignored. This implies complete enumeration of taxonomically defined species in sample units so arranged as to detect heterogeneity of distribution and correlation with the distribution of other species and of environmental factors. The period since the war has seen a considerable improvement in techniques for enumeration of soil animals; these have been reviewed recently.[1,13,16,17,19] Both in the statistical treatment of data from patchily distributed populations and in procedures for removing animals from soil in a quantitative manner progress has been such that it is now usually possible to obtain reproducible abundance estimates for a given group of soil animals in a given soil.

As a result of estimates of this kind we have a general idea of the orders of magnitudes of the numbers of different groups to be expected in a few soil types (all of them temperate or subarctic). Although none of these studies covers the entire soil fauna the combined work of specialists over a decade has resulted in a relatively complete knowledge of the fauna of the Moor House Reserve in the high Pennine Mountains in England[4]. Even in the sub-arctic and mainly acid conditions of this region (the coldest part of England) more than 350 different species were found and this figure would be augmented by at least 25% if it were to include soil mites, and by a further factor to cover non-testate protozoa. In lowland districts and particularly in woodlands and grassland we really have no reliable estimate for the upper limit of animal species, but it seems certain that the inclusion of Protozoa and Nematoda would bring it into the thousands.

A not un-natural reaction to this state of affairs is one of despair. Certainly the qualitative approach, resulting in the construction of elaborate and inevitably over-simplified food webs is a daunting experience, but if we are to approach our third objective and try to assess what the heterotrophic forms are doing in the soil we need to supplement such work by a different approach.

The layman faced by a faulty television receiver or petrol refinery is naturally daunted by the intricacy of their morphology; the mechanic who services the apparatus has, however, learned to look beyond the details of components and connections and to think of the functional unity which provides the logical key to the system. He uses appropriate measuring instruments

(3)

and by concentrating on amplitudes and flow rates of the primary medium (electric signals or petroleum fractions) and comparing these with designed levels it is possible to locate the source of faults in the system. In a strictly analgous way the ecologist is learning to look for the primary processes and to meter them at defined stages in an eco-system. The soil fauna and microflora is concerned with the breakdown of that plant material which has not been consumed by herbivores, plus the excreta and corpses of the herbivore food chain. All this material was originally derived from inorganic substances by photosynthesis, during which complex, energy-containing food materials are created. The soil heterotrophs as a whole restore the simple substances as plant nutrients and carbon dioxide and liberate the energy in the process. As has been repeatedly pointed out[11] the organic litter produced by plants does not accumulate appreciably in the soil and it disappears after one or only a few seasons. During the year the litter in a deciduous woodland liberates some 6,000,000 calories per m^2, enough energy to feed a man for 2½ days. The simplest criterion by which we can judge the contribution of soil organisms to heterotrophic activity is evidently their rate of energy liberation as measured by their metabolic activity. Metabolic activity is, of course, only one biological property of organisms which is likely to effect breakdown rate, as we shall see later, but it has the great advantage of being a universally measurable property of all organisms regardless of trophic roles, of which we are often ignorant. The metabolic activity of a heterotrophic organism can be expressed by a series of equations, thus:- (block letters signify materials of high energy content)

FOOD INGESTED = { FOOD ASSIMILATED = { FOOD STORED IN TISSUES AND REPRODUCTIVE PRODUCTS; FOOD METABOLISED = { ENERGY LIBERATED + excreted breakdown products } } ; FAECES }

The energy flow through a population can be measured at any arbitrarily defined stage and, since the respiration rate of an animal is only equal to the energy <u>liberated</u> it can be argued that this is not the same as total energy flow through a population. This is strictly true, but in the case of most invertebrates the respiratory metabolism is a large and fairly constant proportion of the energy assimilated, and taken over a whole life cycle it usually amounts to about 90%. The proportion of ingested food which is <u>assimilated</u>, on the other hand, is rather variable and is usually progressively less for carnivores, herbivores and decomposers[1,9,24].

Thus in some contexts it is justifiable to equate respiratory metabolism with energy flow but in others it is not. The two can certainly be equated when the objective is an overall comparison between different ecosystems, between different food chains in the same system and between different trophic levels in the same food

chain, because in all these cases error of 10% or even 50% will not upset the kinds of conclusions which can be drawn. In such studies we are still only beginning to comprehend the orders of magnitude to be expected. On the other hand comparisons of efficiency and energy flew through rival organisms at the same trophic level and calculations based on man-managed and experimental systems demand greater accuracy and in such cases we need to take notice of variations in the partitioning of energy in different species and in the different phases of the life cycle of each species. For example, ingestion, assimilation, storage and metabolism have been measured in harvest spiders (Phalangiida)[24] and shown to be shared differently through the life cycle. Moreover Phalangids and other soil invertebrates have distinct diurnal rhythms and it follows that crude short-term repiration determinations can be highly misleading[15,24].

Although, therefore, there are fields in which metabolic rate determinations must be treated very cautiously, nevertheless we already have some quantitative understanding of communities at the higher levels.

The studies of authors in the U.S.A.[20,21,22,27] have provided basic productivity figures from communities other than soil, which serve as a frame of reference with which the soil conditions can be usefully compared. I have attempted such a comparison, based on temperate grassland exploited by cattle, of total decomposer metabolism in soil with estimates of energy flow through plant and herbivore levels[15,18]. The most conspicuous, and to me the most surprising features of this comparison are, firstly, the large amount of energy which is present at one time, waiting as it were to be exploited by the soil heterotrophs and secondly, the large share of total plant-produced energy, of the order of 75%, for the liberation of which these forms are responsible. With regard to the first point, the existence of large quantities of dissolved organic matter has already been recognised in the case of oceans and lakes[6], of non-living particulate matter in oceans[7] and of möder soils consisting almost entirely of arthropod faeces. However, I think that the implications for the energy and nutrient cycles which these vast accumulations of slowly circulating organic materials represent are not yet widely appreciated. Not only plant waste and detritus but also inefficiently utilised herbivore faeces contribute to these reserves, as has been demonstrated in Calanus[5], cows[18], benthic crustacea[8] and Daphnia[26].

The result of this situation is of course that the overall food chain efficiency (when expressed as ratio of energy present in stock to energy available in food) of the soil decomposers is low, or in other words, that there is always a superabundance of organic matter. The commonsense reason for this is surely that detritus is usually a lot less digestible than fresh green matter; the cellulose and lignin contents as well as the Carbon:Nitrogen ratios are usually high and breakdown demands the specialised biochemical properties of microorganisms. The microorganisms in their turn lack organisational complexities which ensure the main-

tenance of a constant internal environment (in the sense of Claude Bernard) and so are largely dependent on the mechanical and other activities of soil animals for establishment and growth. The overall activity of the soil decomposers is therefore dependent on strictly biological inter-reactions of the constituent organisms rather than on simple metabolic considerations.

As a first approach to an understanding of the roles of soil decomposers an analysis of total metabolism is obviously desirable. Unfortunately, even this is difficult. Field measurements of total carbon dioxide output from soil, based on absorption by a gas train[29] do not seem to equate with likely annual total organic matter supplies, perhaps because ventilation changes or soil disturbance lead to excessive figures. Measurements of respiration of soil cores in the laboratory also indicate greatly enhanced microbial activity generally attributed to disturbance, increased aeration and possibly destruction of antibiotic agents[23]. The only available approach seems to be to estimate total energy supplies through litter and detritus, to subtract the known animal metabolism and to attribute the remainder of the metabolism to microbes. If this is done it seems clear that microbial metabolism is much lower than would be expected from population estimates but still accounts for about 90% of the total decomposer metabolism [15]. If these estimates are correct and if it is true that a good deal of potential microbial activity in soil is not realised, then one can appreciate the practical significance of some remarkable experiments which showed that both larger and smaller arthropods can greatly increase the metabolism of soil fungi on the one hand and their reproductive activity on the other[28,30]. The passage of dormant stages through the gut can promote germination, the carriage of spores inside and outside the body can lead to the spread of fungus along the tracks formed in sterile soil by mites and springtails. A number of different mechanisms may be involved here, not excluding breakdown of antibiotic effects and even such simple causes of resting body germination as deprivation of oxygen[12]. Certainly also the grazing activities of small animals must promote microbial activity by liberating nutrients from senescent colonies and replacing these by young active ones, and perhaps even endowing them with gratuitous fertilizers. Finally, of course, the tunnelling activities of earthworms, crustacea and millipedes have a decisive effect on microbial activity. By transporting fresh litter from the surface to deeper regions such forms increase the heterogeneity of soil and constantly provide fresh loci for the activities of soil microbes. The importance of these larger animals has been demonstrated by the results of repeated spraying of an apple orchard with copper[25]. The elimination of earthworms resulted in the development of an acid mor profile in which plant litter accumulated and decomposition by microbes changed to the fungus-dominated type.

At first sight it may appear that my simple vision of the soil as a chemical plant, whose complexity might be penetrated by measurements of energy and nutrient flow, has run into the sand of biological complexity. I want to urge that there is no need for

our understanding to terminate at this stage. The mere fact that the full expression of the metabolic activity of microbes and of animals are interdependent and particularly that the activity of the former appears to be highly dependent on all kinds of "incidental" biological properties of the latter does not prevent our measuring such effects in terms of enhanced respiration rates of soil as a whole. We need much more detailed information on the potential and on the actual activities of soil decomposer organisms of all kinds and particularly we need to devise a series of experiments to bridge the gap between laboratory and field conditions in which the "catalytic" effects of one group of organisms on the other can be measured. The resources of many chemical and physical fields will be required for a study of this kind and it follows that our attitude must at times resemble that of the biochemist investigating a higher organism. But without the appreciation of the biological peculiarities of the component animals we shall be repeatedly faced with metabolic bottlenecks which, from the point of view of the system as a whole, are incomprehensible. In terms of research effort a study of this kind sounds like a tall order; certainly, it represents a considerable challenge to the ingenuity of biologists, but I would suggest that soil science has for far too long been occupied with physical and chemical aspects which are only remotely concerned with the essential properties of soil and that in an increasingly hungry world we now need a knowledge of the immediate factors responsible for soil fertility and plant growth.

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IMPORTED ORGANIC MATTER AND SECONDARY PRODUCTIVITY IN STREAMS

by H.B.N.Hynes (University of Liverpool, England.)

We are moving into an era when ecologists are looking more closely at the productivity of biotic communities, and undoubtedly the International Biological Programme will give great impetus to these studies. In most environments it is obvious that productivity begins with green plants; I want here to show that at least in many streams and rivers much of the initial production occurs not in the water itself but on the land around it. In this respect running water differs from other fairly well-lighted environments, and to some extent resembles dark areas like the bottoms of lakes and the deeper parts of the sea. This suggestion has been made before, e.g. by Scott (1958), and Ivlev and Ivassik (1961) to quote only two recent papers, but it seems worth reiterating, as some modern work does tend to stress the importance of primary production in running water (Odum, 1956).

Some primary production does, of course, occur in rivers, and Odum has shown that streams which are recovering from organic pollution may be among the most productive areas on earth. Moreover, Whitford (1960) found that there are several species of alga which have an inherent demand for current, and others which grow better in running water. This he attributes to the continuous removal of the 'diffusion shell' which represents a barrier to the ready uptake of nutrients. For this reason running water is physiologically richer than still water which contains the same amounts of nutrients; a fact which can be readily seen in the field where, in shallow water, the densest growths are always where the current is swiftest.

Despite this potential fertility, one of the most striking things about streams and rivers is that many support very little vegetation during most of the year. Stony streams have often only a trace of algae, and rivers are often turbid, or deep, with clear sandy or muddy bottoms. Exceptions do occur, such as stable rapids which are often covered with mosses; this seems to be related to the carbon dioxide content of the water, and cannot represent much actual production, as such areas are limited in extent and mosses are notoriously slow-growing. Occasionally there are algal blooms, which may be planktonic, and are thus largely confined to parts of rivers which are artificially impounded, e.g. the Thames and the Ohio; or the blooms may be attached, consisting of diatoms and filamentous algae. The latter are, however, essentially unstable and readily, and often soon, swept away by high water (Butcher, 1946). They thus represent no steady source of primary production, except in the few areas where there are seasons during which no spates occur, or in reaches where the water level is unusually constant. Similarly higher plants produce complete cover on river beds only where the water is shallow and stable and the plants are not much subject to wash-out. Such situations are relatively rare, but when they do occur production may be quite high; Edwards and Owens (1960) report annual productions up to 520 g./sq.m. dry wt. in an English chalk stream. More usually, however, higher plants are absent or occupy restricted areas, and are much subject to wash-out (Butcher, 1933). For instance, in the lower reaches of the Siberian River Angara, which

seems to have rather exceptionally large amounts of plants, only 2.6% of the bed is covered by vegetation, and even there the production (1.2 tons/ha. dry wt.) is not particularly high when judged by terrestrial standards (Greze, 1953).

The only other possible source of primary production is phytoplankton, but this is known to be absent from most small streams and rivers except below lakes, and even there it disappears rapidly (Chandler, 1937). It seems to occur as a regular feature in large and long rivers such as the Volga (Behning, 1929), and there it is possible that it does play a significant role in the food nexe. More generally, however, it is sparse or irregular, and only becomes dense when the water is impounded, e.g. the Nile (Brook and Rzòska, 1954), or the Ohio, which before human interference is known to have had clear water. In many naturally turbid rivers, lack of light alone must prevent much photosynthesis. Yet, despite this lack of considerable and sustained primary production, streams are always full of animals, and even turbid rivers like the Missouri teem with fishes. We must therefore look to the land surface for the source of at least much of the basic production. Indeed, it has long been known that fish productivity in small water-courses is correlated with the type of landscape, and Badcock (1953) found that heavily wooded streams in Sweden had more invertebrates than very similar, but relatively open, streams in Wales. Much earlier, Jewell (1927) noted that streams on the Kansas prairies, which received very little organic debris from the land, were very poor as compared with streams in other areas.

If one tries to imagine the condition of running waters before man had drastically altered them, it becomes obvious that animal communities which relied primarily, or even very largely, on home-grown organic matter could hardly have evolved. Nearly all waters must have been bordered by tall trees, and even those above the tree-line or in very dry areas must have been fringed with relatively dense vegetation because of the shelter and water provided by the valleys. Inevitably therefore all but the central parts of wide rivers were shaded during the growing season, and it is hardly surprising that few plants were evolved which could withstand both the rigours of running water and the lack of light. In this respect it is noticeable that some of the most characteristic stream plants are mosses and Rhodophyceae, both groups which are adapted to low light intensities. It is also significant that periphyton blooms are, in temperate climates, often confinded to the early part of the spring, before they would under natural circumstances be shaded out by riparian trees. Warren et al. (1960) have shown that such algae need 3.2% of daylight in Oregon to pass the compensation point, and they suggest that this is why they are in summer largely confined to exposed riffles. One can indeed readily observe this in streams recovering from pollution, where dense growths of Cladophora or Ulothrix fade out where the water passes under trees.

We must conclude therefore, that debris from terrestrial plants is a major part of the base of the food nexe in running water, and the fact that alders (Alnus), which are very common riparian trees, are nitrogen-fixers may be a significant factor in productivity. Their autumn leaves contain about four times as

much nitrogen as those from other trees (Goldman 1961). I had assumed that autumn leaves would be a poor source of protein, and had thought that its production must depend very largely on bacteria. That protein becomes available somehow is evident from the ease with which many stream invertebrates can be reared on a diet of such leaves. I was surprised therefore when Dr.D.Boulter, of Liverpool University, who at my request analysed a sample of fallen elm leaves, showed that they contained 7.5% air-dry wt. of extractable protein. Possibly alder leaves contain even more.

Let us now examine the zoological implications, and see how far faunal studies bear out the above considerations. Illies (1961) has divided running waters into two categories, which are best considered separately. In rhithron reaches the bottom is rock or stony with gravel, the oxygen content always high, the flow turbulent, and the summer temperature relatively low. In potamon reaches the bottom is sand or mud, the oxygen content may be low at times, flow tends to be laminar and the summer temperature is relatively high. This distinction between 'stream' and 'river' habitats has considerable biological validity, and avoids the terminological problems associated with small, warm, lowland, river-like streams and swift, cool, turbulent, stream-like rivers.

Rhithron habitats usually support a rich fauna of animals even where the stones are almost clear of algae at most seasons. Standing stock is, of course, no indication of production , but Allen's (1951) careful study of trout production in a New Zealand stream showed that the fish alone consumed seventeen times the standing stock of invertebrates. We now know this to be a considerable over-estimate; Hynes (1961) and others have shown that it should, on the basis of the mesh-size used for invertebrate samples, be reduced considerably, and Gerking (1962) showed that there are physiological reasons for further reduction. Even so it seems that the fish alone eat several times the standing stock, probably something of the order of 6 or 7 times as judged by fish-hatchery findings. Fishery production estimates vary considerably, but they are mostly of the order of tens of kg./ha., and this means probably hundreds of kg. of invertebrates, and tons/ha. of primary foodstuffs - all apparently based on very little standing stock of plants. McConnell and Sigler (1959) suggest that in the Logan River, Utah, contemporaneous photosynthesis must account for much of the production of invertebrates, as so much algal growth is lost by attrition; but if this were so the very small amount of plant growth would have to be extraordinarily productive, and one would expect the winter to be virtually without biological activity. As I shall show this is very far from being the truth.

Most of the invertebrate biomass in the rhithron consists of Plecoptera, Ephemeroptera, Trichoptera, Diptera, Isopoda, Amphipoda and Decapoda; and, apart from the carnivores, the great majority of species feed on detritus. This term includes fallen leaves, decaying plants and even wood, which is the chief food of some stoneflies such as Pteronarcys and many Trichoptera. Within each group there are a few species which are algal scrapers, such as Brachyptera among the stoneflies, and the Glossosomatinae among the Trichoptera, but the majority eat at least a great deal of non-living organic matter, and this applies even to the large crayfishes and crabs. The most abundant net-spinning caddis worms,

the Hydropsychidae, which may be so numerous as to cause nuisance as adults, or block hydro-electric tunnels with their nets, are also indiscriminate eaters of anything they catch, as apparently are the ecologically important Simuliidae. Indeed these two groups increase enormously in areas where there is an increase of organic matter in the water. Müller (1956) calculated that Hydropsyche produced 2620 sq.m. of filter/ha. in a stream below a Swedish lake, which largely explains why so little plankton persists in the rhithron. Even some rhithron fishes feed directly on detritus, e.g. Campostoma anomalum and Hypentilium nigricans.

Exactly what is digested by detritus feeders is not known. Ivlev (1945) suggests that it may be micro-organisms, but in view of the apparently high protein-content of fallen leaves, it is possible that much feeding is direct. Nevertheless, Ivlev showed that it was possible to rear Chironomid larvae on filter paper together with suitable bacteria, and Fredeen (1960) reared Simulium entirely on bacteria. Almost certainly therefore, micro-organisms play an important role: how important will doubtless have marked consequences in future research, as it is bound to affect efficiencies of conversion.

How great a quantity of leaves etc. enters a stream is readily apparent in the autumn, when one can actually see tons/ha. all at once, and it is clear that more must blow in throughout the winter. It is less obvious in the tropics, but even there, in Central Africa and the West Indies, I have been struck by the large amounts of plant debris which are to be found in clean stream beds.

The seasonal nature of autumn leaf fall presents the possibility of investigating its significance, which a study of a small trout stream in Wales (Hynes, 1961) will serve to illustrate. It was possible to identify to species a large proportion of the fauna and to work out their life histories. Also, on the basis of abundance, size etc. each species was allotted "index" units which were related to its total biomass. There is not space here to go into details of how this was done; it is sufficient to say that the method was, although arbitrary, quite objective, and was a summation of the percentage compositions of several sets of samples taken at all seasons with both fine and coarse-meshed nets. A total of 1063 index units made up the entire fauna, which could be divided into "herbivores" (941) and carnivores (122), and also into species growing mainly in winter, (553.5), species growing in summer, (203.5), and species for which no seasonal cycle was apparent (306). This last category includes many individual species which were present at all seasons, either because they live longer than a year (Perla) or because several successive generations were not distinguished: it also includes some groups, e.g. Orthocladiinae (210), in which the species could not be identified. Undoubtedly therefore some at least of the units put in the last category belong in one of the others, and this point has to be considered in our analysis.

Considering first the ratio of "winter herbivores" (487.5) to "summer herbivores" (188.5) we get a figure of 2.59, and even if we add all the non-seasonal herbivores (265) to the latter the ratio is still more than 1. Similarly, with the carnivores the

ratio is 66/15 = 4.4, and the second ratio 66/56 = 1.18. This shows therefore that the winter activity in the stream, based almost entirely on allochthonous material, was at least as great and probably twice as great, as the activity in summer, the only period when algal production could have played a significant role. A rather similar picture of much biomass in winter and relatively little in summer is given by Gaufin (1959) for the Provo River, Utah.

We can conclude therefore that the rhithron very often depends to a large extent on allochthonous organic matter, and that possibly it did so even more in the past. Unfortunately inflow of such material is difficult to measure as it involves leaves, sticks, detritus, fine particles and dissolved matter, which all vary with season and rate of flow. Nelson & Scott (1962) have, however, attempted to do this for a small rocky outcrop in a stream in Georgia, and it is significant that even where plant-covered rock provid ed an exceptionally productive localised area, allochthonous material was important. They also showed that dissolved organic matter occurred in large amounts. Very probably this supplies food for bacteria and so also becomes available to stream animals, e.g. Simulium.

The potamon reaches of running water have been much less studied, and they remain a challenge to freshwater biologists, but it is well known that they can be highly productive of fish. The substratum is usually subject to very severe scour and redeposition and so is largely unsuitable for attached plant growth. Plankton also seems often to be unimportant, as has already been discussed. The benthic fauna tends to be very unevenly spread, there being very little in sandy areas, and Mikulski (1961) has found that in the Vistula many of the communities are ephemeral, developing rapidly after spates. Several other studies, in Illinois (Gersbacher, 1937), on the Volga (Behning, 1924) and on the Missouri (Berner 1951), have shown that the fauna consists primarily of Chironominae, Tubificidae, Sphaeriidae, Unionidae, Dreissenia, Prosobranchia and burrowing Ephemeroptera, and often Amphipoda and Isopoda. These are mostly detritus feeders, and it seems very probable that the Pelecypoda, which often form a very large proportion of the biomass, also feed either directly on particulate organic matter, or on bacteria in the water. Unlike the rhithron there does not appear to be any very marked seasonal cycle in temperate climates other than that imposed by seasonal spating and the summertime emergence of insects. The winter-growing insects of the rhithron are not represented, and most of the biomass is composed of groups which are present at all seasons. This is, however, perhaps not surprising as organic matter is brought down continuously from higher reaches and the seasonal cycle is therefore largely smoothed out. It is therefore less easy to demonstrate the dependence of the animal community on allochthonous material, but it is my belief that detailed study will show it to be at least as great as, if not greater than, that of the rhithron. Here not only do most of the invertebrates depend directly on detritus, but many ecologically important fish species feed directly on dead vegetable matter. Such are Dorosoma cepedianum, Rutilus rutilus, Chondrostoma nasus, and Ictalurus lacustris.

I conclude therefore that at least a very large part of the productivity of all running waters is based on photosynthesis which takes place elsewhere, and that without this source of allochthonous organic matter the inherent instability of running water and its consequent inability to grow much in the way of plants, except in specially favoured places, would cause it to be almost a desert habitat.

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Marine Fish Production: A World-Wide View

C.P. Idyll (University of Miami, Miami, Florida)

In assessing the contribution of the ocean to the world food supply the figures are not impressive. It is estimated that the proportion of human food which comes from the seas is only about 10%. Further, while the sea is sometimes regarded as the most likely source of the increased supplies of food required by rapidly increasing human populations, most experts are pessimistic about any substantial increase. The common opinion is that we can expect only about a doubling of present world catches. It is likely, however, that the figures given for marine fish production are too low. It may well be, also, that the sea will eventually yield considerably more than twice the amount of fish and other food products that it now does.

It would be of interest to estimate how much of primary production in the sea ends at or near the apex of the pyramid, as carnivorous fishes. Any assessment of the total quantity of living material at the highest trophic level in even a part of the ocean would require much more information than is presently available, and such an attempt would not be useful at the present state of our knowledge. In a small number of cases, some of which are cited below, the size of the catchable sizes in fish populations has been estimated, and in the future we should be able to increase the number of such estimates, as well as to improve their accuracy. Meanwhile, we can perhaps approach more closely the actual quantity of fish caught by the fishermen of the world, and put the sea in a truer perspective as a contributer to the world food supply compared with the land.

World landings of fish and other marine products from 1947 to 1960, as compiled by the Food and Agriculture Organization of the United Nations, have risen from 17.94 to 41.16 million metric tons. These figures include landings of fishes, crustaceans, molluscs, marine mammals and marine plants. In order to restrict the production approximately to one trophic level all of these catagories except the fishes should be excluded. In addition, landings of freshwater fishes should be subtracted. With these corrections, world landings of fishes in 1961 are reported at 32.89 million metric tons, 79.9% of the unadjusted total.

The figures for fish production compiled by FAO are derived from statistics submitted by national governments. To the extent that the landing figures of the individual governments are incomplete or in error, the FAO total will also be wrong. It is likely that the estimates will always be low. To support this contention, the United States may be used as an example.

The system of collecting fishery production statistics in this country is highly organized and relatively efficient. Despite this it is estimated that landings for the United States as a whole should be increased by at least 10 per cent, or some 515 million pounds in 1961, to approach true commercial fish landings.

In addition to the above error, caused by fish landed but not reported, in the United States there are large quantities caught but not landed. These are discarded, being too small, too expensive to handle, or unsaleable for various other reasons. The U. S. Bureau of Commercial Fisheries estimates that 1.168 billion pounds of fish came in this catagory in 1961 (Power, personal communication). This figure is 23.7% of the total landings for the USA in 1961. The billion-plus pounds caught but not landed include a very high proportion of edible fish, fish that would be consumed readily in many other parts of the world. herein lies one hope for greatly increasing supplies of food from the sea, as economic means are developed for handling presently unused species.

In the United States there are large quantities of marine fishes caught by sport fishermen. The U. S. Bureau of Census has estimated that 1,410,952,000 pounds of fish were caught in 1960 by 6,198,000 anglers. Commercial landings cited are for 1961, and sport landings for 1960, but if we can assume then the size of the sport catch was similar in 1960 and 1961, it represented about 27.4% of the reported commercial catch. Adding to the 5.155 billion pounds of reported catch in the United States in 1961 the quantities landed but not reported, caught but not landed, and caught by sport fishermen, the total catch of marine fish in the U. S. A. becomes 8.249 billion pounds, 60% more than the reported total.

There is no trustworthy basis for estimating the accuracy of reported fish landings for the rest of the countries of the world. The production of the fisheries is probably underestimated in nearly all cases, and in many cases very greatly so. Over much of the world, especially in Asia, Africa and South America, there are large numbers of subsistence fisheries, engaged in by very large numbers of fishermen. These fisheries sustain the protein intake of millions of people. For example, the quantities of fish reported to be landed in the Caribbean are very small (70 thousand metric tons in 1961). But an active fishery takes place in all the Caribbean countries. In these countries either only the most rudimentary system of collecting fishery statistics exists or there is no system, and the landing figures are commonly guesses by an official at a desk in the capital. Production from subsistence fisheries in Africa may

be far from completely reported. For example, Vernon Brock of the U. S. Bureau of Commercial Fisheries in a personal communication cites the case of a small stretch of beach some two miles in length north of Dakar, where an estimated 8000 metric tons of fish are landed a year by canoe fishermen. This and the production of innumerable other similar fisheries may or may not be recorded. In Asia, the Indian fish production is all estimated, and is probably very low. Even in Japan with exceedingly highly developed and controlled fisheries, the full production may fail to be recorded. The catches of Japanese tuna boats may be underestimated, since they are extrapolated from production of what may be a biased sample of boats rather than from records of the companies. The sharp increase reported in recent years has been attributed in part to better collection of data. On the contrary, it seems likely that the increase may in fact be even larger than reported, since the spread of vessels over the world oceans makes collection of data greatly more difficult.

Thus, the total amount of fish over the world which is caught but is not reported is probably very substantial. To attempt to estimate the size of the error over the world is clearly risky. Nonetheless, it is suggested that it is not less than 10 per cent and may be 35% or more, world wide.

But even if total marine landings are still inaccurate, a good start has been made. Furthermore, in some intensively studied fisheries the total landing figures are highly accurate, and this permits estimates to be made of fish population sizes. Estimates of the size of animal populations in the sea are exceedingly difficult to make because of the inadequacy of sampling techniques. It may be that we have a better chance of achieving trustworthy figures for the biomass of fish populations than of populations lower in the ecological pyramid. This is because the spur of the necessity of managing fish populations of economic importance has encouraged the development of techniques for estimating the size of fish stocks, and comparatively accurate assessments of the size of some have been made. The fishery population dynamicist has a considerable advantage over most of his colleagues in the possession of a massive sampling system, the fishery. Some examples may be given of estimates made already, which will serve to show what may be expected in the future.

The sardines of the eastern Pacific (Sardinops caerulea) formerly supported the biggest fishery in the United States. Its importance caused the species to be extensively studied, and the dramatic decline of the fishery during the 1940's and 1950's spurred the research effort even more, consequently, far more is known about the biology of the sardine, and associated animals, and about the marine environment they occupy than about most

other fishes over the world. Among the data gathered are estimates of the total size of the sardine population over several years. The average population of California during the period 1936-37 to 1943-44, based on tagging data, ranged from 9 billion to 16 billion fish. The first figure is certainly too low according to Ahlstrom (Proc. World Sc. Meeting on the Biology of Sardines and Related Spp., 1960).

The North Pacific halibut fishery, engaged in by United States and Canadian fishermen since the 1880's, is a well known example of successful fishery management. The halibut stocks off the coasts of western Canada and Alaska have been the object of intensive study by the International Pacific Halibut Commission for more than 30 years. In this period scientists of the Commission have accumulated enough statistical and biological information about the halibut to enable them to make estimates of the size of fishable stocks. The data necessary were accurate measures of commercial landings, fishing effort in units of gear, growth and mortality rates of the fish. Estimates have been made of the "initial stock size" for the southern halibut stocks. These estimates are for catchable sizes, which in this case are fish mostly over 5 years old. In the period 1950 to 1960 initial stock size varied from 98.0 million pounds to 149.5 million pounds, with an average of 121.8 million pounds (Chapman, et al, Rep't. Int. Pac. Halibut Comm. #31, 1962).

An ancient fishery has been carried out for cod in the North Atlantic Ocean, both in Europe and America. These populations have been extensively studied over many years and among the information available are estimates of total commercial landings and of total annual mortality. According to Wise, (FAO Fish. Biol. Sym. #21, 1961) "A conservative estimate of the biological production of cod may be made by considering the approximately 1 thousand million cod landed each year in recent years and assuming a 50 per cent total annual mortality. Thus about 2 thousand million catchable cod are present in the seas on known fishing grounds each year."

In addition to the magnitude of the exploited populations of fish, it is necessary to have an estimate of the size of the population of young stages before they become available to the commercial fishing gear if a total population size is to be made. Even for a species as well studied as those cited above we are on very unsure ground. For the pre-recruit population of cod, Wise estimates, in a personal communication that there are "over 6×10^{11} pounds of cod of year class 0. This estimate could be either halved or doubled, or even vary by orders of magnitude, with slightly different assumptions. But since it represents in its present form about 100 times the weight of the total catch

even though only one of the 4 to 6 pre-recruit classes the figures become rather meaningless." Wise concedes that his assumptions may be wrong and that his estimate may be too high. In that case the quantity representing the biomass of pre-recruited cod would also be too high. A similar uncertainty about the pre-recruit populations exists for the halibut and sardine populations. In the latter, since recruitment is at an early age (fish in their first and second years), the bulk of the stock before it enters the fishery may perhaps not be as large in relation to the fishable stock as in the case of the cod. For the halibut stock Heward Bell, Assistant Director of the International Pacific Halibut Commission says in a personal communication "So many and some unsafe assumptions had to be made concerning the weight of the pre-recruited stock that we would hesitate at this time to make even a best estimate."

In addition to the three examples given, there are some other fish populations for which estimates of biomass of at least the catchable stock have been made. These include the yellowfin tuna stocks of the eastern Pacific, some flatfish and clupeoid stocks of Europe as well as others. While the aggregate of all of these embraces a small fraction of the fish populations of the sea, and covers only a part of the life history, nonetheless it has been shown to be possible to make good estimates of parts of the biomass at this high trophic level. For certain areas of the sea it is conceivable that the productivity at this level can be estimated with moderate accuracy, the precision of which will increase as technique of fishery population dynamics improve.

Studies of the productivity of marine waters in terms of fish have been conducted most intensively in high latitudes, particularly in the northern hemisphere. In comparatively recent times attention has been directed strongly to tropical waters. It has been the consesus that tropical waters were fundamentally poorer in productivity, since many indexes (phytoplankton, zooplankton, chemical content, fish populations) gave lower values there. Then attention was directed to turnover rates, in contrast to standing crop values. Because of greater light penetration in middle latitudes there is a deeper column of water producing primary plants, and this, along with the possibility of greater growth rates, protracted reproductive periods, increase numbers of generations, and other reactions of organisms to the environment, the differences between high latitude and middle latitude waters appear to be much less than former estimates. It appears, nonetheless, that the production of tropical waters at high tropic levels is less than the production of cold waters. It is possible that this results more from the pattern of production of nutrients than from their total production. Phytoplankton is being produced continuously, or nearly so, in middle latitude waters. At the same time, these

plankters are being cropped continuously by herbivores, since conditions are favorable for their activity over all or nearly all the year. Consequently the phytoplankton is not able to build up a surplus, as it does in high latitudes. The great blooms of diatoms in cold waters are followed by similar spurts of production of herviores, and these in turn by other predators, up to the fishes. The massing of the fishes in time and space provides favorable conditions for commercial fisheries, which depend on heavy concentrations for economic operation. There are few if any such "jerks" of productivity in middle latitude waters, but instead the fish production is thinly spread, to the disadvantage of mankind seeking fish to catch.

A massing of forage plants might take place in the sea if the herbivores interrupted their activities for a suitable period. If this could be induced artificially it is conceivable that a buildup of organisms at various levels, including useful fishes, could result. Very low concentrations of toxins specific for crustaceans and perhaps other herbivores could possibly be employed to accomplish this temporarily. It is open to doubt whether this interference with the marine environment would be desirable even if it were feasible, but it might offer a means of increasing the production of areas of mid-latitude waters which now yield comparatively little human food.

The efficiency of energy transfers in the sea is a problem of considerable theoretical and practical interest. Usually a value of 10% is given for the efficiency of transfer of living substance from one trophic level to the next, with three to five steps usually assumed from plant to man. Thus Sverdrup (FAO Fish. Bull. 1952 6) states that consumers retain for growth 1/10 to 1/20 of their food. Weiss (J. Ag. & Food Chem., 1953, 1) considers five steps necessary in the process through codfish to man, using 10% at each level, or a 100,000 to 1 ratio. Sverdrup, Johnson and Fleming (The Oceans, 1942), beginning with eelgrass, use the 10 times ratio and arrive at cod in three or four stages.

The 10% figure for transfer has been used by many other authors. It seems to be derived from experience with warm-blooded animals. It may represent a rough average figure for mammals and birds that the variation is great, ranging at least from as low as 3% for lamb to as high as 25% for pork (Maynard, J. Nutrition, 1946: 32). It seems certain that cold blooded animals would exhibit a higher efficiency. This may be considerably higher than 10%, and research on this question would be of great interest.

PRIMARY AND SECONDARY ENERGY FLOW IN RELATION TO ECOSYSTEM STRUCTURE. Eugene P. Odum, Dept. of Zoology, University of Georgia, Athens, Georgia, U.S.A.

Heterotrophic utilization of net primary production is examined in five ecosystems that represent contrasting types in the biosphere as follows: (1) A grassland or pasture containing large mammalian grazers. (2) An open water plankton ecosystem. (3) A monophytic stand of the forb Lespedeza cuneata. (4) A tidal Spartina alterniflora salt marsh. (5) A forest. Examples (3) and (4) have been the object of intensive study in the University of Georgia ecological program, especially by E. F. Menhinick, A. E. Smalley, and E. J. Kuenzler; data on the other ecosystems are from the literature. All of the examples selected for comparison exibit the same order of magnitude of annual net primary production (2,000-4,000 kcal/M^2/year) but differ greatly in biological structure and in the manner in which net production is consumed in the community. The energy flow patterns of each of the five ecosystems are presented as field models useful in evaluating how communities adapt to the physical environment.

In the first two ecosystem types a major portion of net production is utilized in what may be termed the grazing food chain. That is, a considerable portion of net production is consumed by animals that eat the living plants, and, in turn, a considerable portion of the growth of the grazers is consumed by predators and parasites that feed upon the living primary consumers. In contrast, most of the energy flow in the remaining three ecosystems listed above follows what may be called the detritus food chain in that most consumption of the net production is delayed until the plants are dead. The term detritus is defined for our purposes as particulate organic matter originating from the dead bodies of plants and animals or from non-living fragments or excretions shed by living organisms. Phytodetritus, or plant detritus, is thus the major energy source for heterotrophic organisms in the Lespedeza, Spartina and forest ecosystems. In aquatic ecosystems phytodetritus is mostly in the form of small particles since bodies of producers are small and rapidly decomposed; on land detritus ranges from tiny particles in the soil to fallen logs. It should be further emphasized that detritus, although originating from dead organic material, is not in itself a dead component since microorganisms are intimately associated with the particles. Better means of determining the ratio of living to dead material in detritus as well as a more accurate means of estimating the relative importance of each component as food energy for detritus feeding animals are needed before energy flow in the detritus food chain can be fully understood. Finally, it should be emphasized that detritus is important not only as a potential energy source but

also as an environmental factor that modifies light, temperature, moisture, and nutrient cycles in the ecosystem.

The estimated per cent of net production grazed in each of the five field models is as follows: (1) 40%, (2) 60%, (3) 1%, (4) 2%, (5) 8%. On this basis we may designate (1) and (2) as "grazing type ecosystems" and (3) to (5) as "detritus type ecosystems". Net production not grazed is consumed in the detritus food chain as described above, or stored in the community. In early successional stages a considerable portion is stored, while in energetically steady state ecosystems (one type of "climax") the detritus is consumed annually at the same rate as produced. Grazing ecosystems have a relatively small standing crop since autotrophs (grass and phytoplankton) are eaten as fast as they grow. As a result the community does not appreciably modify or control the physical factors of the environment although the community metabolism may regulate certain aspects of the chemistry of the environment. In some grazing ecosystems, as, for example, shallow water marine bays, animal biomass may be greater than plant biomass resulting in an inverted biomass pyramid; in such cases benthic animals (such as shellfish) rather than plants provide most of the permanent biological structure in the community. In contrast, the standing crop of plants and organic matter in the detritus type of system tends to be very large, the mesic forest being the extreme case. As a result the community is able to control the physical environment to a considerable extent. In general terms we may say that the habitat in a forest (or detritus systems in general) is strongly biological while the habitat in the sea (or grazing systems in general) is largely physical.

In many terrestrial ecosystems a distinct food chain intermediate between the grazing and detritus energy flow routes starts with seeds, fruit and nuts. Such a granivorous food chain seems to be absent in most aquatic ecosystems. In the Lespedeza stand about 5 per cent of annual net production goes into seeds. Because of its high nutritive value this component of primary production is rapidly consumed by animals such as small mammals, birds, and insects, many species of which depend on this energy source at certain seasons. As a result of selection by man the percentage of net production going into seeds is considerably greater in cultivated grain crops.

To obtain further insight into the detritus type of ecosystem details of the studies on Lespedeza and Spartina ecosystems are presented. Despite the fact that all the primary energy is fixed by a single species of autotroph with but a small part of net production utilized in the grazing food chain, the diversity of species in this food chain is quite remarkable. In a 400 M^2 area of the Lespedeza stand, for example, about

100 species of grazing insects were found at any time the area was sampled during the summer months. The ratio of cumulative number of species/square root of cumulative number of individuals (a diversity index found suitable for type of samples taken) was about 2 in Lespedeza and less than 1 in Spartina, the low diversity in the latter presumedly is probably due to the limiting effect of tidal action. In both monophytic communities the number of species of arthropod predators and omnivores (including ants, parasitic wasps and spiders) was about 1/5 that of phytophagous arthropods. The salt marsh ecosystem is especially interesting because almost all organisms in the grazing food chain are of terrestrial origin, which most species of heterotrophs functioning in the detritus food chain are of marine origin.

Despite the large diversity of herbivores able to feed on a variety of plant parts and plant products in the Lespedeza ecosystem, the grazing rate was very low, as previous indicated. In addition to weather, which is clearly limiting in winter, spring and fall, we have been impressed with the following three mechanisms that seem to play a role in effectively controlling grazing during the favorable growing season in this stable detritus-type ecosystem. (1) Inherent life-history limitation such as limited number generations, diapause, territoriality, etc. (2) Diversity of secondary consumers that include a variety of both specific parasites and generalized predators. (3) A shift in plant net production to organic material unedible or unavailable to grazers as the season becomes favorable for herbivore production.

So far man has depended almost entirely on the grazing or granivorous food chains for his food; all of his highly developed agricultural systems are of these types. Since detritus systems seem to have a greater buffering effect on fluctuations in the physical environment, and, thus, greater inherent stability it might be well for man to consider the possibility of developing cultural systems of the detritus type. Detritus might be utilized directly by man in two ways: (1) cellulose and other undigestable materials might be converted into metabolizable energy by suitable enzymes, (2) or by the culture and harvest of detritus-feeding animals such as is already done in some types of tropical fish and shellfish culture. Or perhaps better still the large particulate material could be converted for direct use and the bacterial-rich small particles fed to animals. The detritus system would seem to be especially desirable in hot climates where a large biological structure is necessary for mineral retention and recycling and where insect damage to crops that have a high nutritive value is inherently great.

ECOLOGICAL AND EVOLUTIONARY ASPECTS OF "BIOLOGICAL CLOCKS"

A symposium organized for the
XVI International Congress of Zoology

by

Colin C. Pittendrigh
Department of Biology
Princeton University
Princeton, New Jersey

DIE BEDEUTUNG CIRCADIANER RHYTHMEN FUER DIE OEKOLOGIE! (THE ECOLOGICAL SIGNIFICANCE OF CIRCADIAN RHYTHMICITY). Jürgen Aschoff, Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen und Erling-Andechs, Germany.

Eine der Aufgaben der Ökologie ist es, die zeitlichen Beziehungen zwischen den Handlungen eines Tieres und Vorgängen in seiner Umwelt festzustellen. Besondere Beachtung verdient hierbei der Wechsel zwischen Tag und Nacht, dem gewöhnlich ein Wechsel zwischen Aktivität und Ruhe entspricht. Diese Tagesperiodik wird dem Organismus nicht rein passiv von der Umwelt aufgeprägt. Sie hat vielmehr bei vielen Arten den Charakter einer selbsterregten Schwingung, die dem Organismus angeboren ist und durch periodische Faktoren der Umwelt mit der Erddrehung synchronisiert wird. Ohne solche Zeitgeber weicht die endogene biologische Periodik mehr oder weniger von 24 Stunden ab; sie ist deshalb von Halberg circadian genannt worden. (1,2,3,4).

Für den Ökologen sind unter anderem folgende Fragen bedeutungsvoll: 1) Wie kann man eine unklare Tagesperiodik verdeutlichen?; 2) Gibt es art-typische Muster der Aktivitäts-Periodik? 3)Ist die Variabilität der Muster regelhaft?; 4)Welche Umweltfaktoren beeinflussen das Muster?; 5) Ist das Muster auch eine Eigenschaft des Genotypus?; 6) Unterscheiden sich ökologisch trennbare Gruppen hinsichtlich ihrer circadianen Periodik?; 7) Gibt es Regeln für die Synchronisation der circadianen Periodik mit der Umwelt unter natürlichen Bedingungen?

1. Nachweis der Tagesperiodik. Er fällt leicht bei solchen Arten, die ausschliesslich während einer Hälfte des Tages aktiv sind: rein lichtaktive oder rein dunkelaktive Arten. Ist dagegen die Aktivität auf alle 24 Stunden verteilt, so lässt sich eine Tagesperiodik oft kaum erkennen. Misst man z.B. die Aktivität der Spitzmaus Sorex araneus in Intervallen von 10 Minuten, so findet man etwa 10 fast gleich grosse Aktivitäts-Schübe ohne jede merkliche Bevorzugung von Licht- oder Dunkelzeit. Bildet man aber von diesen 10-min-Werten der Aktivität das Mittel über viele Tage, so zeichnet sich eine Tagesperiodik ab mit einem Aktivitätsgipfel in der ersten Hälfte der Dunkelzeit und einem zweiten Gipfel zu Beginn der Lichtzeit. Dieses Aktivitätsmuster wird noch deutlicher, wenn man die Registrierungen mehrerer Individuen zusammenfasst. (5). Eine andere Möglichkeit besteht in der Wahl des richtigen Zeitrasters: Lässt die in kurzen Zeitabständen - in 10- oder 20-min-Intervallen - registrierte Aktivität nur Aktivitäts-Schübe erkennen, so können dieselben, zusammengefasst in 1-Stunden- oder in 2-Stunden-Werten, eine klare Tagesperiodik bilden.(6). Hieraus sind folgende Forderungen abzuleiten: Um sicher zu entscheiden, ob eine Art tagesperiodisch ist oder nicht, sollte die Aktivität a) über mehrere Tage, b) an mehreren Individuen und c) mit einem angemessenen Zeitraster registriert werden. Weitere Bedingungen werden in den folgenden Abschnitten behandelt.

2. Typische Aktivitätsmuster. Beobachtet man mehrere Individuen einer Art unter gleichen Bedingungen - im Felde oder im Laboratorium -, so findet man meist recht ähnliche Muster der Aktivität, die sich statistisch sicher vom Muster einer anderen Art abtrennen lassen. (7). Allerdings sind diese Muster nicht

völlig konstant. Sie können sich vielmehr gemäss den jeweiligen Bedingungen ändern, so etwa mit dem Alter, mit den Jahreszeiten, oder mit dem Biotop (siehe weiter unten). Unter gleichen Bedingungen und bei vergleichbaren Zuständen der Individuen wird man jedoch ähnliche Muster erwarten dürfen. Dabei ist natürlich zu berücksichtigen, auf welche Art von Aktivität sich das Muster bezieht: Flugaktivität, Kopulation, Eiablage und Schlüpfen eines Insektes können alle typische, voeinander verschiedene Muster haben, wobei im Extremfall das Maximum der einen Handlung in die Lichtzeit, das einer anderen Handlung in die Dunkelzeit fällt.

Bei überraschend vielen Arten ist das Aktivitätsmuster durch zwei Maxima ausgezeichnet, von denen das grössere meist zu Beginn, das kleinere gegen Ende der Haupt-Aktivitätszeit liegt. Die Häufigkeit dieses Musters bei Fischen, Insekten, Vögeln und Säugetieren hat mehrfach Hypothesen über die möglichen Ursachen veranlasst. Diese Frage wird deshalb im Folgenden noch kurz behandelt werden.

3. Veränderlichkeit des Musters. Die circadiane Periodik ist, zumindest bei einigen Vertebraten, angeboren. Das schliesst nicht aus, dass sie sich erst im Laufe von Tagen oder Wochen nach der Geburt entwickelt. Hierbei kommt es nicht selten zu starken Änderungen des Musters. Die japanische Lanschnecke Fructicicola (Acusta) sieboldiana ist in ganz jungen Stadien eher lichtaktiv, als ausgewachsenes Tier dunkelaktiv. (8). Ähnliche Wechsel von Licht- zu Dunkel-Aktivität (oder umgekehrt) während der Ontogenese sind auch an anderen Arten beobachtet worden.(7). Dasselbe kann für den erwachsenen Organismus im Verlauf des Jahres gelten: Erkinaro (9) hat kürzlich gezeigt, dass Microtus agrestis auf 60° N im Sommer (also in der Zeit sehr kurzer Nächte) fast ausschliesslich dunkelaktiv ist, im Winter jedoch vorwiegend lichtaktiv. Ältere Beobachtungen an anderen Arten sind damit bestätigt worden.(7). Dieser Befund führt zu der Frage, wie sich Tiere der Arktis bzw. der Antarktis verhalten, wenn die Sonne während des ganzen Tages über oder unter dem Horizont bleibt. Leider liegen bislang nur Untersuchungen aus dem arktischen Sommer vor: Unter diesen Bedingungen bleiben viele Arten, besonders Vögel und Säugetiere, klar periodisch; die Ruhezeiten liegen meist früher als normal (vor Mitternacht). Einige Arten scheinen jedoch im Sommer aperiodisch zu werden. Peiponen (10) hat dies für Clethrionomys rufocanus nachgewiesen: Das untersuchte Männchen war im Juli zu allen Tageszeiten etwa gleichmässig aktiv; als die Sonne im August unter den Horizont zu treten begann, bildete sich ein schwaches Maximum der Aktivität in den Stunden nach Mitternacht, das langsam grösser wurde und nach vorne rückte, bis schliesslich am Ende des Monats das Muster typischer Dunkelaktivität aufgebaut war.

Diese Beispiele mögen genügen, um folgende Forderungen zu begründen: Zu Untersuchungen über das Art-Muster gehören Beobachtungen bzw. Messungen an verschiedenen Altersgruppen, zu verschiedenen Jahreszeiten und auf verschiedenen Breitengraden. Selbstredend ist auch auf mögliche Unterschiede zwischen den Geschlechtern zu achten.

4. Umwelteinflüsse. Wie oben bereits erwähnt, kann das Muster einer Art je nach Biotop recht verschieden sein. Das gilt besonders für Insekten. In zahlreichen Arbeiten sind Korrelati-

onen zwischen der Aktivität einer Art und der Beleuchtungsstärke der Temperatur, der Luftfeuchte oder der Windgeschwindigkeit festgestellt worden. Solche Korrelationen besagen aber nichts über einen etwaigen Kausalzusammenhang. Andererseits zeigen Laboratoriumsexperimente, dass schon geringe Änderungen des Milieus das Muster einschneidend ändern können. Die Elritze Phoxinus phoxinus ist z.B. bei künstlichem Belichtungswechsel im einfachen Aquarium lichtaktiv, wird aber rein dunkelaktiv, wenn man ihr im Aquarium einen Unterschlupf anbietet. (11). Allerdings sind solche Befunde nur sehr bedingt auf natürliche Verhältnisse übertragbar; man sollte jedoch beim Vergleich verschiedener Biotope immerhin mit berücksichtigen, welch unterschiedliche Wahlmöglichkeiten sie den einzelnen Arten bieten.

Wichtiger sind die mikroklimatischen Einflüsse, insbesondere der Temperatur und der Luftfeuchte. Der selektive Wert des Erwerbs einer circadianen Periodik liegt unter anderem darin, die Aktivität eines Organismus optimal an die im Verlauf des Tages wechselnden Lebensbedingungen anzupassen. Zahlreiche Autoren haben z.B. auf den Vorteil der Dunkelaktivität für alle jene Arten hingewiesen, denen hohe Verdunstungsraten gefährlich werden können. Pittendrigh (12) hat darauf aufmerksam gemacht, dass selbst nahe verwandte Arten - Anopheles bellator und Anopheles homunculus - im selben Biotop etwas verschiedene Muster haben und (tagesperiodisch wechselnd) verschiedene Höhen über Grund bevorzugen, in enger Anlehnung an die jeweiligen Feuchtigkeitsverhältnisse und entsprechend ihrem verschiedenen Feuchtigkeitsbedarf. Trotz solch enger Korrelationen mit den für die Adaptation wichtigen Umweltfaktoren sind es häufig andere - und meist der Licht-Dunkel-Wechsel - , die die phasenrichtige Synchronisation der circadianen Periodik gewährleisten. Einzelheiten des Musters werden dagegen von nahezu allen mikroklimatischen Faktoren beeinflusst. Das gilt besonders für das zweite, meist kleinere Maximum der Aktivität, das je nach den Aussenbedingungen scharf markiert sein oder völlig fehlen kann. Die von Larsen (13) gefangenen Nachtschmetterlinge hatten in allen Nächten mit Minimal-Temperaturen über 13° zwei Maxima, in Nächten mit tieferen Temperaturen nur ein Maximum.

Ebenso wichtig wie die abiotischen sind die biotischen Einflüsse. Das Muster einer Art richtet sich unter anderem danach, wie sie selbst hinsichtlich Alter und Geschlecht zusammengesetzt ist, wie auch danach welch andere Arten sich im selben Lebensraum befinden. Leider erlauben die zerstreuten Beobachtungen über all diese Wechselwirkungen biotischer wie abiotischer Art noch keine Generalisationen.

5. Fixierte Muster-Besonderheiten. Der offensichtliche Adaptive Wert der Tagesperiodik sowie bekannte oder vermutete mikroklimatische Einflüsse haben viele Untersucher zu der Annahme geführt, dass bestimmte Einzelheiten des Aktivitäts-Musters von bestimmten Umweltfaktoren verursacht seien. Insbesondere fehlt es nicht an Versuchen, die beiden Maxima der Aktivität auf bestimmte Werte der Helligkeit, der Temperatur oder der Luftfeuchte zurückzuführen. Alle diese Faktoren verstärken jedoch häufig nur ein auch ohne sie vorhandenes Muster. Bei vielen Vögeln sind die beiden Maxima sehr ausgeprägt und scheinbar eng mit bestimmten Helligkeitswerten verknüpft. Bringt man die Vögel aber in einen künstlichen, rechteckigen Licht-Dunkel-Wechsel

so findet man unter Umständen genau dasselbe Muster; Dämmerung ist also nicht die Ursache der beiden Maxima. Noch mehr: Selbst unter völlig konstanten Bedingungen - also ohne jeden Licht-Dunkel-Wechsel - kann das typische Muster mit zwei Maxima erhalten bleiben. Im Dauerlicht beträgt die Periode des Tieres bekanntlich nicht mehr genau 24 Stunden; sie ist vielmehr eine Funktion der jeweiligen (konstanten) Beleuchtungsstärke. Mit wachsender Beleuchtungsstärke wird die Periode bei Vögeln kürzer zugleich wird die Zeitspanne, während der der Vogel aktiv ist, länger. Es lässt sich nun zeigen, dass die beiden Maxima auch bei stark unterschiedlicher Länge der Aktivitätszeit stets dieselbe Lage (relativ zu Beginn und Ende der Aktivität) einhalten. Das spricht dafür, dass nicht nur die Periodik als solche im Genotypus fixiert ist, sondern dass auch bestimmte Einzelheiten des Musters fest eingebaut werden.

6. Circadiane Regel. Die rein licht- und rein dunkel-aktiven Arten lassen sich leicht voneinander trennen. Zuweilen ist jedoch (siehe Abschnitt 1) die Aktivität derart über alle 24 Stunden verteilt, dass die Zuordnung zu einer der beiden Gruppen Schwierigkeiten macht. Es mag deshalb nützlich sein, nach neuen funktionell-taxonomischen Kriterien zu suchen. Eine Möglichkeit besteht darin, die Periodik unter konstanten Bedingungen zu messen. Nach den bisherigen Ergebnissen sieht es so aus, als wirke Licht auf die Periode licht- und dunkelaktiver Arten (entsprechend den Wirkungen auf die gesamte Aktivität) im entgegengesetzten Sinne: Mit wachsender Beleuchtungsstärke wird die Periode dunkelaktiver Arten länger, die lichtaktiver Arten kürzer; gerade umgekehrt ändern sich die Aktivitätszeiten. (Aschoff in 2). Es wäre wichtig, zu wissen, ob diese Regel allgemein gültig ist und ob sie z.B. auch dann zutrifft, wenn eine Art - etwa im Verlauf des Jahres - zwischen Licht- und Dunkelaktivität wechselt. Die Abhängigkeit der circadianen Frequenz von der Beleuchtungsstärke trägt möglichenfalls zur Synchronisation der circadianen Periodik bei. (Aschoff in 4).

7. Phasenrichtige Synchronisation. Unter den Zeitgebern, die die circadiane Periodik mit der Erddrehung synchronisieren, spielt der Licht-Dunkel-Wechsel die wichtigste Rolle. Nach einer experimentell gut gestützten Theorie sind die Übergänge zwischen den beiden Beleuchtungszuständen die eigentlich wirksamen Faktoren (Pittendrigh in 2). Unter natürlichen Bedingungen sind das die Zeiten um Sonnenaufgang und Sonnenuntergang. Diese Annahme steht in Übereinstimmung mit Feldbeobachtungen. Die Aufmerksamkeit der Ökologen wurde schon früh von der Tatsache gefesselt, dass der Beginn der Aktivität vieler Arten im Lauf des Jahres eng an die Sonnenaufgangs- bzw. untergangszeiten gebunden bleibt. Als Beispiele seien das Flughörnchen (DeCoursey in 2) und der Cardinal (14) genannt. Eine genauere Durchsicht der Literatur zeigt jedoch, dass es sich bei diesen beiden Arten eher um Ausnahmen als um Regelfälle handelt. Viele Arten ändern den Abstand zwischen Aktivitätsbeginn und Sonnenauf- oder untergang von Monat zu Monat. Für Vögel lassen sich folgende drei Gruppen bilden: a) gleichbleibender Abstand, b) Aufwachen im Sommer relativ früher, im Winter später (bezogen auf Sonnenstand -6°), c) umgekehrt wie b). Mit anderen Worten: Man findet keine allen lichtaktiven Vögeln gemeinsame Regel für das jahreszeitliche Verhalten bei Aktivitätsbeginn. Das gleiche gilt für die Zeitpunkte der letzten Aktivitätsäusserungen am Abend. Berechnet man jedoch

aus Beginn und Ende der Aktivität die Mitte der Aktivitätszeit und setzt diese in Beziehung zur Mitte des Tages (12 Uhr), so ergibt sich ein einheitliches Bild: Im Sommer liegt die Mitte der Aktivitätszeit maximal weit vor 12 Uhr, im Winter minimal vor oder sogar nach 12 Uhr. Das Gegenteil hiervon scheint für dunkelaktive Arten zu gelten.

Die Verschiebung der Phase der Aktivitätsperiodik gegenüber dem Zeitgeber ist in nördlichen Breiten weit ausgeprägter als in mittleren Breiten (siehe auch Abschnitt 3, Arktis). Die tägliche Lichtzeit (die Photoperiode) spielt offenbar eine ausschlaggebende Rolle. Will man die Ergebnisse von Beobachtungen auf verschiedenen Breitengraden miteinander vergleichen, wählt man deshalb zweckmässiger Weise nicht gleiche Monate sondern gleich Lange Lichtzeiten zum Ausgangspunkt. Nach Angaben in der Literatur lassen sich von 10 lichtaktiven und 10 dunkelaktiven Arten Beginn und Ende der Aktivität für wenigstens zwei verschieden lange Lichtzeiten berechnen. Die Änderungen der Aktivitätsanfänge gegenüber Sonnenaufgang bzw. Sonnenuntergang sind völlig uneinheitlich. Die Mitten der Aktivitätszeiten gehorchen jedoch alle der eben genannten Regel: Mit wachsender Lichtzeit (d.h.: zum Sommer hin) Vorverlegen der Aktivitätszeit-Mitte bei lichtaktiven, Rückverlegen bei dunkel-aktiven Arten.

Es kann nicht der Sinn dieses Vortrages sein, mögliche Theoretische Deutungen für die hier geschilderten Regelhaftigkeiten zu geben. Es liegt nahe, einen Zusammenhang mit der circadianen Regel zu suchen (15), jedoch sind sicher auch andere Interpretationen möglich. Die Absicht war, den Ökologen auf einige Fragestellungen aufmerksam zu machen, wie sie sich aus neueren Erkenntnissen über die circadiane Periodik ergeben, und ihn zu weiteren Messungen unter natürlichen Bedingungen anzuregen.

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TIME-COMPENSATED SUN-ORIENTATION IN ANIMALS. Georg Birukow, I.Zoologisches Institut der Universität Göttingen, Germany.

The time-compensated sun-orientation found in a variety of animals is based on their ability to derive compass directions from the position of the sun or any other celestial body, by taking into account the diurnal movement of this reference point according to its azimuth curve, and associating it to the time of the day. One of the essential prerequisites for this ability is an accurate internal clock furnishing local time. For perfect orientation other parameters of the apparent movement of the sun have to be considered which depend on the time of the day, on the season, and on the latitude. Also, the direction of the apparent sun's movement (clockwise or anticlockwise) differs on the Northern and on the Southern Hemispheres; still more complicated is the situation in the tropics where the sun changes its direction of movement twice a year.

Another basic condition is the existence of an orienting taxis mechanism linked to the internal clock. Because many animals orient themselves to artificial light sources as readily as to the actual sun, the performance of such orienting mechanisms can be studied under controlled experimental conditions. This study comprises general problems of physiological clocks which as yet proved to be not essentially different from those in other circadian rhythms, as well as problems of orientation, of seasonal periodism, of ecology, evolution and ontogeny. It is also closely related to the problems of homing in animals.

I. Phylogenetical aspects.

Time-compensated sun-orientation may have evolved on the basis of more simple orientation mechanisms which effectuate the optical localisation of a stimulus source. The basic patterns are positive and negative phototaxis which are derived from reactive turnings of an animal in order to establish symmetrical stimulus conditions on both eyes, as in tropotactical orientation, or of a mechanism controlling the location of the image within the fixation area, as in telotaxis. Menotactic, or light-compass orientation may have evolved from the basic orientation by the addition of a spontaneous turning tendency which is compensated by the effects of the stimulus. These basic patterns of orientation enable an animal to establish a straight forward course of locomotion in one direction, the bearing of which depends merely on the location of the stimulus source, the sign of the phototaxis, and in menotaxis on the amount of turning tendency. The term "light-compass-orientation" for the basic, non time-compensated menotaxis is somewhat misleading, the course is not necessarily related to a compass bearing.

These relatively simple mechanisms of orientation may become parts of the "circadian organisation" of animals. The photo - taxis of the unicellular Euglena shows a clear 24-hours rhythmicity; the same is true for a number of lower and higher invertebrates which orient by phototaxis as flatworms, some larvae of butterflies and some beetles. Further, in menotactic orientation of the dung-beetle the angle of orientation to the

actual sun or to plane polarized light spontaneously varies through 24 hours. Such overt rhythms of orientation seem to indicate that on this level of organisation the taxis mechanisms may get coupled to the internal clock, the orientation being influenced by different internal states of the organism which are regulated by the internal clock. Although the overt rhythms of orientation often seem to lack any functional meaning, they demonstrate clearly two essential features of the time-compensated sun-orientation: the ability to " measure time", and to "measure angles" as well.

II. Types of sun-compass-orientation.

1. Spontaneous directional orientation. Young migrating birds fly in one general direction in autumn and in the opposite in spring. When displaced perpendicularly to their migration routes they take a parallel course in the previous compass direction. This type of orientation, first demonstrated by G. Kramer in caged migrants, is predominantly based on the position of the sun or at night of the stellar pattern. In invertebrates such one-directional orientation exists in isopods , amphipods, some beetles and wolf spiders which orient themselves with reference to the site of relevant biotopes using the sun or the polarisation pattern of the blue sky as celestial cues. At night the amphipod Talitrus saltator can orient to the moon. In migrating insects the role of the sun or of the sky-polarisation is as yet disputed.

In some cases the spontaneous, probably fully congenital orientation in one direction is related neither to the migration direction nor to the site of a relevant biotope. This type of orientation which does not make any sense to us at present exists in mice (Apodemus flavicollis and others) , birds (mallards, starlings, terns) and may perhaps also affect the homing of pigeons. The preferred direction (e.g. NW in mallards) generally does not depend on the home site, on the time of day or on the season; mallards fly in the same direction even under clear night skies orienting themselves probably to the stars. Overcast sky makes the orientation fail. In invertebrates similar "nonsense" orientation has been described in beetles (Geotrupes silvaticus) and in the pond scater Velia.

2. Directional training. K. von Frisch and G. Kramer were the first to show that bees and birds can be trained to compass directions. Further evidence by means of various methods of reward offered in a particular direction is at present established for fish, lizards, turtles, and also for mice(as yet unpublished results). When mallards are trained to a compass direction at day time, they look for food in the same direction at night: stars appear to be significant celestial cues again. The direction training method has been extensively used to answer the question how much of information, given by a celestial body, is really utilized by an animal, and what role must be attributed to the internal clock mechanisms. Another important problem is raised by the question whether or to which extent time-compensated celestial orientation is "innate" or due to experience. At present the role of the stellar or solar compass orientation in homing animals receives particular attention. True navigation or goal orientation (Griffin's "Type III") which establishes homing, must be clearly distinguished from the basic

directional orientation by means of a sun-compass, though the latter is probably part of the first, according to the "map-and-compass" concept of G. Kramer et al. Apparently the solar or stellar compass may only help the animal to keep its course, after having determined the relative home direction in a still unknown way.

III. Internal and external factors which influence the sun-compass orientation.

1. Shifting of the internal clock. Like in other circadian functions, the internal clock which regulates the orientation can be reset by shifting the phase of the light-dark regime. If the artificial day in which the animals are kept begins 6 hours later or earlier than before, which corresponds to a east or west displacement along the same degree of latitude, then the expected compass bearing will differ by 90° to the right or to the left of the original direction of orientation. Such experiments, first performed in direction-trained birds, have been confirmed in a number of other animals which use the sun-compass either in trained or spontaneous compass-orientation, and also in homing pigeons.

2. Orientation at night. In nearly all vertebrates and invertebrates the sun-compass is operative throughout 24 hours. This can be proved by using "artificial suns" or by testing phase-shifted animals outdoors during their "subjective night". So far, two different types of compensation have been found. In Type I (bees, various species of fish, lizards, mice) the animals compensate a clockwise movement of the sun at night, in Type II (Talitrus, some other arthropods) a counterclockwise movement. It seems that under certain conditions a hybrid type of night orientation may appear (pigeons, pond scaters).

3. Estimation of the sun's altitude. So far, the altitude of the sun seems to be of no concern except in fish and perhaps in the dung beetle. Although sun-compass orientation works by means of azimuth determination predominantly, it cannot be excluded at present that some additional information may be obtained from the altitude, too. In stellar orientation this factor may be of greater importance.

4. Diurnal, seasonal and latitudinal variations. Some animals (fish, lizards, probably some arthropods) allow for such variations. In lizards quantitative computation of azimuth is clearly affected by the length of the day in which the animals live, and by the hour of the day. Because day length and azimuth curve of the sun are functions of latitude and season, the length of the day alone cannot be utilized for correct orientation at different latitudes, e.g. in birds which migrate from N to S. As to latitudinal displacements, but few quantitative data are available at present, and the results are often ambiguous. Fish and pigeons trained to compass directions in the Northern Hemisphere, and then flown to the equator, referred to the sun as if it were the sun of their home and did not allow for the respective local sun-movement.

5. Innate and learned components. Some animals (fish, some birds, lizards , mice) first learn the absolute azimuth-angle for one time of training (angle constant orientation), and relate it to the complete diurnal and nocturnal azimuth curve only after additional training during different times of the day (time-compensated orientation). Others (bees, most birds, some fish) can "switch in" the complete azimuth curve of the sun after being trained to a single azimuth-angle. There is little experimental evidence with unexperienced animals raised in deprivation of the sun-sight. Generally it seems that the ability to compensate the direction of the sun's movement at all is inherited, whereas the actual direction of the movement, clockwise on the Northern Hemisphere or anticlockwise on the Southern, must be learned. Thus bees can adapt to the actual sun-azimuth curve when they are displaced from the Northern to the Southern Hemisphere or vize versa. Unexperienced lizards native to Middle Europe can be trained to an artificial sun which moves anticlockwise or even through 360° in 12 hours , i.e. half the normal time. Identical species native to different latitudes often show a different behavior; this is probably due to an adaptation to local astronomical conditions. Similar differences in spontaneous orientation during the night have been recently described in the wolf-spiders of the genus Arctosa native to Italy and to Finland.

IV. The time-compensated sun-orientation and the homing problem.

In homing birds (pigeons) the sun-orientation seems to provide the compass-element of a " map-and-compass" bi-coordinate orientation. Despite some more or less valuable theoretical approaches towards a celestial navigation hypothesis including the problems of clock-synchronisation and of reference clocks utilized by long-distance travelling birds, the question remains whether the homing ability can be explained on the basis of pure solar or stellar navigation. There is clear evidence that many animals (birds, some mammals, toads and newts when migrating to their breeding sites) can home without using any celestial cues. Homing surely involves more complex mechanisms than one-direction orientation, therefore the effects of shifting the individual clock are much less clear in homing animals. Further studies in homing birds, especially pigeons, will surely have to deal much more with the part which sun-compass orientation plays in homing at various distances.

Some recent, very fruitful theoretical approaches tackle the problems of homing and orientation mathematically along the lines of information theory and cybernetics. Furthermore some instrumental aspects of animal navigation may be derived from comparing technical missiles guidance systems with the still unknown sensory mechanisms of true homing. The basis of the "map" would then be comparable to the inertial guidance systems which, in animals, would require a "sense of inertia" of otholiths and cupula-endolymph systems (J.S. Barlow). The solar and stellar components of homing would better fit the star-tracking guidance systems which use celestial bodies for the determination of the flight program. So far, such theories can neither be rejected nor proved until further experimental

evidence, and a much broader basis for comparison are available. In all these hypothesis again we are confronted with the clock-problems because Inertial-Celestial-Systems require correct absolute time. Such a reference clock would also be required by travelling birds in order to determine position with respect to the home. On the other side internal clocks of animals are known to underly phase-shifts, and to deviate from the exact 24-hours periodicity. Thus, at present the problem of homing on a inertial or a pure astronomical basis remains unsolved.

" TIME SENSE OF BEES "

Martin Lindauer (Zool. Instit. der Universität Frankfurt a. M., Germany)

Introduction:

Time as a biological measure can be used in two different ways in the life of bees:

A. In periodic seasonal or daily rhythm decisive activities are carried out by collaboration of the whole community

B. Thanks to an efficient "time memory" individual actions become related to an important, biological "mark" of time.

A. Periodic annual and daily activity in the social community.

1. Broodcare, swarming, migration - in their annual cycle.

Aristotle mentions the bees indicating the solstice by their activity: Ἡ μέλιττα δοκεῖ τὰς τροπὰς σημαίνειν τῷ ἐπὶ τὰ ἔργα βαδίζειν.

In fact we find brood-rearing and swarming in their culmination towards the summer solstice: at the end of January or February egg-laying starts, increases tremendously until June, falling down then more and more July to autumn. The rearing of drones, which begins about April, and the replacement of young queens in May/June are inserted into this cycle harmoniously in special annual periods. Swarming in conclusion goes hand in hand with this breeding cycle, as it is connected with the overpopulation in the hive and depends on the appearance of drones and young queens (FREE, 1958); MARTIN (1963) succeeded in producing artificial swarms throughout the whole year under controlled conditions; even there the annual culmination in the summer solstice could not be suppressed (Fig. 1)

Short mention may be made of those mysterious migrations of the giant honey bee Apis dorsata in South East Asia. Twice a year the whole colony leaves its domicile as a migratory swarm, leaves its giant honey-comb behind empty and migrates hundreds of kilometers, in order to settle somewhere in another land for the next 5 - 6 months. In the region of Kandy in Ceylon, the colonies leave their low-country domiciles in August/September and migrate up to Haputale, 2000 meters above sea-level, where they then populate the rocks and the bee-trees - always the same ones. Then in March/April they return to the valleys.

2. Periodic daily activity.

Important activities in the colony, like brood-rearing, building of combs, regulation of temperature, continue

day and night without interruption and without rhythmic peak periods. Collector-bees on the other hand adapt their activity to the weather conditions,to nectar and pollen supplies, and - naturally - to dawn and dusk. Nevertheless beneath this exogenously controlled activity there lies concealed a periodic daily rhythm: a group of collector-bees, which gathers at a feeding-table under experimentally completely identical conditions, shows at noon every day a decrease in collecting-energy and particularly in its alarm activity, i.e. its tendency to dance is noticeably lower. This "midday rest" lasts about from 12 to 14 o'clock (LINDAUER, 1948, BOCH, 1956), and it is regularly followed by the "Vorspiel" i.e. the orienting flights of the young bees (Fig.2).Mention may be made of the compulsory midday rest of bees in the tropics, where the sun-compass does not work when the sun passes through its zenith (see below). One may remember that our native bees had their old home in these regions.

The remarkable thing in all these annual and daily periodic activities is that they occur in harmonic collaboration of many individuals. One should realize that these individuals represent a very heterogeneous material according to their age, physiological conditions (e.g. the activity of their nursing-glands and wax-glands) etc. Besides, each of these groups is exposed to varying external factors: young bees, contrary to field bees, stay in the darkness of the hive day in, day out, and are almost completely withdrawn from periodic climatic factors. Moreover: the individual bee has only a short lifetime (in summer only a few weeks), and yet it induces some work which will bring about the periodic annual event, as for example the departure of a swarm, only after weeks or months.

B. Time-Memory

The true time-memory is of another kind: important biological actions are associated with a particular hour or minute of the day. BELING (1929) fed bees between 10 and 11 a.m. for example. After a few days of training the feeding table was left empty the whole day, nevertheless the marked collector bees visited the place at their usual feeding-time. They had noticed not only the beginning of this feeding-time but also just as exactly the end (Fig.3). Even three or four feeding-times a day the bees can learn in this way (WAHL 1932, 1933) and they can combine several feeding-times with different feeding-places (FINKE 1961) (Fig. 4). RENNER (1957 1959) has proved convincingly that primarily an endogenous rhythm underlies this memory of time: Bees which were transported from Paris to New York, came to the feeding table in New York corresponding to Paris local time. Furthermore,bees which were shifted from New York to California searched according to New York local time and in that direction whose sun azimuth would have corresponded to the New York training time (Fig.5). Therefore: no exogenous factors which are correlated with the earth'rotation, like magnetic field or the altitude of the sun's position and its directly correlated

azimuth could be employed in these experiments.

C. Suncompass-oriention

A special achievement of bees' timesense is their ability to combine fixed periods of time with the sun's azimuth and to make use of them for compass orientation. The first proof of this ability was given by v. FRISCH (1950, 1952): a group of bees is trained 200 m towards south, in a strange area; they are allowed to visit there a feeding place only on one single afternoon. The colony is transported overnight to another district,and the following morning the south-trained bees are faced with a choice of four feeding tables, 200 m in the south, north, east and west respectively. The bees come spontaneously to the south, thus showing that they have taken account of the apparent movement of the sun from afternoon training to the next morning; they orientated "azimutgetreu" and not "winkeltreu": the previous afternoon they had flown always left of the sun, now in the morning they had to keep right of the sun in order to reach their goal (v.FRISCH, 1952, v.FRISCH and LINDAUER, 1954), (Fig.6).

The so called "Marathon dancers" give us further evidence for sun-compass-orientation: By illuminating and heating one can sometimes induce forager-bees or scout-bees to start dancing out of collecting time or swarm-time in the closed hive. Even then they use the sun as compass memorizing the exact sun position at the actual dancing time - without sight toward the sky.

MEDER (1958) caught his bees on an air field at the feeding table and kept them in a dark cage for several hours. After being released the bees returned in the correct compass direction to the hive,even when no landmarks could offer any help in finding their home.

A final proof for calculation of sun motion has recently been provided by NEW, D. and NEW, J. (1962). They found that even when the sun is passing through its zenith, so that bees can no longer measure the angle of the sun directly (LINDAUER, 1957), some bees succeded in calculating the course of the sun further, through pure extrapolation (Fig.7). Efforts to analyze the physiology of the time sense of bees had up to now little success. Contrary to the findings of KALMUS (1934) and GRABENSBERGER (1934 a, b), neither RENNER (1957) nor WERNER (1954) was able to establish any disarrangement of the internal clock by means of euchinine, tyroxine or adrenaline. Only in the case of cooling treatment (cooling at 4° C) did the bees come to the feeding table several hours too late. As RENNER (1961) however quite correctly emphasises, this does not prove that the time sense mechanism had been slowed by the cooling; the sensitiveness to an external timer can also have been reduced. Thus the problem is still unsolved. The following discussion perhaps offers a new experimental way.

D. Measurement of short intervals of time.

The bees have not only to measure long intervals of time, days or hours; more frequently they are engaged in calculating external or internal events in seconds or thousandths of a second. When a bee flies from the hive to the feeding place, it maintains a fixed speed above the ground; it can be proved that the optical pattern of the ground serves for the measurement of speed (HERAN, 1954, HERAN, LINDAUER, 1963). Testing of speed however demands the exact measurement of short time moments. In the same way mechano-receptors can only provide information when the deformation in the unit of time is taken into account. Furthermore the sequence of impulses in receptors and in the nervous system occurs at characteristic intervals of time (Fig.8). The time pattern of these impulses is a main code for sensory communication. If these sequences of short time intervals are cumulated and stored in the central nerve system, the concept of a "memory of time" - i.e. memory of longer time intervals - would be put on a concrete basis.

We know at least one more situation in the life of the bees, where evidence for measurement of short intervals of time is given and that it is of the greatest biological importance: the "wagging time" in the bee dance: v.FRISCH and JANDER (1958) have calculated statistically that the best signal for showing distance in the wagging dance is "wagging time", (Fig.9); that is the short period which the dancing bee takes to complete its rectilineal wagging line. It varies from about 1/4 - 4 seconds, indicating distances of 100 meters to 6 kilometers. As this "wagging time" shows the best correlation with the distance, the authors consider it probable that even the young beginner takes this duration of time as a signal indicating distance. That means however that when the young newcomer after being informed about the location of the goal by a dancing collegue flies off to gather, it has to calculate the distance to its goal with an extremely accurate measurement of time, even distinguishing fractions of seconds.

In this connexion it is particularly remarkable that this measurement of small time intervals is standardised in different races and species of honey-bee. v.FRISCH (1953) had found that the Italian bee shows a slower dance rhythm than the Krainer Bee, and BOCH (1958) extended this finding to six different races in Europe and Africa. He showed that such "dialects" in the language of bees can lead to slight misunderstandings when two different races are mixed together to form a bastard colony - thanks to their racial specifique "time sense".
These racial differences in the "clock timer" are even more evident with the Indian bees Apis indica, Apis dorsata and Apis florea, as is shown by the comparison in Fig.10. It will be a difficult, but very attractive task of future experiments to discover the physiological mechanism of these short time calculators. It seems quite possible that the varying calculation of identifiable moments of time, as provided by the peripheral sense organs, may prove to be parameters specific to race and species. Here however there is still many a hard nut to crack.

ENDOGENOUS TIDAL AND LUNAR RHYTHMS. James T. Enright, Max-Planck-Institut für Verhaltensphysiologie, Seewiesen and Erling-Andechs, Oberbayern, Germany.

Introduction: A great many natural populations show a significant coordination in their physiology or behavior, synchronized with a relatively constant phase relationship to the tides or moon. Observations of such tidal and lunar periodicities under field conditions have been well summarized, for example, by Caspers (1951) and Korringa (1957).

The existence of many of these field rhythms has been established beyond doubt, and explanations for the phenomena often seem to be self-evident: e.g. the inhibiting or stimulating action of moonlight on activity of nocturnal organisms; and the influence of tidal immersion on feeding behavior of intertidal invertebrates. Nevertheless, until very recently, the interpretation of tidal and lunar periodicities has been often associated with a "somewhat mystical atmosphere", as Hauenschild has phrased it. The origins of this mysticism lie at least partly in field observations and preliminary experiments which have, in some cases, strongly suggested that an observed synchronous event was not a direct response to the more obvious, concurrently acting stimuli like moonlight and tides. Such difficulties have then led a number of investigators to seek explanations in terms of response to lunar influences via gravitation, cosmic radiation, geomagnetic field or other environmental factors not usually recognized as biologically effective. These latter hypotheses have sometimes produced an impression that tidal and lunar rhythms cannot be explained within the framework of present biological knowledge.

During the last ten years, however, a small number of quantitative laboratory studies have provided a new sort of experimental foundation for possible interpretations of tidal and lunar rhythms. An important impetus to the recent research has been the accumulation of data on circadian rhythms, and the accompanying recognition of the widespread potentialities of organisms to undergo spontaneous, endogenous oscillations.

The purpose of this review is a thorough examination of these few recent, significant experimental studies, in an attempt to answer the question, "How much is experimentally known about tidal and lunar rhythms?"; and to distinguish between established conclusions and tentative hypotheses. As a first step in a generally skeptical approach, all presumed rhythms which have been "revealed" by numerical techniques related to spectrum analysis, using data drawn from an array of diverse organisms, will be here disregarded, together with the interpretations based on such studies. In a complete spectrum analysis, a particular frequency becomes of interest only when a sharp peak in the plot of amplitude against frequency appears there. That a measurable amplitude corresponding to a 24.8 hour period can be demonstrated in data offers no evidence that this presumed "lunar-tidal component" rises above background noise. Real but obscured lunar rhythms could give results similar to those obtained from the analyses; there is, nevertheless, no assurance that the "rhythms" demonstrated by a line-spectrum treatment of continuous spectrum data are more than artifacts.

Tidal Rhythms: One of the few investigations which produced clear, quantitative data on the persistence of a tidal rhythm

under laboratory conditions was the study by Rao (1954) of mussels of the genus Mytilus. Rao reported that the filtering rates of these animals showed fluctuations paralleling the local tides, with a rhythm which persisted under constant conditions for four weeks, with unchanged phase relative to the tides. The unaltered timing relationship between the rhythm and the actual local tides, after prolonged exclusion of all simple lunar and tidal influences, could lead one to speculate about the possible importance of unrecognized environmental variables with a similar frequency; but Rao's finding that translocated mussels adapted to local tidal phase during exposure on a pier piling, while those maintained in the laboratory did not, argues against this interpretation. As a result of his observations, Rao concluded that the rhythm had an "intrinsic (or endogenous)" origin. Two aspects of his results provide noteworthy contrasts with the properties usually characteristic of endogenous circadian rhythms: 1) the average period of the reported rhythm was extremely close to a true natural tidal interval, a long-term correspondence seldom seen in circadian rhythms; and 2) the period of the rhythm was apparently less sensitive to prevailing light and temperature conditions than has been documented for circadian rhythms. Because of these interesting properties, it is particularly regretable that extensive attempts to confirm Rao's findings have, to date, not been successful (Pickens, cited in Enright, 1963).

In a second important study of an overt tidal periodicity, Naylor (1958) described the persistent rhythm of locomotor activity shown by Carcinus maenas. Freshly-collected crabs, when kept in laboratory aquaria, showed bursts of intense activity centered around times of local high tide, a rhythm which could be followed for up to 5 or 6 days in the same individuals.

For freshly-collected animals, high tides at night were correlated with greater activity than those occurring during daylight hours. Naylor interpreted this observation as evidence for two endogenous rhythms, one with tidal and one with diurnal period. Since an appropriate remodification of amplitude, however, was not demonstrated in individual crabs kept under constant conditions, an alternative hypothesis is that the initial correlation was an externally imposed amplitude modification carried over as a repeated pattern within one, approximately-25-hour, endogenous rhythm. The persistence of the 24-hour "component" in the laboratory was not demonstrated. Naylor (1960) also found that under prolonged light-dark conditions, in the absence of tidal factors, the activity of this species showed a weak synchronization with the light-dark regime, suggesting that light is a potential but normally latent zeitgeber for this species.

A third recent study of tidal periodicities (Enright, 1963) dealt with the persistent rhythm of swimming activity shown by an amphipod, Synchelidium sp., when held in laboratory aquaria under constant light and temperature conditions; and also described superficially similar rhythms in an isopod, a mysid, and a decapod from the same beach. The first-observed activity peaks showed a strong timing correlation with the tides on the beach of collection (S= ± 21 min for Synchelidium sp.). No evidence could be found for a diurnal amplitude modification of the rhythms. Although two activity peaks per day, one corresponding to each tide crest, were always observed, the evidence strongly suggested that the period of the fundamental rhythm is double-tidal (25 to 27 hours), rather than that each activity peak corresponds to a

full oscillation of the system.

Marked deterioration of the synchronization within the experimental populations occurred within a few day in all experiments; and the periods of the rhythms observed in all four species were consistently longer than that of the tides. This latter observation provides the essential evidence, for these species, against the importance to the rhythms of some unrecognized tidal zeitgeber acting under laboratory conditions.

The primary inadequacy of the three cited studies of tidal rhythms is that the exact nature of the normally effective synchronizing agent has not been demonstrated. It seems evident, for Mytilus and Synchelidium that the zeitgeber was some factor operative on the shore and inoperative in the laboratory. Experiments with Synchelidium argue against natural changes in light or hydrostatic pressure as the normal zeitgeber for this species, but the hypothesis proposed, that wave-induced mechanical stimuli normally synchronize the rhythm, has, as yet, insufficient experimental support. An array of superficial similarities between tidal and circadian rhythms suggest the hypothesis that an endogenous tidal rhythm may be a manifestation of a circadian rhythm modified to permit entrainment by zeitgeber other than light and temperature.

Lunar Rhythms: Three recent laboratory studies of lunar periodicities have been successful in isolating the entraining agents. The first of these was the demonstration by Hauenschild (1960) that the monthly reproductive rhythm of the polychaete, Platynereis dumerilii, can be synchronized by a program of periodic night-time illumination, indicating that moonlight is the natural zeitgeber. Further experiments also provided some evidence that the monthly swarming peaks, once initiated, can persist without restimulation for at least two cycles-- hence, that this species probably possesses an endogenous rhythm with a period of approximately thirty days.

The persistent rhythms in Hauenschild's experiments were only weakly defined; much clearer results were obtained with Dicyota dichotoma. The semi-monthly spore discharge of this algal species, as shown by Bünning and Müller (1961), can also be initiated and synchronized by dim night-time illumination. The persistence of the rhythm for at least three cycles under constant conditions was very clearly demonstrated, as well as the fact that the period of the observed rhythm was consistently two or more days longer than a natural semi-lunar cycle-- providing decisive evidence against the influence on the rhythm of some pervasive lunar zeitgeber in the laboratory.

Both Hauenschild and Bünning have attempted to explain their results with the hypothesis that the long-cycle periodicity was the result of the superposition of endogenous tidal and circadian oscillations within the organism. Hauenschild's experiments to test the hypothesis produced negative results; Bünning's similar experiments were qualitatively in agreement with the hypothesis, but quantitatively, less than half of the predicted effect was realized. There is, thus, no convincing demonstration that any organism simultaneously shows both an endogenous tidal and an endogenous circadian rhythm.

The objection of Hauenschild, however, that "the concept of one oscillating system, which has been applied to circadian rhythms, seems not to be useful to explain a periodicity taking place not within hours but within weeks", is not persuasive. The human menstrual cycle represents a feedback system also

coupled with reproduction, which shows a long-cycle self-sustained oscillation, often of considerable precision. The human rhythm differs from an endogenous lunar rhythm, however, at least in that it is apparently not synchronizable by the action of moonlight or other lunar factors.

A third recent and fruitful study of a lunar rhythm is described by Neumann (1963) : the laboratory entrainment of the hatching and reproductive rhythm of the chironomid, Clunio marinus. On the shore, this insect hatches and breeds during the few hours preceding extreme low tides. Neumann has demonstrated that the restriction of hatching to particular days of the month can be experimentally achieved by a program of periodic night-time illumination with the intensity of moonlight; and that the time of day of hatching is directly related to the timing of diurnal illumination, and can be reset to any desired local time by altering the light program. The interaction of the two rhythms can then result in a hatching closely correlated in timing with the tides on the shore, without any involvement of the tides in the rhythm, except in an evolutionary sense.

Other recent laboratory studies having a bearing on tidal and lunar rhythms include: 1) Creutzberg's demonstration (1961) that the tidal rhythm of swimming shown by migrating elvers can be entirely explained by a direct, sensitive response of the fish to odors originating periodically from river outflow--hence, that neither endogenous rhythms nor unknown geophysical variables need be involved in an explanation of a field rhythm in which the animals superficially seem to be well isolated from actual tidal stimuli; and 2) the demonstration by Brown (1962) that orientation of a fresh-water flatworm, Dugensia doratocephala, to a three-point light source shows an oscillation of moderate amplitude with an approximately lunar period, a rhythm which could still be seen after about 4 months under laboratory conditions. The ecological significance of this population synchronization remains obscure.

A final and interesting instance of a presumed lunar rhythm occurs in the orientation of talitrid amphipods to the moon. Experiments with both Talitrus saltator (Papi and Pardi, 1959; 1963) and Orchestoidea corniculata (Enright, 1961) demonstrate that these animals can usually use the position of the moon in order to orient and move in an ecologically appropriate direction, i.e. toward the waterline on the shore, $\pm$ 90°. It is further claimed by Papi and Pardi that the amphipods possess an endogenous lunar rhythm which persists for several weeks under constant conditions, and which enables the amphipods to compensate for changes in lunar position in a manner similar to the well-documented phenomenon of time-compensated solar orientation. This contention is, however, as yet, based on only meager evidence. A small number of experiments with Talitrus saltator show deviations from the average orientation which tend to support the hypothesis; other observations with Orchestoidea corniculata seem, on the contrary, to suggest that after being kept in darkness for several hours, this species shows a constant angle of orientation to the moon, regardless of lunar position. Since the latter results have been criticized on grounds of experimental methods (Papi and Pardi, 1963), a final decision on the existence of an endogenous, long-persistent lunar rhythm in talitrids must await further data.

Summary: The following points represent a brief answer to the question of how much is experimentally known about tidal and

lunar rhythms: 1) Spontaneous biological periodicities with approximately tidal, approximately semi-lunar and approximately lunar periods have been demonstrated to persist for at least a few cycles under constant laboratory conditions. 2) Because the periods of these rhythms in most cases depart consistently from the environmental periods, there is no reason to doubt that their persistence is independent of possible subtle environmental stimuli directly linked to lunar position; these rhythms seem truly endogenous. 3) Periodic night-time illumination, i.e. moonlight, is apparently the zeitgeber for three lunar and semi-lunar rhythms studied. 4) An interaction of a moonlight-induced lunar rhythm with a diurnal rhythm can result in close coordination of a population with the tides, without the direct involvement of the tides in synchronization; indirect synchronization has been achieved by at least one species.

Equally important to this review, however, is the question of how much is not yet known about tidal and lunar rhythms. A first answer is that the present isolated studies provide too little data for meaningful comparative treatments of the subject. There are some indications, for example, that habitat groupings may prove at least as homogenous in their rhythms as taxonomic groupings, but far too few organisms and rhythms have been experimentally investigated to permit any confidence in such generalizations.

Furthermore, the presently-available results do not even fully explain the rhythms shown by the animals studied. To cite only two of the remaining problems, definitive experiments with tidal rhythms are needed to identify the zeitgeber-- which may differ in nature from one species to the next; and experiments are needed to determine whether moonlight is a sufficient agent to explain the semi-lunar (rather than lunar) reproductive rhythms shown under field conditions by *Clunio marinus* and the Atlantic populations of *Platynereis dumerilii*.

The recent accumulation of data on endogenous rhythms, supplemented by direct responses of organisms to concurrent stimuli like moonlight and tidal immersion, now permit the construction of tentative but plausible hypotheses for the interpretation of the many field observations of animal activities synchronized with lunar and tidal phase. But an ability to erect plausible hypotheses is no ground for complacency. The testing of these hypotheses will offer major research problems for years to come; and the recent progress offers no assurance that all important mechanisms have been uncovered or explored.

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PHOTOPERIODISM AND BIOLOGICAL CLOCKS. Colin S. Pittendrigh, Dept. of Biology, P. O. Box 704, Princeton University, Princeton, N. J.

Photoperiodism is discussed as a general phenomenon; as a mechanism whereby animals and plants recognize the seasonally appropriate time to switch from one metabolic steady-state to another. The way in which the measurement of time is effected in photoperiodism is an unsolved problem. Particular attention is given to Bunnings (1937) suggestion that daily rhythms are causally involved in photoperiodism. That suggestion is evaluated both in the light of the existing experimental evidence and in terms of its evolutionary plausibility.

The hypothesis is reformulated in such a way as to permit a more definite statement of the problem and of the needed experimental tests of its validity. It is suggested that photoperiodism in the classical sense is to be analyzed as one aspect of the mechanism of entrainment of circadian systems by the environmental light cycle.

This formulation is followed by a brief statement of what is meant by circadian system. The principal point here is to emphasize that the usually, easily, observed phenomena of daily rhythmicity (leaf movement, locomotory activity, etc.) are only reflections of a more profound circadian oscillation that pervades the whole physiological organization of living systems. Examples are given to support this general statement; they include recent evidence of changes in the state of the protein population of cells.

The question is then raised as to what is meant by the entrainment of such a system; and some details are given of recent advances in understanding, specifically, how light cycles entrain the circadian system. The point of principal interest here is that the photoperiod is the crucial parameter in determining the peculiarities of the steady state of the circadian system entrained by any light cycle.

It is shown that when circadian systems are entrained by two distinct light pulses in each cycle they manifest peculiarities of response that parallel closely recently discovered (Adkisson) peculiarities in the induction of diapause by photoperiod in the moth *Pectinophora gossypiella*. These peculiarities in the response of circadian systems to entrainment by two light pulses in each cycle are noted as being very relevant to any evaluation of experiments in the photoperiodic field that utilize light-breaks of the dark period. Models are offered as to how circadian systems could effect the time-measurement involved in photoperiod.

The question is raised as to how general are the effects of light (in the visible region) on proteins (as enzymes) in general. Finally some evolutionary considerations on this and other aspects of photoperiodism are discussed.

THE ONTOGENY OF BASIC RESPONSE PATTERNS

Sponsored by the Section on Animal Behavior
of the American Society of Zoologists

A symposium organized for the
XVI International Congress of Zoology

by

David E. Davis
Department of Zoology
Pennsylvania State University
University Park, Pennsylvania

Principles of Ontogeny of Behavior Patterns

J. P. Scott

Roscoe B. Jackson Memorial Laboratory

A basic problem in the study of the development of behavior is that of the nature of behavioral organization. Many organizational processes are involved, including the general genetic organization of the species, (meaning the population of genes available for intercombination), the processes of cell growth and cell division, the processes of tissue differentiation, and the processes of learning. The final organization of behavior is dependent upon all of these processes, none acting completely apart from the others, but all having considerable possibilities of independent variation.

This symposium is primarily concerned with the development of the basic behavior patterns which are characteristic of different species of animals. The problem has received most attention in vertebrates, particularly birds and mammals, although some studies have also been done in insects. It is often assumed that basic behavior patterns in the lower animals are organized almost entirely by genetic factors and that experience has little effect. On the other hand, we have evidence that behavior is modified by experience even in one-celled animals and that at least a transitory type of learning capacity is present.

Most studies have been concerned with patterns of behavior related to certain major systems of behavior. Those which have received the most attention are sexual, agonistic and care-giving behavior. All of these are primarily patterns of social behavior, and their development is consequently closely related to the social environment of the species concerned.

Certain basic methods of study have been developed by workers in the field. The primary methods of observation and description may be supplemented by experimental cross-fostering between species, which has the effect of altering the social environment in which behavior develops, and isolation, which has the effect of eliminating the normal social environment. Other experimental methods include altering the early experience of the animals in some way.

From this descriptive and experimental work, several general principles may be derived. In the first place, since the mechanism of transmission of inheritance is accomplished by means of chromosomes and genes, behavior cannot be biologically inherited as such. As with morphological characters, development must be epigenetic rather than preformationist.

At the same time, the development of behavior is dependent upon various pre-existing sorts of organization, as follows:

1. The gene pool peculiar to the species. Development in a given species is dependent upon the particular combination of 50,000 or so genetic units characteristic of the species population. Genetic organization varies according to the viable mutations present in the population but undergoes permanent changes only quite slowly through the process of evolution.

2. The egg. As chromosomes from the male join those from the female, genetic factors are reorganized in each new individual, within the species limits. The egg itself is already organized as a cell and has acquired polarity, probably through its position in the ovary. It forms the first environment with which the genes must act.

3. Prenatal environment. In mammals the egg develops within an organized prenatal environment which is ordinarily held quite stable by the physiological process of homeostasis. Various sorts of disturbances of this environment may modify the course of development. In birds, incubation provides a similar stable prehatching environment outside the body.

4. Social environment. In social animals, the social environment into which the young animal is born or hatched is likewise preorganized, and present evidence indicates that this organization is highly stable in evolution.

5. Physical environment. We ordinarily think of the physical environment as fluctuating in random fashion, but it is, in fact, highly stable in some respects such as gravitation, and we have only recently acquired the means of varying this factor. It may be highly variable in some other respects such as temperature, humidity, and barometric pressure, but this does not apply to animals living in the sea, where there is a highly stable chemical environment much less subject to temperature fluctuations.

6. Biotic environment. Finally, the young animal develops within a pre-organized biotic environment. In many ways this is the least stable of any of the above, over short or long periods of time.

By varying each of these organized systems, the function of each as it affects the development of behavior can be experimentally determined. While these systems can to some extent be varied independently, none of them exist independently of the others and any assumption of independence is unjustified. Yet, under many conditions, these various organized systems are so related

to each other that behavior will develop with a high degree of reliability.

Closely related to organizational processes is the theory of critical periods: that drastic changes in organizational processes can be made at times when these processes are proceeding most rapidly. One of the most important critical periods directly related to behavior is the critical period for primary socialization, or imprinting.

The general biological principle of adaptation applies to the development of behavior. Development must be adaptive and, because an animal may live in different environments at different periods in life, adaptation may change in development. Likewise, the biological principle of variation applies to development. There is not only variation between individuals, but the behavior of any one individual must be variable in order to be adaptive. This leads to the principle of differentiation; in order to be adaptive, behavior must be differential.

Differentiation still remains a somewhat vague concept, but certain phenomena are well-established. In embryological development, the growth of cells and their histological differentiations are opposed to each other. Similarly, in the differentiation of behavior through the process of learning, the improvement of adaptation, or problem solving, is opposed to habit formation. Finally, function or use has a highly important effect upon differentiation both of cells and behavior. The general process of learning can be thought of as a life-long process of functional differentation, taking place both within and between individuals.

The physiological basis of the development and differentiation of behavior is directly related to the organization of the nervous system and related endocrine phenomena. This problem has been approached on three levels. First, the study of the gross morphology of the nervous system, determined by early growth, has led to the theory of localization of function. While this principle holds in a general sense, recent experiments indicate that there is considerable plasticity of function and consequent vagueness of functional boundaries, at least in the brains of the higher animals. Second, the study of the cellular organization of the nervous system, dependent upon the process of histogenesis, has led to the study of the connections between cells and a "telephonic" theory of function, and more recently to a computer theory. Again, there is considerable foundation for this theory, but no adequate physiological basis to explain the instantaneous formation of new connections between cells. Finally, there is considerable evidence that the

actual process of learning must be a biochemical one and hence an intracellular phenomenon. Only this will explain the continually increasing complexity of neural organization and also the retention of pre-existing organization which is seen in a complex brain.

ONTOGENY OF SOCIAL BEHAVIOR PATTERNS IN JUNGLE FOWL.

J.P. Kruijt

Zoological Laboratory, Groningen, Netherlands.

This study is concerned with the development of social behavior in Burmese Red Junglefowl (Gallus g. spadiceus), starting on the day of hatching and including the adult stage. It attempts to describe and analyse the appearance and changes of social behavior patterns performed by chicks which are kept in small groups, usually without a mother-hen. It is hoped that such a study will contribute to a better understanding of principles underlying the ontogeny of social behavior.

A special purpose of this study is to relate the results with a hypothesis developed by N. Tinbergen and other ethologists concerning the causation of courtship and threat in animals. This hypothesis states that courtship of many fish and birds is caused by simultaneous activation of three more or less incompatible tendencies: to attack, to escape and to behave sexually. At least three phenomena are the result of the interactions taking place when conflicting tendencies are aroused simultaneously: redirection (change of the object or animal to which a motor pattern is directed), ambivalence or compromise (combination of components of different tendencies into one posture or sequence of movements), and displacement (occurrence of motor patterns not belonging to the primarily activated tendencies).

As a result of the interactions underlying these phenomena, the activated patterns of attack, escape, and sexual behavior become transformed and combined in various ways, and they are mingled with functionally irrelevant behavior. The resulting mixtures are subject to evolutionary changes: the patterns become ritualized, that is, adapted to serve as signals in the communication between individuals.

In recent years these ideas have proved very fertile in understanding the otherwise enigmatic rituals accompanying courtship and threat in animals. Moreover, ethologists are becoming gradually aware of the fact that multiple motivation of behavior is a very common phenomenon (Tinbergen, 1960). Thus, the study of the interactions between different, simultaneously aroused behavior systems, is of general importance in the analysis of behavior.

In small groups of junglefowl chicks, escape behavior is

present soon after hatching. It consists of withdrawal, squatting, and certain vocalizations, and is released by strong tactile, visual and auditory stimulation. It is only rarely released by companion chicks during the first days.

Aggressive behavior develops out of hopping, a locomotory pattern present in chicks of a few days old. It consists of a series of little jumps in which the feet are pushed off simultaneously and the wings are flapped. Initially it is not released by, or directed to other chicks. Gradually it becomes oriented to other chicks and as a result little fights start to occur. In these fights, escape may be released in one or both individuals, as a response to tactile stimulation delivered by the attacking opponent. If this happens, the immediate continuation of fighting is inhibited: simultaneous activation of attack and escape is apparently not yet possible.

When the chicks are 3 weeks old, aggressive pecking and kicking are added to the fights. In kicking, the attacking bird leaps at the opponent, flaps the wings, throws both legs forward and hits the opponent with the feet, moving both legs alternately and with high frequency and amplitude. By the addition of aggressive pecking and kicking, fighting becomes more effective and in consequence escape is now more often released in one of the opponents during fighting. Contrary to the earlier stage, this no longer inhibits the continuation of fighting; fighting is now continued despite simultaneous activation of escape. In other words, the motivation of fighting is now ambivalent.

The development of ambivalence occurs in two stages, each of which is characterized by the appearance of new movements in association with fighting.

In the first stage (3 to 7 weeks), ambivalence only occurs during actual fighting. As a result of simultaneous activation of attack and escape, five types of irrelevant head movements start to occur during fighting, each of which is caused by a specific ratio of attack and escape. These movements are ground-pecking and its intention movement (recognizable as feeding movements), head-shaking, preening, and head-zigzagging (a combination of head-shaking and intention ground-pecking). The occurrence of these movements can probably be considered in part as displacement and in part as redirection.

In the second stage (7 to 12 weeks), fighting becomes more vigorous; aggressive pecking and kicking occur frequently. The ambivalence of attack and escape is extended to the walking patterns of approach and withdrawal, preceding and following fighting. This coincides with the appearance of another

irrelevant feeding movement during fighting: ground-scratching. Further, side display and waltzing now appear during fighting. They are probably ambivalent combinations of attack and escape, in which legs, body and wings are involved. For example, the downward extension of the outer primaries in waltzing can be understood as a compromise between aggressive wing-flapping and folding of the wings under the influence of escape.

Early male sexual behavior is observable from the age of one month. It is released by other sitting chicks; it is always incomplete and consists of approach to the rear, mounting, sitting and trampling. Trampling consists of alternating leg movements of high frequency and small amplitude, performed while the mounting bird sits on the other. Early male sexual behavior is usually not accompanied by strong overt aggression or escape. Side display and waltzing do not occur when young cocks perform sexual behavior, even in the stage that these displays occur during fighting. It appears that simultaneous activation of the sexual tendency and strong aggressive and escape tendencies is not yet possible in this stage.

During later stages, (from 12 weeks on), it becomes evident that sexual behavior may be continued despite simultaneous activation of strong aggression and escape: conflicts between fighting and copulatory behavior start to occur. Side display and waltzing now become associated with sexual behavior and two new displays start to appear during social contacts; these displays are caused by simultaneous activation of all three tendencies.

The first of these is tidbitting. It consists of irrelevant feeding movements (ground-pecking and ground-scratching), to which a high rhythmically repeated call is added. The feeding movements appear to be caused in the same way that these movements are caused during conflicts in fighting of earlier stages. The calls are due to the addition of the sexual tendency to this conflict. The calls can be traced back in ontogeny to vocalizations of chicks of a few days old; they are then uttered in feeding situations which also release escape.

The most complex display that finally develops is nesting. It occurs in cocks of nearly 4 months old, when they are able to perform complete copulations. It consists of walking to a corner (possibly an escape component), sitting (probably a copulatory component), stamping with the legs (a compromise of aggressive kicking and sexual trampling) and head-zigzagging (also present during actual fighting). No evidence has yet been obtained which necessitates the postulation of an independent set of causal factors for nesting: nesting may be explained

entirely as the resultant of the interactions of the three conflicting tendencies during this stage.

It can be concluded from this study that the development of social behavior in junglefowl follows the course of increasing interaction and integration of the causal factors underlying attack, escape and sexual behavior. The origin and form of courtship and threat patterns can all be explained on such a view.

After a cock has performed his first successful copulations, overt aggressive behavior toward the female disappears more and more from the courtship situation. Conflicts between aggressive and sexual behavior, if they occur at all, apparently become more and more covert. This suggests that experience with the female influences the final organization of the cock's courtship behavior and indicates the importance of knowledge of the development in attempting to analyse the causation of social behavior.

The influence of social experience on the organization of social behavior is studied in a more general way by raising cocks from the day of hatching in visual isolation of conspecific birds. These isolated individuals behave highly abnormally as compared with group-raised cocks; for example, they develop aggressive behavior directed to parts of their own body. Although most of the species-specific displays develop, they are often released and directed abnormally. Aggressive and escape behavior appear to be less often integrated into composite behavior, indicating that the developing interactions between attack and escape are at least partly due to social experience.

If cocks are kept in isolation from the day of hatching until more than one year old, the ability to perform functional sexual behavior is usually irreversibly lost, whereas comparable isolation in group-raised cocks does not affect this ability. It is possible that this difference is also due to abnormal interactions between aggressive and escape tendencies in the cocks that were socially deprived from the day of hatching.

Thus, although the exact nature of the mechanisms is still unknown, social experience appears to be an important factor in the development of the complex adult organization of social behavior.

TOTAL PATTERNS, LOCAL REFLEXES, OR GRADIENST OF RESPONSE?

BY ZING-YANG KUO

Due to the influence of Charles Sherrington's spinal dog and Pavlov's conditioned reflex of the salivary gland, the reflex concept had dominated the thinking in neurophysiology and psychology nearly for the first thirty years of the 20th century. The movement of behaviorism, led by John B. Watson had made great use of the concept, that is, reflexes were considered as the basic units of instincts and habits. In the early twenties, even Kuo himself in his attempt to repudiate the concept of instinct was not free from the traditional concepts of reflexes (Kuo called them 'The Units of Reaction').

The inevitable assumption of the reflex concept was that the development of behavior was a process of combination of reflexes into more complex behavior (both prenatal and postnatal). But there had been no experimental evidence to support this assumption, nor was it logically sound.

In 1929, G.E. Coghill published a book summarizing the results and his interpretation of his painstaking studies on the development of behavior in Amblystoma in correlation with the histological development of the nervous system, in which he challenged the then current concept of reflex with reference to the development of behavior. Coghill attempted to reverse the sequence of the developmental process, that is, instead of the more extensive patterns of behavior being the result of an integration of local reflexes, Coghill insisted that local reflexes were individuated or freed from the domination of the more massive patterns. Later on, W.F. Windle and his associates, based on their studies on the mammalian fetus and the bird embryo, questioned the Coghillian sequence and proposed to revert to the pre-Coghillian or behavioristic view of development. The controversy was joined by Joseph Barcroft, Davenport Hooker and Leonard Carmichael, with a rather non-extreme interpretation of their own embryonic findings. In 1932 and 1939, Kuo published three papers on this controversy, pointing out that in a given stage of development, whether the response was localized or generalized depended upon a number of factors, physiological as well as environmental, and that both sides of the controversy on the developmental sequence were really beside the point.

However, not only the Coghillian and the anti-Coghillian views, but also Kuo's own were based on the accepted proposition that local reflexes did exist, whether they existed before or after the appearance of total patterns.

It is the purpose of the present paper to present a rather untraditional theory. This theory may be briefly stated as follows:

Behavioral development is neither a process of integration of local reflexes, nor that of the individuation of local reflexes from total pattern. The developmental process is a continuous pro-

cess, a continuous stream of activities which always involves the whole organism. At no moment there are any local movements, independent of the other parts of the organism. In this continuous activities of the whole body, there are always different degrees of intensity in different body-parts as well as in different internal organs. The intensity of activities in some parts may be so subdued or so reduced as to become invisible to the naked eye so that the observer is apt to be misled to consider the more intense or the more predominant activities of certain body-parts as independent local actions.

These differences in various degrees of the intendity of activities involved in the different body-parts and in the different physiological systems are what I am now proposing to call 'Gradients of Response'.

The study of the development of behavior is not a study of reflex, instincts or maturation but a study of the gradients of of different body-parts and different physiological systems in response to a given stimulus in a given environmental setting. As development proceeds, the response gradients are organized and reorganized into more or less definite and stable patterns in accordance with the demands of the environmental settings. In this process of organizing and reorganizing, the activities of certain body-parts are being subdued in various degrees, while some other parts intensified. Thus, a new pattern of response gradients is formed.

As a matter of fact, the formation of response gradients into definite and stable patterns is what has been popularily known as 'Learning Process'. The formation of patterns of response gradients involve two phases: spacial and temporal. In most cases, the learning process, in man and animal requires both spacial and temporal patternization of response gradients.

When the psychologist says that the learning process is complete, or a certain habit is formed, he means in our present theory that the gradient patterns have reached a certain degree of stability. We shall call such a stability the Equilibrium of Gradient Response.

In a given stage of development, the response of an organism to a new situation is determined, to a very great extent, by the accumulation of its previously acquired gradient patterns. ('Early Experience'). Such accumulated gradient patterns of the past may tend to facilitate the formation of a new pattern as required by the new environmental settings, (transference) or to interfere with it.

In the acquisition of a new gradient pattern in a large number of cases, there may be a time limit beyond which the new acquisition may encounter different degrees of difficulty.

When a developing organism is brought into a new environmental setting which requires a new gradient pattern of response, the equilibrium of the previously acquired gradient patterns may

undergo, in different degrees, some disturbances. The duration of the disturbance, or rather, the ability of the organism to restore the equilibrium of its original gradients as well as to regroup the response gradients into a new pattern to meet the demands of the new situation, depends upon, other things being equal, the stability and flexibility of the original gradient patterns and the intensity and complexity of the new stimulating situation. Such an ability of the organism must be measured with quantitative and precision methods so that we can devise objective and precise criteria to determine the degree of normality or abnormality of behavior of the organism in question.

This, I would like to propose to those who are interested in psychiatric problems as a new and quantified approach to the major problems of the behavior abnormality in men and animals.

There are a large number of factors which determine not only the pattern of the response gradients of the organism in a given moment but also the relative intensity of the activities of the individual elements of which the gradient pattern is composed: 1. The developmental history of the organism. 2. Its blood chemistry at the moment of stimulation. 3. The nature and intensity of the stimulating object. 4. The nature of the environmental setting. 5. The position of the body at the moment stimulus is applied. 6. The nature and the quantitative aspects of the immediately preceeding response.

The elements of the response patterns vary very greatly in their intensity of activities (thus forming a gradient pattern). Some of them are very predominant, some less so, others are so subdued as to become imperceptable to the unaided eye. In our ontogenic studies of behavior in the past, we have overlooked the existence and the role of the apparently invisible activities of the parts and almost exclusively observed and recorded the more predominant ones. We have not devised automatic and quantified technique to detect and record such less predominant and even invisible gradients of response. We have not made much use of precision instruments, electronic devises etc. except the motion picture and the tape recorder, either in giving stimulus or in recording response. This is especially true in our investigation of prenatal behavior. Nor have we given sufficient thought to the biochemical problems involved in behavioral ontogeny. Very few ontogenists have applied the recent advances of biochemistry and electronin science and their technics to their investigation of behavior development. Nor have we made sufficient or adequate exploration into the field of physiology of the internal organs in correlation with our observations of the gross behavior of the developing organism. We seemed to have been contented with our work on the purely gross behavior level, grossly observed.

The work on the embryology of behavior accomplished in the 20's and 30's including that of my own should be considered at best as pioneering work. These behavior embryologists including myself had barely scratched the surface of the ontogeny of the basic response patterns. We have left the problems of the biochemistry and physiology of the internal organs in their correlation with embryonic behavior practically untouched. We

seemed to have reached the end of the road as further studies required adequate training of biochemistry and biophysics, which none of us had.

Unfortunately, the developmental work in the last 20 years has been concentrated mainly on the post-natal level and on level of gross response, especially on imprinting and 'early experiences', leaving those problems of biochemistry and biophysics of embryonic behavior behind, if not entirely ignored. The study of behavioral ontogeny as a branch of developmental biology includes both prenatal and post-natal periods. With the recent advances in biochemistry, nuclear physics and electronics which were not available to us twenty-five years ago, we must make the best use of our present knowledge of these sciences and their precision and quantified techniques. I cannot overstress the importance of devising refine and quantified techniques in our analysis, not only the patternization of response gradients but also the analysis of the stimulating object and environmental settings. Recent researches on the gross activities of the developing organism on the effects of early experiences on later behavior etc are important, not in themselves, but as the first step leading to the more refined and quantified study of the biochemical and biophysical aspects of the gross movements. This is the main purpose I propose to serve in this paper.

ANIMAL NAVIGATION

A symposium organized for the
XVI International Congress of Zoology

by

Donald R. Griffin
Biological Laboratories
Harvard University
Cambridge, Massachusetts

A review of orientation studies and their relevance to the problems of bird navigation

G.V.T. Matthews (Wildfowl Trust, Slimbridge, Gloucestershire)

The term 'navigation' may properly describe behaviour whereby, in unknown country, an animal moves in a directed manner towards a goal beyond the range of the senses involved in regular, direct contact with the environment. Some birds appear to have a complete form of navigational ability in that, when displaced in any direction from a previously known area, they return in numbers and at speeds that cannot be attributed to chance. Others, especially young birds, have a more limited form of navigation which nevertheless enables them to reach an area typical for their population but unknown to themselves. These move for a certain distance in a fixed direction, and when displaced continue to move in that direction and so fail to reach the normal area.

In the past fifteen years the nature of the stimuli from which the bird derives information on its position relative to its goal, or on the simple direction of the migration axis, has been investigated by studying their initial orientations. These were given by the bearings on which the birds disappeared from sight after release or by the directions they took up when confined in a round cage. There were tacit assumptions that homeward orientation indicated awareness of the goal position, and that orientation coinciding with the migration direction implied intention to proceed along that axis. The birds' behaviour and the environmental factors were under simultaneous observation, open to manipulation, and their interaction could be easily studied. Those factors encountered in the course of a migration or homing journey would on the contrary be largely unknown.

Migration orientation in cages by day was shown to derive from the sun's position. To extend the period of observation beyond the migration season the technique of training birds to feed in one direction was introduced. Analysis of this trained orientation showed that it was at an angle to the sun's position in azimuth, varying according to the time of day. The necessary internal 'clock' was accurate but readily changed in phase with a new day/night rhythm. Whether adjustments are made for variations in azimuth speed, both diurnal and seasonal, is not clear.

Migration orientation in cages by night was observed under starry skies and then under the artificial stars of a planetarium. When stars of the 'wrong' season were presented disorientation or conflict behaviour resulted, suggesting that a complex interpretation of the star pattern and one not confined to circumpolar stars, was concerned. Claims that certain individuals were able to obtain a geographical

'position' from the planetarium stars were strongly criticised.

Homeward orientation of free-flying Manx Shearwaters displaced from their nests was reported, as it was for Homing Pigeons. In the latter there was a conflict of evidence regarding the speed with which the orientation was taken up. On the one hand it was claimed to emerge slowly, within a matter of minutes, on the other to be almost immediate. Since both forms broke down with overcast skies, the time elapsed was critical for deciding whether the birds could be interpreting the sun's position in both altitude and azimuth to obtain information on their geographical position relative to that of their goal. However the swiftly orientating pigeons homed much better from one direction and it became apparent that they had a strong directional bias in their initial orientation, to the north in the main stock used. Such orientation was therefore not necessarily relevant to the navigational process, though it might facilitate it in some way.

Similar irrelevant directional tendencies were reported in Common Terns removed from their nests and in migrating Mallard and other wildfowl. In a non-migratory stock of Mallard a very strong north-west tendency was demonstrated to occur throughout the season, regardless of age, sex, topography or distance and direction if displacement. Few of these birds homed from any distance and the orientation was apparently maintained for a short time since subsequent recoveries were scattered at random. The orientation was not a simple escape tendency and did not seem to be concerned with the reassembly of a scattered flock. Different stocks of Mallard had different 'nonsense' orientations and the indications were that such differences were congenital.

Although the biological significance of such 'nonsense' orientations are still obscure they impinge directly on the study of bird navigation. In the first place they provide a constant orientation for study in the field and so free from the artifacts resulting from confinement in a small cage. Moreover the same species, Mallard, can be studied by both day and night. The main conclusions of the laboratory work concerning orientation at a changing angle to the sun's position in azimuth have been confirmed and light thrown on the way the regulating mechanism works. Orientation by the stars has been obtained throughout the season and without the need for a knowledge of the time. This argues for the determination of direction from the alignment of the constellations. Even the moon's position in azimuth can

apparently be used for orientation when the stars are hidden.

In the second place the existence of 'nonsense' orientations has emphasised the need for extreme care before there is assumption that navigational ability has been demonstrated. Separation of different stocks released at the same point is no longer sufficient evidence. A minimal requirement would seem to be that birds from the same stock, preferably the same individuals, show homeward orientation from at least two very different directions. Nevertheless there is still sufficient critical experimental orientation evidence, coupled with long distance homing returns, to assert the existence of complete navigational ability in some birds of certain species. As far as understanding this ability is concerned we are but little in advance of the situation ten years ago.

GOLDEN PLOVER MIGRATION, ITS EVOLUTION AND ORIENTATION.

E. G. Franz Sauer, Department of Biology, University of Florida, Gainesville, Florida

The Golden Plovers consist of two species, Pluvialis apricaria and Pluvialis dominica which inhabit the boreal circumpolar region. Both species have been split into two subspecies. The migrations of these plovers between their breeding grounds and wintering quarters are, at least, race-specific and reflect a long evolutionary history. It may date back to Pre-Pleistocene times when the Subfamily Charadriinae originated probably in the tropics or subtropics. From there, those birds would have spread northward that gave rise to the common ancestral form of the Golden Plovers which lived in Siberia. This ancestral form of the Golden Plovers, which also gave rise to the Black-bellied Plover at the beginning of the Pleistocene, might have shown the following evolutionary steps during the glacial period. Some birds escaped the glaciers by moving west along the border of the tundra toward Europe. There, after isolation from the Siberian ancestral form, Pluvialis apricaria probably speciated during an interglacial period; later it split into the two subspecies apricaria and altifrons. Another branch of the ancestral form probably moved eastward into the Bering Sea refuge. The advance-guard of this branch might have spent the last glaciation in the Alaskan refuge from which it subsequently invaded the arctic regions to the east as Pluvialis dominica dominica. The remaining population expanded westward as Pluvialis dominica fulva after the glaciers receded. In postglacial times Pluvialis apricaria(altifrons) ranged eastward and met Pluvialis dominica (fulva) in western Siberia. By then, both forms were specifically distinct, but possible hybridization between the two has been reported recently.

An attempt is made to demonstrate that the present migratory patterns of the Golden Plovers reflect the historic movements of their ancestors. While the assumed preglacial northward movements of the earliest ancestors of the Golden Plovers are probably of great significance in relation to the origin of their migratory movements and orientation mechanisms, the glacial and postglacial south-north movements would have enforced, re-established, and modified the original pattern. The movements to the east and to the west may reflect earlier range expansions of the ancient population and they are most likely indicative of the movements of these plovers during the glacial and interglacial periods.

In an analysis of the mechanisms of orientation on which the recent migration of Pluvialis dominica fulva is based, it

became evident that these birds are capable of a time-compensated visual celestial orientation. The visual clues obtained from the sun during daytime and from the starry sky during night are sufficient to orient the migration of these birds toward their seasonal goals. Ten time-shifted and geographically displaced birds, that had been under experimental control from soon after hatching, gave evidence in controlled cage-experiments that their celestial navigation is based on a bi-coordinate celestial grid, unless, during daytime, the sky is overcast to a degree which forces the birds into an azimuth orientation whereby they probably neglect the altitudinal component of the sun. The experiments also gave proof of the existence of at least two sensitive phases during which the birds learned the geographical positions of their breeding and wintering areas. With this ability to modify their race-specific and evidently genetically determined pattern of migration by learning, these Golden Plovers reach a level of efficiency which is beyond pure instinct and which is of great adaptive value for their flight across the Pacific Ocean.

ACOUSTICAL ORIENTATION

Donald R. Griffin, Harvard University

Sound waves are used for orientation by several groups of mammals, ranging from whales to bats. In addition to acoustic communication between individuals, many species achieve by means of echoes an effective sort of communication with their environment. This type of orientation, conveniently called echolocation, was first discovered in bats, but it has since been found in cave dwelling birds, in certain terrestrial mammals (including blind men), and it has reached a highly developed form in marine mammals. At first echolocation was assumed to be merely a warning system enabling the animal to avoid collisions with stationary obstacles. But recent experiments have shown that it is also used by insectivorous bats to locate flying insects and by fish-catching bats to locate fish. It is not yet clear whether fish are located by Noctilio leporinus even when they are entirely below the surface or only when they produce some disturbance of or projection above the surface.

While echolocation is effective only over relatively short distances, the entire lives of most bats of the suborder Microchiroptera are conditioned by its advantages and limitations. While we still know much less about echolocation in the order Cetacea than in bats (because of the obvious difficulties of experimental research with the former), the powers of discrimination displayed by porpoises such as Tursiops when relying on echolocation are quite sufficient to demonstrate that this mode of perception is also of major importance in the behavior of these mammals. Within the past few years many significant investigations of echolocation have been concerned with the acuity and information handling capacities of the echolocation systems that have evolved in bats and in marine mammals as part ot their adaptation to various ways of life.

Recent investigations by my colleagues (D. Cahlander, D. C. Dunning, N. Durlach, J. Friend, A. D. Grinnell, D. Hayman, C. Michael, C. Slater and F. A. Webster) have shown that small objects such as fine wires and insects as small as Drosophila are located by bats at distances of 0.5 to 2 meters. The distance of detection has been determined by analysis of the vocal reactions of flying bats, in which the repetition rate of their pulsed orientation sounds increases on approach to a small or important object such as insect prey, obstacles difficult to dodge, or a surface on which the bat is preparing to land. These adjustments of the orientation sounds can best be appreciated through sound films in which the motion of the bat is slowed and the orientation sounds are reproduced synchronously with the same proportional change in time base. This procedure, which will be demonstrated at the symposium, not only allows one to see

important details of the orientation behavior of the bats which normally occur too fast for the human visual system to resolve, but also to hear in literal translation the otherwise inaudible orientation sounds. Not only the interfemoral membrane but also the wings themselves are used in an almost prehensile fashion to grasp insects that are then transferred a fraction of a second later to the mouth.

Recent experiments have demonstrated at least some of the capacities for discrimination between similar objects that bats can achieve by echolocation. For example, some Myotis lucifugus have learned to distinguish reliably between Tenebrio larvae (which they catch after the mealworms have been tossed into the air) and spheres or disks of approximately the same dimensions. In one experiment disks that were larger or smaller than a mealworm were interspersed with actual mealworms in an irregular sequence, and after several days of practice the bats caught mealworms (2 to 3 mm diameter, 16-23 mm length) in 93% of 278 trials, while thin disks (0.5 mm X 4.25 -- or 15.9 mm) were touched only 31% of 249 trials. In the same experiment thick disks (3 mm X 15.9 or 17.8 mm) were touched in only 22% of 250 trials.

This and similar experiments indicate that these bats can discriminate between small moving targets on the basis of their shapes, or at least on the basis of differences between the properties of their echoes. This discrimination was equally successful in the dark, and since the targets moved through the air relatively slowly they generated no sounds that could permit their identification by passive listening. Olfaction was rendered most unlikely as a basis for this discrimination because of the speed of the interception maneuvers, and disks smeared with the body fluids of mealworms were not caught appreciably more often than unscented disks. These discriminations were achieved by echolocation despite the fact that both mealworms and disks rotated or tumbled as they moved through the air.

Finally the large and specialized auditory areas of the brains of bats have been studied by neurophysiological methods by A. D. Grinnell. The results show considerable specialization for processing information from echoes of the bats' high frequency orientation sounds. Especially significant are cells that respond selectively to pulses of sound arriving from a certain direction and cells that are more sensitive to a second pulse of sound if it follows a first pulse by a few milliseconds. Such mechanisms may facilitate a bat's reactions to faint echoes following an emitted pulse of orientation sound.

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