NASA TECHNICAL TRANSLATION NASA TT F-11,780

NASA TT F-11,780

FURTHER INVESTIGATIONS ON THE SEXUALITY PROBLEM

R. Hertwig

GPO PRICE $ _____

CSFTI PRICE(S) $ _____

Hard copy (HC) 3.00

Microfiche (MF) .65

ff 653 July 65

Translation of "Weitere Untersuchungen uber das Sexualitatsproblem".
Verhandlungen der Deutschen Zoologischen Gesellschaft auf der sechzehnten Jahresversammlung zu Marburg, den 5. bis 7. Juni 1906.
Wilhelm Engelmann Press, Leipzig, pp.90-112, 1906

FACILITY FORM 602

N68-30530 (ACCESSION NUMBER)

(THRU)

32 (PAGES)

1 (CODE)

(NASA CR OR TMX OR AD NUMBER)

04 (CATEGORY)

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 JUNE 1968

NASA TT F-11,780

FURTHER INVESTIGATIONS ON THE SEXUALITY PROBLEM*

R. Hertwig

ABSTRACT. The sexuality problem, as it relates to protozoa, Dinophilus apatris, daphnids, and Rana temporaria, is discussed from the standpoint of the development of the genital organs of these organisms, by which their sex is identified. The effect of the nuclear-plasmic relationship is also considered, and a mathematical expression is developed for the effect of this relationship on sexual development.

The investigations, which I would like to report here, relate to the /90† lecture which I gave last year at our meeting in Breslau on the sexuality problem. At that time I was able to report on work which was undertaken partly by myself and partly by several of my students, in order to determine whether influences which have an effect on the nuclear plasmic relationships in protozoa could modify the sexual ratio in multicellular animals (cold and /91 heat, hunger and abundant feeding, prolonged autogenous development). Several different animals were used as investigational subjects: Baron von Malsen used *Dinophilus apatris*, Issakovitch used daphnids, and I used *Rana temporaria* and *Rana esculenta*. In my experiments the influence of the state of maturity of eggs had shown itself to be particularly effective. When I produced a premature transfer of eggs into the uterus by a ligature belt on *Rana temporaria* females or by means of pairing of females of *Rana esculenta* from the area of Munich with Italian males of the same species, only a small percentage of the eggs developed. These produced exclusively males. Likewise I obtained almost exclusively males and to be sure in great quantities, when I artificially fertilized eggs which were postmature, i.e. which had remained for a long time in the uterus, so that females separated from males began to lay eggs spontaneously.

In the spring of 1905 I began my experiments once again and in fact with the support of my private assistant, Dr. H. Prandtl, who took over the most tedious care of the cultures for the most part. Since only a portion of the comprehensive material has been collated, I will restrict myself today to representing the results which we have obtained from postmature eggs.

---

*From a lecture given by Prof. R. Hertwig, Munich; Transactions of the German Zoological Society at their Seventeenth Annual Meeting at Rostock and Lubeck, 21-23 May, 1907.

†Numbers in the margin indicate pagination in the original text.

To further clarify the following I must give some introduction on the development of the sex glands of the batrachia. These developed left and right of the vertebral columnas a genital ridge which begins at the upper end of the kidneys, bordered here by a fat body, and caudally stretches perhaps to two-thirds of the length of the kidneys. The originally thin thread acquires rose garland shaped swellings, 10-12 in number, by developing spaces in it. As far as I have been able to follow its origin, these are invaginations of the coelom which, however, are later completely pinched off. The spaces are known to be maintained in the female for a long time and produce pockets which can be found in the interior of the ovary, while in the male they developed into testicular canals and the longitudinal canal which connects these, but are first narrowed to solid cell strands by the rapidly growing genital cells.

In the conversion of the genital strands into testes or ovaries, males and females can be distinguished with the magnifying glass at a very early stage. In female toads and frogs (Bufo rulgaris, R. temporia, R. esculenta) /92 the genital anlage develops in typical cases along its entire length into a wrinkled, folded and lobed appearing organ. In toads the foremost portion which abuts on the fat body becomes the so-called Bidder organ, a typical ovary, which is remarkable however in that it does not function and produces no ripe eggs, which is why many authors have unjustly considered it an ovary.

In the male sex there are very unusual differences between toads on one hand and frogs (especially R. esculenta) on the other hand. In toads the foremost end of the genital ridge develops without exception into the Bidder organ, thus a seeming rudimentary ovary. I found that the rest of the genital glands in young male animals which were caught in the wild at the end of August developed into a seeming cord along its entire original length, which is sharply distinct from the Bidder organ and is differentiated from it not only with loupe examination, but also with microscopic examination of cut sections. With loupe examination the differentiation is based on less thickness, while no large cells clearly recognizable by their blastocysts are detected microscopically. I have not yet had time to examine the fully formed glands of the male. According to the results of Knapp whom we must thank for the most recent and most exact description of the Bidder organ and its relationship to the testis, the circumstances vary according to the individual male and in fact similarly between the the various types of toads. In the majority of males the testis comes close to the Bidder organ as a short pressed body; in other male the same pressed body lies further caudally on the kidney from the Bidder organ separated by more or less intervening space. This in-between space is taken up by a kind of secondary Bidder organ apart of the genital ridge in which no eggs have developed. I assume from these findings that the genital ridge in toads can develop in variable fashion beyond the stage which I studied. Either the third of the genital ridge which touches the Bidder organ develops into a testis which atrophies in its caudal portion, or the portion which becomes the testis develops in the direction of an ovary and produces the secondary Bidder organ and the portion which would otherwise atrophy becomes a testis.

According to this the cryptohermaphroditism apparent in male toads would /93 be variably separated and accordingly also the intensity of the tendency in the genital glands to develop towards the female direction. This tendency is predominant over the genital ridge in its complete extent when the polliwogs develop into female frogs, while in typical males it is dominant only over the foremost end

of the anlage and stimulates the development of the Bidder organ, while the rest of the anlage experiences a less striking growth and disappears at the back end by means of regression, f o r m i n g testes in the portion bordering on the Bidder organ. The masculine genital ridge shows a tendency in comparison to the female toward rudimentation, which proceeds from the caudal end to the foremost end. If this rudimentation is not very energetic then the hindmost end of the genital ridge remains and becomes a testis, and the portion which would otherwise develop into a testis becomes a secondary Bidder organ, of variable size, according to individuals. This is a question of the significance of incomplete developmental stages studied; I hope however to be able to reinforce these findings with a collection of material. There is some support in my experiment with the testicular development in frogs.

Rana esculenta is the most suitable for studying the stage of development of the genital glands. In this species the difference between males and females can be seen very early. In normal cultures many polliwogs whose hind extremities are still minute, but which are already showing pentadactyl differentiation of anlage can be determined sexually with confidence; it would be desirable however in investigations where the determination of the sex is one of the problems, to allow the culture to continue for a time until the hind extremities have become a powerful paddling organ. Even then there are some forms among which a macroscopic investigation of the sexual glands is not sufficient to differentiate; this delay can extend so far in some individuals that they have to be kept for a long time even after metamorphosis, in order to be certain. I have found this delay in the development of sex glands in cultures for which I have no reason; they occur, as we will see, always in animals which are raised from postmature eggs, but it appears to me also, however, from prematurely ripe eggs.

Rana temporaria is much more unfavorable for sex determination. Other /94
circumstances are apparent, to which Pfluger has referred, which are in and of themselves very interesting for the discussion of the sexuality problem, but which make recognition of the sex at the present time very difficult with our present insufficient knowledge.

I will now discuss the typical sex differentiation as it occurs in Rana esculenta. In this case only the foremost portion of the genital ridge develops which corresponds approximately to the three or four foremost rose garland shaped swellings, into testes which atrophy in the rear. The hind end can be seen in young testis anlage to string itself out and residues of the rose garland shaped swellings can still be recognized. The testis itself is a flattened body, superficially constricted at first, later developing into an oval rounded body, which is usually separated from the fat body by a clear constriction, but sometimes merges without constriction. Since these bodies are of very variable size in animals of the same culture, in some cases twice as long as in others, I assume that the length of the genital ridge used in development was variable fom the very beginning.

Let us compare the testicular development of Rana esculenta with that of the bufonides in which case we can say that in the case of the latter the rudimentation which was mentioned earlier in the sexual ridge of the male animals has made even further strides. The entire length in bufonides has disappeared whether the foremost or hindmost becomes a testis, and only that portion which is female under all circumstances in toads and which--if we refer to

differentiation of male sex organs in this sense--offers the strongest resistance to the progressing rudimentation, i.e. the region of the Bidder organ, is converted into testis.

And now we come to the most characteristic sexual relationships of Rana temporaria, in which species, which offer difficulties in sexual determination are unbelievably frequent. I must refer here to earlier investigations on sex determination in frogs. When Born studied frog larvae under various conditions of nutrition he found in all of his cultures approximately the same sex ratio and indeed a ratio which differed very markedly from that of nature. In toto he cultured 1,443 animals, of which 1,371 females and 72 males, thus approximately 95% females and 5% males, while in nature he found roughly equal numbers of males and females. He draws significance from his findings in the sense that some special quality in his kind of culture must have brought about this enormous displacement of the sexual ratio. Pfluger /95 contradicts this interpretation; first he tried to explain Born's results from a markedly increased mortality of the male sex. Born had raised only 1,443 frog larvae from 8,000 frogs; the rest--almost 5/6--had died. There only need have been 2/6 of the females and 3/6 of the males and the unusual sex ratio would be explained. However when Pfluger himself cultured frogs in a mass fashion he came to another conclusion. In cultures from Rana temporaria from the neighborhood of Bonn he found a sex ratio of 35% males to 65% females, from the neighborhood of Utrecht 13% males and 87% females, and from the region around Konigsberg 51.6% females and 48.4% males. He found these same percentage figures when he tested for the sex of frogs from corresponding stages of development and corresponding origin, which however had grown up in the wild. Since it is extremely unlikely that the mortality of males and females in different areas is different he looked for another explanation. In all areas the results for animals three or more years old was very close to the 50:50 ratio. Since this equalization in the three necessary years before sexual maturity in frogs cannot be well explained by varying mortality, Pfluger suggested the hypothesis that the excess of females which was derived from determinations of freshly metamorphosed frogs, has its cause in a more or less developed tendency towards hermaphroditism. Among the 65% of animals in the Bonn region which were characterized as females about 15% were hermaphrodites which became males later. A confirmation of this hypothesis was seen in the fact that even a year after the metamorphosis a normal sex ratio was achieved.

As many other investigators, who have concerned themselves with the sexual differentiation and development of frogs, I have come to a conclusion which confirms the Pfluger opinion on the basis of my own investigations. In the course of my investigations I was completely surprised at how sparse the number of undoubted males there was. I determined until now only a few smaller cultures among whom, however, all animals were cultivated sometime beyond metamorphosis in the presence of abundant food. I found in one such culture 43 females to 18 males, in another even 47 females to 8 males, and of 59 trapped in nature which had apparently metamorphosed some weeks before I found 37 females and 23 males.

As I studied the females more closely, however, I found that the ovary was developed in its normal length in only a portion of the animals and had the /96 striking wrinkles of the normal ovary, while in the others I found that the ovary was more or less regressed in its hindmost portion so that only a half to a third of the genital ridge had developed. At microscopic examination many of the eggs were in a stage of regression; in other cases the eggs could not be found,

while the central space, however, was distended and had sent diverticulae towards the periphery, so that I got the impression that the central space had spread at the expense of the regressing ova material. The genital cells which surrounded the space showed an indifferent character, or, to be more precise, a condition which did not enable me to decide whether they were eggs or sperm cells. Certainly this is a case of indubitable rudimentation of sexual glands in contrast to typical females. This is, however, not as definitive as in typical males. I assume that in such forms the sex glands next develop into nonfunctional ovaries, in which then the eggs regress, while the newly growing section of sex cells supplies sperm material. These frogs with rudimentary ovaries correspond without doubt to those forms which Pfluger had called hermaphrodites. I do not think this t e r m is appropriate for expressing the essence of the phenomenon. I believe that this characteristic can be better articulated if the word protogynaecia is used. It is certainly correct that in amphibia there is a persistent cryptohermaphoditism which is not unusual (aside from toads and the Bidder organ), in so far as rudimentary eggs lie imbedded in the center of testes, as has been observed by various investigators including myself in sharks, river crayfish, and other forms of animals which have separate sexes. However it is not certain whether this cryptohermaphoditism is a residuum of the above described protogynaecia or whether it is independent of that.

If I now consider animals with rudimentary protogynaecia as males, the above mentioned counts would be somewhat different; there would be 10-15% fewer females and correspondingly more males. Nevertheless, the count of females would still be large in comparison to the sex ratio of mature animals; it must therefore be assumed that many forms characterized as typical females must transform into males.

The problem which Pfluger asked on the basis of his statistical investigation requires further investigations. The transformation of an ovary anlage /97
into testiswhich has become apparent through my observations must be pursued step by step. Apparently it takes place in the time between metamorphosis and the following summer. I have already started some of my students working on this question.

After these very detailed remarks which, however, were necessary for understanding what follows I will now proceed to a discussion of my investigations on postmature eggs. I used both Rana temporaria as well as Rana esculenta.

Three pairs of Rana temporaria which were brought to me on the 21st of March for whom of course I cannot give the beginning date of their pairing nor the duration of their pairing in the wild, were separated on the 25th of March. One female began to lay eggs on the 31st of March. I used the egg material which was remaining in the uterus--there were about 500 eggs--for artificial fertilization. I will designate these in the following as A. On the night of the 2nd to 3rd of April a second female laid her eggs and that material remaining in the uterus was fertilized early on April 3 (material B). On April 3 the third female which showed no tendency to spawn was killed and all the material was fertilized (material C). Material B we can ignore, since about 300 eggs decomposed prematurely; of 130 hatched animals about 90 were non-viable; on March 17 there were only 40 animals still alive which also very soon died. Of the the two other remaining materials first we will discuss C and then A. I assume from the circumstance that one of the females showed no voluntary disposition to spawn that the eggs, although they were used later

for artificial fertilization, had not been retained in the uterus as long as A. The results of my investigation also are in agreement.

Material C was divided into two portions after fertilization, one portion raised in the heat at 20° C. and the other portion at a temperature of 10° C. The warm culture died very quickly so that not a single larva was obtained while the cold culture developed unfavorably as well; 120 eggs did not develop into larvae; of 340 hatched larvae only 74 achieved metamorphosis, in contrast to the culture results of Born; nevertheless this was a favorable result. Of the 72 frogs whose sex was tested 38 were clearly males, 23 clearly females, and 11 showed the circumstance in the sexual organ which I referred to as rudimentary proterogynaecia and later male quality. Under all circum- /98
stances I attempted to obtain a large displacement of the normal sex ratio in favor of males.

Material A yielded an even greater postmaturity than the material C. Of the 460 eggs remaining in the uterus after spawning I obtained 266 larvae, of which 218 were raised to metamorphosis. The sexual apparatus of these animals had remained extraordinarily behind in its development. In 169 examples I found only a genital ridge or the rose garland form of sexual glands, in 30 individuals a beginning stage, and in 19 individuals a clearer stage of transformation of the genital ridge to ovary, but only in the region of the upper part of the genital ridge, as in proterogynic forms. If I now consider that Rana temporaria characterized by a striking tendency for eggs in the genital glands to develop early, that in the case in question the animals farthest along in sex development could only have reached the stage designated as proterogynaecia of the sexual apparatus, I come to the conclusion that the entire culture was male.

My experiments done with Rana temporaria permit one objection, i.e. that the condition which brings about a departure from the norm of the sexual apparatus of frogs cultured from postmature eggs is not caused by postmaturity of the eggs but rather is a characteristic of the eggs in question from the very beginning. From the very start, this objection had little merit. For considering the many cultures which I and o t h e r s have reported which all yielded the previously described preponderance of females, it would hardly be possible that in my experiments with postmaturity of eggs I obtained twice and, including the case of last year which was referred to, even three times females which had only eggs predestined to be males. Nevertheless it was desirable to carry out further experiments to meet this objection by so arranging the experiment that I could compare the apparent sexual ratio of a normal oviposition with the sex ratio of postmature eggs. For such an experiment R. esculenta offer the best preconditions, in that it does not lay its eggs all at once but rather intermittently in small piles. The spawning procedure therefore can be interupted after a portion of the eggs have been laid, males and females separated and artificial fertilization performed several days later. In this way the sex ratio of the eggs which were laid in the /99
natural way and those of the postmature artificially fertilized eggs can be determined and compared to each other.

In such experiments, designed and performed for the first time, a certain amount of experience must be obtained; and so it went for me. In two experiments the intervals between the first and last fertilization were too great. In the first fertilization I obtained one time absolutely none and the other time only two larvae appropriate for culture, the latter being males. Two

other experiments gave me so much difficulty at sex determination not only with the first fertilized group, but also the late fertilized eggs, that I had to delay the investigation until I had become more familiar with the different circumstances of the sex organs of frogs. Unfortunately there is a paucity of usuable investigations in this area. There are indeed investigations on the development of the ovary from the genital ridge, however there are no investigations on the corresponding stages of testis development. The latter has been cited for many years as the next investigation to be done, but as much as I am familiar with the literature and the handbook of embryology vertebrates I find no references to the work having been done. However, as long as the developmental history of the testis is not exactly described, then the investigations of the developmental history of the ovary with respect to its earlier stages are unreliable. For then there is a possibility that the early stages of testis development were described as early stages of ovary development. After all, as I have mentioned above, the sex differentiation of frogs is a very difficult problem.

I was fortunate in a fifth experiment and I will confine my report from this point on to that experiment. First, however, a few words on the usual sex ratio of R. esculenta. I will explain this on the basis of an especially favorable culture experiment for which a nest taken from nature was used. This contained embryos just before hatching, and was raised partly at room temperature and partly at 30° C. The sex was determined for 786 partly metamorphosed and partly premetamorphosis animals (taken early because of the early differentiation of the sex glands). The animals were killed and the sex was determined; the determination yielded 352 females, 380 males, and 54 proterogynic forms.

The female which I used for the experiment on postmaturity laid 140 eggs naturally on June 1 and was separated from the male. On June 3 in the /100
evening it laid a large quantity of eggs and I decided on June 4 to try to fertilize these artificially. Because the male which belonged to this pairing got out and among the other males, I cannot say with certainty whether the male which I selected by eyesight for artificial fertilization was the same male or not. I found remaining in the uterus about 400 eggs, of which over half did not hatch. Of the larvae which hatched about 70 died prematurely; 97 could be sex-determined, therefore about 1/4 of the starting material. Of the 140 first fertilized eggs 79 developed, therefore about 4/7, so far that they could be killed for sex-determination. Since at that time I was still of the opinion that Rana esculenta could be sexed in larvae with well developed hind legs, I did not wait for completion of metamorphosis in about 2/3 of the material, only later raising them to complete metamorphosis. Investigations on sex ratio of the cultures gave the following results.

Of 79 animals of the normal culture, 47 were females with developed ovaries, 32 typical males; it was a question therefore of a culture with a relatively high percentage of females. Of the 97 animals of the postmature culture only one animal had sex glands which gave me an impression of ovaries. But at microscopic investigation I found no eggs, so that I was of the opinion that this animal had begun to develop into a female and then, however, the eggs had regressed and it was in the midst of transformation to a male. The rest of the 96 I considered to be males; for all those which metamorphosed and also a portion which did not metamorphose there can be no doubt; for the sex glands in this case had the form of a typical even though minutely small testis.

Among the rest only the foremost portion of the genital anlage which bordered directly on the fat body, developed, and this often only as a thin thread. I consider it entirely impossible that from such an anlage anything could develop except the testis. Moreover, the anlage in older animals, which were able to complete metamorphosis, developed in fact into testes.

From these cultural results which I have reported I can draw two conclusions.

I. Postmaturity of the egg leads to rudimentation of the sexual glands. This rudimentation expresses itself in two ways, 1) timewise in so far as the sex glands develop much more slowly in comparison to the rest of the body, 2) /101 spatially in so far as the hind end of the sex glands often is not present or perhaps regressed prematurely, concerning which further, more extensive experiments are necessary. This rudimentation of the sex glands is so striking, especially in our case of the culture of Rana esculenta discussed above in which the young frogs demonstrated an especially vigorous growth and were significantly larger than normal animals at a corresponding stage of development. We have before us a size difference which similiarly occurs between cold and warm animals. On the average the frog larvae raised in the cold are also significantly larger than those which were raised at higher temperatures. The fat body which lies in the region of genital glands behaves in this regard like a part of the body; it develops enormously in most cases of animals with rudimentary sex glands.

II. Egg postmaturity lead to development of male animals. This statement cannot be expressed with the same certainty as the first. For of the three cultures discussed in this lecture specifically that culture, culture A of Rana temporaria, which contained the most individuals, was not without flaw. There was no single example in this culture which could have been characterized for certain as a male by the presence of typical testes. Only because, with few exceptions which bore the character of protogynaecia described in detail above, all individuals demonstrated the same characteristic, a high degree of developmental inhibition of the sex apparatus, I assumed that all individuals were of the same sex in which case it could only be the male sex. This interpretation fits into the framework of the phenomena. As I have already discussed, the development of the testes from the genital ridge demonstrates a greater rudimentaion in comparison to the development of ovaries. In male animals the fat body in general is much more developed than in females and also the body size more significant, which is true also for animals with a developmentally inhibited genital ridge. The more significant body size of males at corresponding stages, especially at the stage of metamorphosis, is a more striking phenomenon, since it is known that the size relationship of both sexes is later reversed, in that females surpass the males in growth, so that fully grown females are significantly larger than fully grown males. If we now further consider that the eggs of a very mature female yielded almost exclusively males in the previous year then we can defend the statement with great certainty, that there exists between postmaturity of eggs and development /102 of male progeny some kind of connection.

How can this relation be explained? There seem to be two a priori possibilities. In accordance with one explanation the result would rest on selection of postmaturity: it would then be stated, which in fact corresponds to the prevalent opinion that there are female and male determined eggs.

It would have to be assumed further that female eggs are more sensitive to the damaging effects of postmaturity and therefore die earlier than male eggs. This explanation would be plausible since in all postmature cultures the mortality is high enough to explain entirely the disappearance of the female; it is not probable insofar as in general experience suggests that the female sex is more resistant at earlier stages than later in cultures of normal eggs, in which a high mortality prevailed likewise, in which case it is found that the female sex is not more decimated than the male.

According to the second possibility, which I favor, the disappearance of females in postmature cultures would rest on the fact that eggs which under normal conditions would have yielded females, experienced an alteration by means of postmaturity so that now they yield male animals. In order to make my preferred explanation understandable I must refer to the theoretical discussions on the problem of sexuality which I discussed last year. There is a conflict here that the development of an egg, either into a female or a male or even into an hermaphrodite, rests on the appearance, separated or simultaneously, of specific male or female substances from which the egg derives an inalterable character from the beginning. The assumption of such specific sex substances would be a hypothesis which has no real basis. According to my interpretation, the difference in the sexes rests much more with the different regulation of the cell, i.e. on a different exchange relationship of its most important components, the nuclear substance in the protoplasm.

We can now view the interpretation as sufficient, that for every organism there is a certain mean size ratio of nucleus and protoplasm, which I have named "the nuclear-plasmic relationship" and which I have expressed as the following quotient, K/P (mass of the nuclear substance/mass /103
of the protoplasm). We know further that the nuclear-plasmic relationship in mature eggs is displaced in favor of the protoplasm and in spermatozoa in favor of the nucleus. In this difference in cell regulation rests the only common marker for all the individual cases of sexual differentiation. That this different cell regulation finally comes about and leads to different sexes is a consequence, on one hand of the characteristic of the egg cell which yields the organism and, on the other hand undergoes influences which have an effect on the egg cell and further on its descendants the blastomeres and the body cells; in this regard those influences ought to be considered which derive from the spermatozoa, moreover those influences which are affected by temperature and nutrition after fertilization. Now there will be egg cells in which from the very beginning there is such a firmly fixed nuclear-plasmic relationship that no alteration is possible in its sexual characteristics, be it through fertilization or through other types of effects. In this regard I might mention the small eggs of the rotatoria, daphnides, dinophilus, etc., which always yield only males. On the other hand, there will be eggs, however, and indeed this is apparently the rule, which are responsive to a greater or lesser degree to sexual determination. Eggs with a high degree of labile sexual equilibrium appear, as we know, to include the eggs of amphibia. Such eggs will clearly yield the same sex if they have the same nuclear-plasmic relationship from the beginning and are raised under equivalent conditions; if they are exposed, however, to influences which have varying effects on the nuclear-plasmic relationship, then males or females will derive from them corresponding to the type of effects. In this way

there arises the possibility of sexual transformation, that eggs which would have yielded one sex under normal conditions change their sex determination under especially strong influences. We know that unfertilized bee eggs yield males and that they are transformed by fertilization to female eggs. Just as such transformations can be shown in one direction, then a priori a transformation in the opposite sense, transformation of female eggs to male eggs, is possible.

According to my opinion which is based on the characteristics of sexual products, eggs which are relatively poor in nuclear substance would yield females, in constrast chromatin-rich eggs would yield males. We can express this thought in the following formula: $\frac{K}{P} = ♀$; $\frac{K + k}{P} = ♂$. Thus /104 it would be unimportant whether at the beginning of development the nuclear-plasmic relationship given in the above formula was actually present or only in the form of anlage. By anlage I mean that nucleus and protoplasm are synchronized in their activity to each other in such a way that the nuclear-plasmic relationship relevant for males and females can be achieved in the course of development, even if they are not apparent at the time of fertilization from a strictly quantitative point of view.

If I had made the assumption above that eggs of frogs experience a sex transformation by means of postmaturity by which female eggs are converted to male eggs, then it must be assumed that an actual or at least potential displacement of the nuclear-plasmic relationship occurs in favor of the nucleus by means of postmaturity. In my lecture in Breslau I have already traced this change to an induced tendency in the egg to parthenogenetic development. Parthenogenesis is an autogenous development and, similar to autogenous development among protozoa, will favor an increase of the nuclear substance at the expense of the protoplasm; thus I can refer to previous discussions on this point. Quite clearly the mode of influence will extend to all eggs, not only those disposed towards the female but also those disposed towards the male. When the former change their nuclear-plasmic relationship from $\frac{K}{P}$ to $\frac{K + k}{P}$, then the latter will experience a conversion from $\frac{K + k}{P}$ to $\frac{K + 2k}{P}$; they will reach the limit of normal developmental possibility and exceed this in many cases. For we know that a cleavage process presupposes a high degree of nuclear-plasmic tension, an emormous difference of nuclear mass and plasma mass, which is equilibrated gradually by means of a large number of divisions. If the nuclear-plasmic tension is insufficient from the beginning because of an overwhelming accumulation of nuclear substance (actual nuclear hypertrophy of the fertilized egg), or it is equilibrated too early during the cleavage process by means of an overenthusiastic growth of the nuclear substance (potential nuclear hypertrophy of the fertilized egg), then development must come to a standstill; there must be inhibitions of development corresponding to the degree of nuclear hypertrophy sooner or later. In fact the postmature eggs which have not achieved normal development /105 demonstrate the markers of developmental inhibition in the described fashion,

especially clearly at the stage of gastrulation.[1] On the occasion of my lecture in Breslau I have already referred to the developmental inhibiting effect of an increased nuclear-plasmic relationship, to be sure from another point of view at that time, i.e. to explain why animals arise from the small eggs of rotatoria and dinophilus, which are not only male but also rudimentary. I believe that these effects of different nuclear masses on the course of ontogenetic processes have received too little attention in the scientific evaluation of results of developmental physiology.

My arguments might be reproached for their very hypothetical nature. I myself would not like to dispute this, but at the same time I would like to suggest that all these hypotheses which I make are subject to experimental proof. I myself have taken the opportunity and encouraged several of my students, to attack individual problems and to undertake more exact studies than it has possible for me so far. I would be very happy if other colleagues in this area would participate in these investigations.

This spring as well as on other occasions I have begun new investigations with Dr. Prandtl on the relationships between the sex ratio and the degree of maturity of eggs, which take into account the following points of view. If early maturity of eggs on the one hand and postmaturity of eggs on the other hand favor the development of male progeny, then the optimum for the female sex must lie between both extremes. The tendency to yield the female sex must thereby demonstrate an ascending and descending curve. Now, since experience shows that in a normal oviposition in a nest both sexes are represented, then I am forced to the assumption that eggs of one frog exist in various stages of maturity and thus behave very differently at any given moment.[2] These /106
sexuality curves of individual eggs which are not accessible to investigation will therefore combine to make a cumulative sexuality curve of the total egg mass of a frog which can be controlled experimentally. If two fertilizations,

---

[1]The question whether developmental inhibitions are caused by nuclear hypertrophy is subject to experimental investigation. In this case it is a question of the developmental situation in the cell which can be brought about not only by autogenous development but also by the effects of cold. Investigations in the Munich Zoological Institute are in process in both directions which have led to reproducible results

[2]That the eggs of one and the same nest of a frog are different from one another derives from the results of extremely large numbers of cultures which I have raised. If the larvae of a single nest are placed under very favorable and equal conditions and cultivated at such an abundant nutritional level that there can be no question of lack of nurishment, it is completely impossible to obtain homogeneous cultures, several larvae lagging behind in growth and others hurrying ahead so that onset of metamorphosis occurs over several months. It also appears completely impossible to me to raise all individuals of a culture. I believe that even the different eggs of one and the same frog adapt to various conditions of existence, just as exists between the eggs of different female frogs.

derived from different times, are tested for their sexual ratio, there is no certainty that a correct impression of the periodic changes of sex ratios is obtained; for example two corresponding points on the ascending and descending parts of the curve could be obtained and thereby an equal sex ratio, although a change in the egg material has taken place in the meantime. Therefore we have tried to obtain not only two but three and four fertilizations in intervals of 24 hours from a single pair of frogs. We have been successful in this for 7 different pairs of Rana esculenta so that now we can figure on having the necessary material for solving the question. With the help of this material and from the way in which the sex ratio is displaced it will certainly be possible to decide whether the decline in the numbers of females with post-maturity is based on a transformation of female eggs to male eggs or mortality amoung the female eggs. I think that I can decide on the former possibility with great probability already and indeed with reference to the result which was obtained in the summer of 1904 with slightly overripe eggs. This is the culture which I was able to report on in my lecture in Breslau. This one yielded the most favorable results of all the cultures begun at that time, so that the sex ratio could not be explained from the mortality of one specific category of forms. In this case there was an enormous percentage of male animals. I found only 13 females vs. 317 males.

A year ago I was not aware that postmaturity resulted in a rudimentation of sex apparatus. Moreover I was accustomed to find among females of Rana esculenta well before metamorphosis a richly wrinkled ovary, so that I called the compressed sex glands of the appropriate postmature animals of cultures taken far beyond metamorphosis testes without question. With /107
reference to my experiences on the rudimentation effect of postmaturity therefore I have found it necessary to recheck the cultures again. Since time has been short I have been able to review only 213 forms designated as males in the few days before Pentecost. Among these there were 131 typical males, a few examples having had still the elongated sex glands without the characteristic folding of the ovaries, therefore showed a typical inhibition formation, the rest, perhaps 33%, having the structure of proterogynic forms: only the upper end of the genital ridge developed, this being slightly folded; at microscopic investigation eggs in the stages of regression and a large central space. I believe that the peculiarity of the discovery confirms my opinion that postmaturity brings about a sexual transformation of eggs, that the 33% of animals with abnormal sex glands were females in transition to males and that in fact my results given in Breslau over sex ratios in the culture were right. And so I have the certain hope that the exact investigations begun this year will yield a confirmation of my opinion concerning the sex transformability of egg cells.

The investigations carried out in the past year on the sexual difference of mature and postmature eggs of Rana esculenta went in a still different direction from the starting point of the experiments. In the case discussed I had no certainty that the males used in both fertilizations were the very same; therefore all possibilities were extant, that the use of another male might have caused the apparent change of the sex ratio. That led to the question whether the spermatozoa at all could have such a high degree of influence on sex.

This question is discussed considerably in the literature. Usually it has been attempted to solve this on the basis of statistical analyses and

usually without results. The prevalent interpretation which is supposedly justified, however without sufficient grounds, denies any possible sex-determining influence on the part of the spermatozoa. An attempt to decide the question on the basis of experimantal investigation, which would certainly lead to that end, has not been made until now, as far as I can determine. And yet the decisive experiments are easy to perform in this case; all that has to be done is to fertilize the eggs of the same frog or female fish with sperm /108 from different males and to test the sex ratio of parallel cultures so obtained.

We have undertaken simultaneously a double experiment. We used the eggs of two females, one of which came from Schleissheim, the other from Lochhausen in the Dachau bogs; the egg material of the first might be designated S and the second L. The sperm which were used for the fertilization were taken from the three males from Schleissheim and three from Lochhausen; they might be designated by s^1, s^2, s^3 and 1^1, 1^2, 1^3. Ten fertilizations were carried out and therefore ten cultures started. The combinations used derived from the following summary.

$S.s^1$	$L.s^1$
$S.s^2$	$L.s^2$
$S.1^1$	$L.1^1$
$S.1^2$	$L.1^2$
$S.1^3$	$L.s^3$

I must still remark that the Schleissheim frogs were significantly behind the Lochhausen frogs in sexual maturity and differed from them in size and general habitus so that in crosses of both forms I can refer to them as "bastards" in short. The phenomenon that all of the cultures begun with Schleissheimer females developed much more poorly than the corresponding ones deriving from Lochhausen females can be explained on the basis of insufficient maturity; in one case the mortality was greater and in the second case the development very delayed. In addition to this influence derived from the eggs there was another influence on the part of the spermatozoa. Eggs of one and the same animal developed significantly differently according to whether they were fertilized by one or another male. That we are not dealing with chance phenomena, but rather with a very energetic influence derived from the sperm is derived from the fact that the stratification dependent on the sperm with regard to favorability or unfavorability of development recurred in the same fashion in Schleissheim and Lochhausen egg material. Thus $L.1^2$ and $L.s^2$ developed particularly well closely followed by $S.1^2$ and $L.s^2$, while poorer cultures included $L.1^1$ and $L.s^1$, but above all $S.1^1$ and $S.s^1$. Of the Lochhausen cultures $L.s^3$ developed the worst while $S.1^3$ gave mediocre results. These relationships would best be explained by referring to a graph, in which the results of June 2 are summarized; in the upper row all of the results of the cultures until now are entered including dead eggs or larvae, and in /109 the lower row, those larvae still alive on June 2.

$L.1^2$	$L.s^2$	$L.1^1$	$L.s^1$	$L.s^3$	$S.s^2$	$S.1^2$	$S.s^1$	$S.1^3$	$S.1^1$
59	117	96	124	212	100	215	150	221	249
606	557	369	317	310	227	217	182	361	187

The lesser total counts of the Schleissheim culture can be explained from the fact that a rather major portion of eggs fell to the ground during artificial fertilization and thus were not used. The following results may be derived from the table, part of which has already been discussed:

1) that the Schleissheim egg material in contrast to the Locchausen material was numeriacally less;

S material of all cultures	living	1,174
	dead	935
L material of all cultures	living	2,159
	dead	608

2) that crossing Schleissheim and Lochhausen animals was unfavorable. For let us ignore cultures S.1[3] and L.s[3] in order to compare two bastard cultures with two normal cultures, so that we obtain for the ratio of bastard cultures to normal cultures the following counts:

	S		L	
	Bastards	Normal	Bastards	Normal
dead	436	250	241	155
living	578	409	874	975

Furthermore, the same results emerge from the fact that the best Lochhausen culture is a normal culture L.1[2] $\begin{cases} 59 \\ 606 \end{cases}$, and likewise the best Schleissheim culture S.s[2] $\begin{cases} 100 \\ 227 \end{cases}$. Likewise, the worst cultures in both cases are bastard cultures L.s[1] $\begin{cases} 124 \\ 317 \end{cases}$, S.1[1] $\begin{cases} 249 \\ 187 \end{cases}$.

3) the result that, completely ignoring the effect of bastardization, the spermatozoa of certain males had an unfavorable effect. Of the Lochhausen male the one designated as 1[1] apparently had a particularly unfavorable effect.

$$L.1^1 \begin{cases} \text{dead} & 96 \\ \text{living} & 369 \end{cases}, \quad S.1^1 \begin{cases} 249 \\ 187 \end{cases},$$

of the Schleissheim males s[1] and s[3]

$$L.s^1 \begin{cases} 124 \\ 317 \end{cases}, \quad S.s^1 \begin{cases} 150 \\ 182 \end{cases}, \quad L.s^3 \begin{cases} 212 \\ 310 \end{cases}.$$

An interesting question arises here whether, considering favorability /110
or unfavorabilty of the development, both egg and sperm have equal shares, whether this share in influence is greater on the part of one sex cell or the other, furthermore, whether influences derived from bastardization, unfavorability of the eggs or spermatozoa are relevant during all periods of development and equally much. The results which we have obtained with the ten cultures cited above, suggest that the latter is not the case.[3]

[3]Since June 2 it can be stated that many cultures have taken an especially unfavorable direction of development, so that for example, culture L.s[1] which fell from 317 to 102 living animals, S.s[1] (from 182 to 44), L.s[3] (from 310 to 97). In general it is always more relevant that eggs fertilized by Schleissheim males developed unfavorably (aside from S.[2]), apparently because they were behind in sex development.

Yet, they are insufficient to decide the difficult question how the results reported so far have value only for orientation and not only for frogs, but must be tested on other animals as well.[4]

Nevertheless the result is now already secure, which by the way is completely in agreement with experience in medicine, that the characteristics of the spermatozoa have a great significance for the healthy course of development. Under these conditions it may be considered that the characteristics of spermatozoa also exercise a sex-determining influence. Hopefully the experiments begun so far will give us further information.

P.S. I would like to utilize this oportunity to respond to several critical remarks which Wilson made in "Science" concerning my Breslau lecture. Wilson considers the sexuality theory which I set forth to be untenable. To this point I only have one thing to remark, that I did not use the designation "theory" myself. I only discussed the chain of thoughts which led me to my experiment. Whether these are confirmed and lead to the development of a theory, only the future can tell.

Wilson introduces among other things an observation made by him in the field as a fact which speaks against my theory, that the fertilization with spermatazoa, which have one chromosome too few (the accessory chromosome) produce male animals among the bugs. I do not understand this objective. /111
I have suggested in my Breslau lecture how I interpret the influence of parthenogenesis on sex among frogs which is so unrecognizable that parthenogenesis belongs to the "autogenous" developments, that these favor the formation of the male sex. An amphigenetic development must approach a parthenogenetic one that much more, the more the formation of the male sex is favored, the more the effect of the spermatozoa is weakened. That would occur in oligopyrenic spermatazoa and to the the highest degree in apyrenic spermatozoa, in which indeed a typical fertilization could not occur at all, but rather one equivalent to a parthenogenetic process. If in bugs the male animals arise from eggs which are fertilized by spermatozoa without the accessory chromosome, i.e. oligopyrenic spermatozoa, then this is a confirmation of my interpretations, not a contradiction.

Discussion:

Dr. Escherich (Strassburg):

refers to the most recent experiments of Miss Fielde on parthenogenesis in ants. In contrast to Reichenbach she came to the result that ♂♂ develop exclusively from parthenogenetic eggs of workers and from ♀♀, that therefore the same relationships occur in ants as in frogs. On just what these divergent results of Reichenbach are based (perhaps fertilization of the workers?) must be illuminated by further experiments.

---

[4]Fish might be the most suitable animals for these experiments. I have agreed with my colleague Hofer to extend the experiments jointly to fish, also concerning sex determination.

Prof Hertwig:

considers it not ruled out that females could arise from parthenogenetic eggs of ants, just as in daphnides and aphides many generations of parthenogenetic females arise without any males occuring. Apparently eggs in different groups of insects are regulated in different fashion. Among the frogs it would be sufficient to omit fertilization once, in order to get male animals, while in daphnides and aphides it is necessary to obtain the cumulative effect of many partenogenetic generations, in order to convert the nuclear-plasmic relationship characteristic of the female egg into female (sic).

Prof Schulze (Berlin):

refers to the fact discovered by Korschelt that in Dinophilus apatris /112
the small egg spores in protoplasm become males which supports the interpretation of the lecturer.

Prof Hertwig:

agrees with the speaker and emphasizes that the special interest which refers to Dinophilus apatris on the sex problem, has stimulated him to entrust one of his students, Baron von Malsen, with the experimental investigation of sex ratios in Dinophilus.

Dr. Gross (Giessen):

states that partenogenesis in insects does not always yield males but rather in many cases only or overwhelmingly females. In a parthenogenetic culture over three generations of Liparis variety the count of males grew smaller and smaller from generation to generation.

Prof. Hertwig:

repeats in connection with these remarks, which he had made in a discussion with Dr. Escherich, that the genesis of females from parthenogenetic eggs of and by itself does not contradict his observations; in contrast, a major flaw would exist here if it were to be confirmed that the count of males decreased or of females increased in successive parthenogenetic generations. At first this result would seem to stand in contradiction to all of the experiences made thus far in insects.

Translated for the National Aeronautics and Space Administration under Contract No. NASw-1695 by Techtran Corporation, PO Box 729, Glen Burnie, Md. 21061