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FINAL REPORT

THE UTILIZATION OF HABROBRACON AND ARTEMIA AS EXPERIMENTAL MATERIALS IN BIOASTRONAUTIC STUDIES

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General Introduction

The fundamental purpose of these investigations was attained when both Habrobracon adults and Artemia cysts survived the orbital space flight of Biosatellite II to provide the data summarized on pages 17 to 40 of NASA volume SP-204, 1971, THE EXPERIMENTS OF BIOSATELLITE II, edited by J. F. Saunders. The present final report addresses itself to questions raised by the results of the flight and to matters of concern in future space flight research with invertebrate organisms. It is organized into three separate parts, each containing sections on specific methods, results, discussion, conclusions and references.

Respectively the three parts concern

PART I

HABROBRACON FEMALE FECUNDITY AND FERTILITY STUDIES

Introduction

In the reproductive performance of female braconids striking contrasts were revealed between the results from the actual biosatellite flight and those from experiments when the recovered vehicle was subjected to the forces of simulated launching and recovery (Grosch 1970). Second week decreases in egg production due to the radiation damage of cells in mitosis were minimized for the females irradiated during space flight. Figure 1 from page 95, volume 9 (1970), Mutation Research, reproduced here, demonstrates that females irradiated for two days during orbital flight laid as many eggs during the second week as the unirradiated ground-based controls. Furthermore after the 10th day their oviposition records exceeded control values. As shown in Table I-1 the hatchability of eggs deposited by Biosatellite II females was excellent. Explanations are sought for the space flight's cancellation of the characteristic radiation-induced decrease in egg production and for the exceptionally good hatchability of eggs derived from most of the cell types in the irradiated ovarioles. Eggs from only two classes of cells showed enhanced embryonic lethality: (a) those poised in meiotic metaphase during their mother's orbital flight, and (b) those from oocytes beginning vitellogenesis. Depending upon cell type and criterion of effect, enhanced, null, and antagonistic effects were demonstrable for cells of the braconid ovary.

All braconids survived every experiment in which the biosatellite capsule was used, and their life spans after such experiments were excellent. This signified good somatic fitness for the wasps and demonstrated their compatibility with the other biological specimens packed into the capsule. No modifications in shape and size of the Habrobracon package have been suggested by subsequent investigations.

Although the postflight experiments at Ames Research Center failed to provide an explanation of the space flight's antagonism to radiation damage, certain aspects of the flight could not be reproduced. Weightlessness is an obvious aspect, but another which could be important is the immediate transition from one phase of flight to another. In the Ames simulation there were unavoidable delays in shifting from treatment with one type of equipment to another. Periods of half an hour or longer intervened between vibration and centrifugation, and in turn between centrifugation and the start of irradiation. Avoiding such delays could be an important consideration if disturbed cell contents can normalize within seconds or minutes under the influence of normal gravity.

In our research using equipment on the North Carolina State University campus, we have devised a procedure which avoids time lapses.

The system involves a battery powered rotator or clinostat on a cart which enables transport of experimental groups of wasps from the vibrator to the irradiator without a pause in the stress situation. The rotating device is turned on simultaneously when the vibrator is turned off. The present report summarizes the results of preliminary experiments accomplished in this study and emphasizes the final series of experiments in which female braconids were put through a time-sequence of simulated flight stress profile: vibration and centrifugation g-force (to simulate launch stresses) — gamma irradiation during gravity compensation (i.e., horizontal clinostat rotation) — centrifugation g-force and vibration (to simulate re-entry stresses).

Preliminary investigations included data from preflight experiments begun in 1965 at the Ames Research Center (Status Reports of NsG-678, 1966). Additional sets of experiments were performed at North Carolina State University on females subjected to centrifugation up to 20 g and to vibration frequencies as high as 120 cycles per second (Grosch 1966). These experiments established that the results obtained with rotating devices and vibration equipment are reproducible. Furthermore, the same patterns of reproductive performance obtained from the Ames experiments were obtainable on the North Carolina State University campus in Raleigh.

At no time were prolonged studies of reproductive performance made off campus. Braconids were returned to Raleigh, North Carolina in hand luggage on commercial air flights from Florida, Hawaii or California in the course of the various experiments. A correspondence between results from treatments on campus with those subjected to post-treatment transportation by jet plane ruled out any unsuspected influence of the conditions experienced during commercial air flights. Perhaps most important was the demonstration of a moderate radiation protection of oocytes and oogonia caused by brief periods of vibration and centrifugation. But the radiation induced valley reflecting the vulnerability of the mitotic series attendant differentiation from oogonia to oocytes and associated cells always remained. The purpose of the final set of experiments was to determine if rotation during two days of protracted gamma irradiation would cancel out the destruction of potential eggs and improve hatchability of the eggs laid, when applied in combination with optimum simulation of the flight stress influences to which Biosatellite II organisms were exposed.

Materials and Methods

Habrobracon carrying wild type genes for all aspects of reproductive behavior and performance have been employed for all NASA supported experiments. For experiments reported here, virgin females of wild type stock No. 33 were dissected from their cocoons just before eclosion. After treatment each female was placed in an individual stender dish

Table I-1. The hatchability of eggs laid by Habrobracon females from the Biosatellite II experiments. Means ± standard errors.

Gamma Radiation (R)	Days of Oviposition						
	3	4	5	6	7-10	11-15	16-21
Ground-Based Control Wasps							
1.7	97.3±1.6	98.2±1.2	95.0±4.2	92.9±2.4	91.9±2.8	80.8±2.8	85.7±3.7
371	96.5±1.7	96.5±1.6	86.0±2.6	96.4±1.9	85.7±3.2	83.3±3.9	87.3±3.6
729	97.7±1.0	92.6±2.1	81.1±2.5	95.8±1.2	83.7±4.3	84.8±3.7	90.5±1.7
1245	91.2±2.2	88.1±3.5	61.5±7.0	69.4±7.3	87.1±3.4	78.9±3.2	91.1±1.9
2431	93.1±1.7	82.1±3.9	59.3±7.1	62.3±7.5	85.1±4.2	83.6±3.1	86.0±3.5
Flight Wasps							
0.7	85.4±3.1	87.7±2.4	90.4±6.4	95.7±1.2	96.1±1.2	88.9±1.5	91.0±2.3
356	28.0±6.0	81.2±3.9	91.4±4.4	93.9±1.2	93.9±1.5	89.5±2.7	90.8±1.6
589	17.9±7.0	81.0±4.3	75.0±4.4	95.2±2.5	94.2±2.1	88.0±2.4	76.6±1.6
1272	37.7±7.7	75.2±3.9	85.0±4.7	94.1±2.8	94.0±1.7	86.0±1.9	84.4±1.3
2425	40.9±7.5	70.7±3.8	91.2±3.5	96.9±1.5	92.5±1.8	86.5±1.9	89.3±1.7

containing two paralyzed (stung) host caterpillars (Anagasta kuehniella). The eggs deposited on the hosts were collected daily for 20 days and their hatchability determined after incubation in mineral oil. Unhatched eggs were categorized by stage of death according to the scheme of Von Borstel and Reckemeyer (1959). After each egg collection the more flaccid host was replaced with one freshly prestung.

Synchron Timing Motors (Herbach and Rodaman, Inc., Philadelphia) were employed for clinostat rotation and centrifugation. In the former category the speeds of rotation tested were 6, 60, and 180 RPM. For centrifugation at the desired level of 8 g, a radius of 7.53 cm was used with a 300 RPM motor, and a radius of 1.99 cm with a 600 RPM motor. These motors were mounted on a sheet metal stand in turn attached to a 3/4 inch plywood base. A single screw in the center of the base enabled attachment of the unit to the vibrating platform. To hold capsules of wasps a circular lucite plate was secured to the motor shaft by a set screw.

The vibration facilities of the Mechanical Engineering Department of the University were used. Of the systems available the one finally selected consisted of a Model Ea 1250 Exciter and Vibramate Vibrator, a Model 2120 MB Power Amplifier, both manufactured by M. B. Electronics of New Haven, Conn., along with a Random Noise Generator Type 1402 obtained from Bruel & Kjaer, Naerum, Denmark.

Fast transitions were made possible by powering the rotators by a light weight AC source, "terado Trav-Electric" Model 50-160 (terado Corp. St. Paul, Minnesota). This consisted of a 12 volt battery, battery charger, meter and DC-AC Inverter that produces 117 V AC at 60 cps. A three-way switch enabled instant exchange of power supplies from the battery-inverter to a wall outlet during the 2 days of irradiation. Once packed into capsules and attached to a lucite disc, a sample of wasps could be carried through an entire sequence of treatments without interruption. A styrofoam box was adapted for transporting rotators with wasp samples between rooms and buildings. Temperature was monitored with a maximum-minimum thermometer.

Gamma radiation was supplied as a beam from the port of a Cobalt 60 source at the dose rate of 48 R per hour. The rotating sample was positioned in a predetermined location before the port was opened.

A Ph.D. thesis by Philip D. Buchanan at North Carolina State University, 1970, was based upon the effects of each influence alone and in combinations with 2-day gamma ray exposures. An adequate range of rates of rotation were tested in combination with mechanical vibrations and 2-day gamma ray exposures, but characteristic radiation induced decreases in oviposition and lethality persisted. Additional experiments summarized for a status report for January to June 1970 on NASA grant NGR-002-023 demonstrated that either vibration or centrifugation prolonged

through 2 days of irradiation enhanced the radiation damage. Both egg production and hatchability were lowered up to 10% for all daily data by centrifuging females at 300 revolutions per minute (6 g) during a 2 day gamma ray exposure (total dose 2200 R). Longitudinal random vibration ranging through a frequency scale of 10 to 10,000 Hertz caused a consistent daily decrease in egg production of about 33% when delivered to the mothers during a 2 day exposure to gamma rays (total dose 2200 R). Consistent with the theory of induction of chromosomal aberration, prolongation of the centrifugation beyond the 2.5 minutes corresponding to the peak period of acceleration for the Biosatellite is likely to be deleterious. On the other hand, 1 minute of vibration exceeds the period of high vibration in the Biosatellite profile. However, since aerial retrieval prolonged the period of vibrational stress, a determination of the effects of 15 minutes of vibration was a matter of interest (see Group 1 below).

A further detail of concern if all environmental aspects of the Biosatellite experiments were to be checked, was the possible influence of a cooling interlude. Not only were the temporary laboratories at Hickham field cold during the unloading of Biosatellite II, but Habrobracon females were stored temporarily in a 5° C refrigerator after an initial period of oviposition. This well-tested holding procedure for adults enabled the technicians to turn their attention to the groups of males. In retrospect it seemed desirable to perform a test for detectable influences of cooling in combination with other stresses experienced by the females (see Group 11 below).

The plane of clinostat rotation made no striking difference in the pattern of egg production for females rotated during 2 days of irradiation (Grosch 1971). As shown in Figure I-1 the characteristic radiation induced valley was obtained. Table I-2 gives hatchability data summarized with respect to cell types at the time of treatment and maternal physiology at the time of oviposition. With adult wasps free to assume any position they may prefer within their container these results were not unexpected.

The final experiment combined the selection of treatments considered optimum in this sequence:

Vibrate	Centrifuge	Rotate 60 RPM			Centrifuge	Vibrate
10-10,000 Hz	600 RPM force 8 g	Before	During Co-60 2300 R	After	600 RPM force 8 g	10-10,000 Hz
1 min.	2.5 min.	8 min.	2 days	8 min.	2.5 min.	1 min.

Environmental conditions included a temperature of 24° C and a relative humidity of 65% which corresponds to the limit specifications set for the biosatellite based upon the limitations of other organisms employed.

Table I-2. The hatchability of Braconid eggs laid by samples of virgin females exposed to Co^{60} gamma rays for two days with and without rotation in two different planes.

Cell type at time of treatment	Differentiated Oocytes	Mitotic Transitional Cells	Undifferentiated Oogonia	Undifferentiated Oogonia
Period in days	1 - 5	6 - 10	11 - 15	16 - 20
Maternal physiology at oviposition	Youth	Prime	Middle age	Senility
Samples				
1. Radiation	84.4 ± 1.2	76.8 ± 1.6	76.7 ± 1.5	68.9 ± 2.3
2. Radiation and Vertical Clinostat Rotation	86.5 ± 1.2	72.7 ± 1.7	73.4 ± 1.5	70.2 ± 2.4
3. Radiation and Horizontal Clinostat Rotation	85.3 ± 1.2	68.0 ± 2.1	67.1 ± 2.0	67.5 ± 2.8
4. Control	94.3 ± 0.8	85.9 ± 1.4	92.6 ± 1.7	82.9 ± 3.1

This final experiment was comprised of 11 groups, 20 wasps each, treated in the following ways:

Group	Treatment
1	Complete sequence. The length of each vibration period was 15 minutes.
2	Complete sequence. The length of each vibration period was 1 minute.
3	No vibration. Otherwise identical to Group 2.
4	No rotation during irradiation. Otherwise identical to Group 2.
5	Eight minute pause before and after rotation and irradiation. Otherwise identical to Group 2.
6	Control (untreated).
7	Radiation only.
8	1 minute vibration--2 days irradiation--1 minute vibration.
9	No rotation during irradiation. Otherwise identical to Group 2.
10	Neither rotation nor pause before starting rotation and irradiation. Otherwise identical to Group 2.
11	Identical with Group 2 except that females were refrigerated at 5°C for six hours after metaphase and prophase classes of eggs were laid.

Data were obtained for three criteria of damage:

1. Life span as a measure of somatic fitness.

2. Daily egg production as a measure of cell proliferation and gross nuclear damage leading to resorption, and

3. Egg hatchability as a measure of localized damage to chromosomes and genes. The stage of embryonic development achieved before death, important for diagnosing lethal genetic action, is recorded at the time hatchability is scored.

In order to study eggs derived from all the cell types in an ovariole, daily egg collections were made for 20 days after unpacking the treated females from their capsules. For H. juglandis this takes the study well into senility for the wasps.

The series of cell types in the wasp ovariole provide cytological material especially suitable for making comparisons and contrasts of the mode of action of destructive and mutagenic influences. In contrast to the multiplicity of structure in other insects, there are only two ovarioles (egg tubes) per braconid ovary and these function synchronously. Each ovariole contains a single developmental sequence ranging from oocytes in first meiotic metaphase to interphase oogonia. Between these two extreme cell types is a series of 16 to 20 oocytes in ever earlier prophase each

enclosed with its group of nurse cells within a follicle. Five successive mitotic divisions are required to produce a cyst of interconnected cells from a single oogonium. Thus, overall the sequence is comprised of differentiated, transitional, and stem cells. Eggs deposited at any determined time subsequent to treatment of the mother can be traced back to the cytological condition of the cell type treated. Different characteristic alterations in the pattern of egg production enable distinguishing cell destruction from mitotic delay. Induced genetic lethality is measured quantitatively by embryonic deaths. Further analysis is facilitated by the ease of recognition of the stage of embryogenesis achieved before death. Since normal males are produced parthenogenetically there is no block against embryonic development in unfertilized eggs with only a haploid chromosomal complement. Thus recessive lethality as well as dominant lethality is expressed in the offspring of virgin females.

Results

Life Span. In days, the mean life spans and associated standard errors for the females of the 11 groups are:

Group		Group		
1	17.7 ± 1.4	6	Control	16.3 ± 1.4
2	19.4 ± 1.0	7	Radiation Only	16.0 ± 1.4
3	19.4 ± 1.3	8		17.0 ± 1.6
4	17.3 ± 1.1	9		18.8 ± 1.3
5	20.3 ± 1.0	10		16.4 ± 1.9
		11	Refrigerated	15.5 ± 1.1

Values equal to or higher than the control value attest to the somatic fitness of all experimental groups. The lowest mean was obtained for the Group 11 females subjected to a 6 hour interruption of incubation after 36 hours. After refrigeration at 5°C they were returned to host caterpillars and kept in the same incubator with other groups for the remainder of the 20-day study. The highest mean obtained with Group 5 came from the sample allowed an 8 minute pause after the "launching" and before the "recovery" influences.

Oviposition. Figure I-2 presents a summary of egg production based upon the daily averages per female of the live wasps comprising each group. Occurring within the shaded area is an intertwined cluster of curves from Groups 1,2,3,4,5,7, and 11, not reproduced individually because of the cluttered appearance. The seven curves are very similar in shape, and lying within plus or minus a standard error of each other do not differ significantly. The valley encompassing days 5 through 9 with the nadir on day 6 is due to the characteristic radiosensitivity of mitotically active cell types. Potential eggs have been lost by destruction of the cells

from which they were to be derived. Only the modest radioprotective influence of vibration in the absence of any other influences (Group 8) ameliorated the cell damage reflected in the induced valley.

Group 10 showed poorer egg production than other groups during the first four days, while Group 9 showed better egg production during the last two weeks than the other irradiated groups. The daily differences between the points plotted for Group 10 and Group 9 are statistically significant. This contrast indicates that in combination with other factors, rotation at 60 RPM during irradiation is more damaging to potential gametes than the radiation delivered to a stationary capsule of wasps.

Hatchability. The success in completing embryonic development has been summarized by calculating the mean egg hatchability and associated standard error for the five day periods corresponding to cytological states at the start of the experiments. Eggs laid during days 1 to 5 were oocytes accompanied by nurse cells during the course of treatment. Those laid on days 6 to 10 were derived from mitotically active transitional cells, and the eggs laid after the 10th day were derived from the oogonia of treated females. Eggs deposited after the 15th day are considered separately from those laid from days 11 through 15 because females have entered their senile decline by day 15.

Table I-3 gives the entire summary. To facilitate comparisons, pairs and groups of data from the table have been plotted on separate graphs. Groups 1 and 2 provided nearly identical patterns of hatchability as shown in Figure I-3. Figure I-4 contrasts control hatchability with that of Groups 9 and 10. This makes evident the superior hatchability of eggs from wasps not rotated during irradiation (Group 9) when compared that for wasps rotated during irradiation (Group 10). Figure I-5 compares the hatchability of eggs from Group 8 with the equivalent data from Groups 3, 4, and 5. Again as in egg production there was a modest protective effect of vibration before and after irradiation (Group 8).

Stages of Death. The morphology of dead embryos formalized into stages of death (Von Borstel and Reckemeyer 1959) enables a trained observer to distinguish the effective lethal stage. It may be attained during cleavage (Stages 1, 2), at yolk consolidation and midgut formation (Stage 3), or in the ultimate steps of larval differentiation (Stage 4). Inability to complete the emergence from the shell is a 5th Stage. A summary in terms of the percentage of total deaths occurring in each stage is presented in Table I-4. Since there were very few deaths during hatching, Stage 5 deaths will receive no further consideration.

As shown, most of the lethality expressed itself in either of two stages, either during Stage 1, 2 or during Stage 4. Stage 3 deaths accounted for a modest percentage of deaths during the first 5 days, but decreased to low values for subsequent periods. The bar graph, Figure I-6,

provides a basis for visual comparisons in the different groups and in the successive periods of reproductive life. Most evident is the relatively low percentage of Stage 1 death in all experimental groups for eggs laid from the 6th through the 10th day.

In comparison with results from the first 5 days, the control group decreased only 7.2%, while Group 7 (Radiation Only) fell 24.5%. Most of the other groups decreased 13 to 20%. However, Groups 5 and 8 increased in the percentage of Stage 1 deaths during days 6 to 10. Furthermore, during the subsequent period, days 11-15, the percentage of Stage 1 deaths was even higher but, in general, for the last periods of life, the proportion of Stage 1 deaths was higher than during the first 5 days for most groups.

The reciprocal relationship between the percentages of embryos dying in cleavage (Stages 1 and 2) and of those dying during final differentiation (Stage 4) has been emphasized by preparing a composite illustration from data plotted for all the experimental groups. A complex entwined bundle of lines results when connections between individual data points are drawn. For clarity this is better represented by the outline of the bundle of interlaced lines as shown in Figure 1-7. Only the 3 points drawn for group 8 were outside the clusters of plotted means. All other mean values for treated groups lie within plus or minus one standard error of each other, indicating that the additional stresses caused no significant differences from the results of radiation alone. Except for the eggs deposited from the 6th to 10th days there was a high proportion of Stage 1 deaths and a low proportion of Stage 4 deaths. At the time of treatment the cells from which this group of eggs was derived were undergoing mitosis, a process which can screen out many chromosomal defects.

Discussion

In order to duplicate the Biosatellite II results it will be necessary to obtain egg production and the hatchability of irradiated groups at rates equal to or better than control values except during the first and third days on host caterpillars. The females performing in such fashion should exhibit a tendency to live two days longer than the controls. Neither the simulated flight experiments at Ames Research Center (Grosch 1970) nor the efforts reported above have succeeded in duplicating the space flight results. Ground-based simulations of flight stress profiles produced data showing the usual radiosensitivity of cells progressing through mitosis and transitional differentiation, expressed as a trough during second week egg production. In addition the hatchability of deposited eggs was poor. Analysis of the degree of embryonic development achieved before death adds another contrast. Stage 1 deaths, absent from the Biosatellite wasp data for the last two weeks of oviposition are conspicuous in the present experiments and predominant during the last two weeks of effective reproductive life.

Table I-3. A summary of mean hatchability with standard errors for eggs laid by control wasps (Group 6) and 10 other groups of females after receiving various combinations of vibration, rotation, and irradiation. Data are pooled by 5-day periods of egg deposit which reflect the age of the female at the time of oviposition as well as the cell categories at the time of treatment. The later the day of egg deposit the earlier its precursor cell type at time of treatment.

Cell types	Oocytes	Transitional	Oogonia	
Maternal Age	Youth	Prime	Middle Age	Senility
Days of Oviposition	1 - 5	6 - 10	11 - 15	16 - 20
Treatment Groups				
1	93.3 ± 7.7	90.1 ± 1.9	82.0 ± 2.7	64.0 ± 4.9
2	89.3 ± 2.2	90.9 ± 1.8	83.6 ± 2.5	84.9 ± 3.5
3	77.7 ± 1.9	57.6 ± 3.7	67.9 ± 3.6	61.3 ± 4.6
4	76.9 ± 2.7	65.0 ± 3.5	66.7 ± 3.0	70.6 ± 4.5
5	78.1 ± 1.7	46.4 ± 3.9	40.4 ± 3.2	43.3 ± 4.6
6	77.2 ± 2.7	47.9 ± 4.1	50.7 ± 4.0	59.6 ± 4.8
7	73.6 ± 2.8	54.2 ± 3.8	47.5 ± 3.9	49.2 ± 3.8
8	87.9 ± 2.5	94.9 ± 0.9	88.0 ± 2.8	78.7 ± 3.8
9	89.9 ± 1.8	92.5 ± 0.9	82.2 ± 4.5	68.9 ± 4.7
10	94.3 ± 1.2	92.3 ± 1.7	78.4 ± 3.4	72.9 ± 4.4
11	76.4 ± 2.9	72.4 ± 2.7	59.6 ± 3.7	39.0 ± 7.1

Table I-4. The unhatched eggs of Table 1 classified according to the percentage of embryos dying during cleavage (1,2), yolk consolidation and gut formation (3), final differentiation (4), or hatching (5).

	GROUP 1				GROUP 2				GROUP 3				GROUP 4			
Days	1,2	3	4	5	1,2	3	4	5	1,2	3	4	5	1,2	3	4	5
1-5	56.4	20.6	23.0		64.3	14.3	20.6	0.8	70.2	21.1	16.9	0.8	65.6	14.1	19.8	0.5
6-10	42.8	2.3	54.4	0.5	43.6	2.5	53.9		38.4	5.2	55.2	1.2	43.4	3.4	53.2	
11-15	70.9	3.5	24.0	1.6	71.7	4.4	23.8		44.5	4.8	30.2	0.4	72.9	5.1	21.7	0.4
16-20	72.6	3.4	24.0		80.0		20.0		72.6	1.7	25.7		69.3	3.2	27.6	
	GROUP 5				GROUP 6				GROUP 7				GROUP 8			
1-5	38.0	22.5	39.5		37.0	16.7	40.7	5.6	63.6	24.3	12.1		45.8	24.3	29.2	0.7
6-10	46.1	3.0	50.9		29.8	7.0	63.2		39.1	14.2	46.8		51.4	6.2	42.5	
11-15	67.9	4.4	27.2	0.5	54.6	12.1	33.3		68.2	4.0	26.5	1.3	80.9	0.5	18.6	
16-20	69.3	2.6	28.2		44.3	5.2	49.5	1.0	72.1	4.7	23.3		50.9	7.3	41.8	
	Group 9				GROUP 10				GROUP 11							
1-5	64.9	18.0	17.1		64.3	8.6	27.1		71.8	16.8	11.4					
6-10	42.9	2.2	54.9		52.3	9.8	37.9		45.8	5.4	48.8					
11-15	66.8	6.2	26.0	1.0	77.1	0.9	22.0		79.5	6.8	13.3	0.4				
16-20	61.4	2.5	34.8	1.3	67.1	2.0	30.9		68.0	1.0	30.9					

In the experiments at North Carolina State University, female wasps were subjected to rotation during two days of gamma irradiation in addition to the simulated stresses of launch and recovery. Such a procedure has been recommended by botanists who have had marked success using clinostats to counteract the unilateral stimulation of plant organs by gravity. However, in preliminary studies a rotation rate of 1 RPM failed to provide patterns of fecundity and fertility similar to those after space flight (Grosch, 1971). Since it was possible that we were not rotating at a rate adequate for small insects, rotations at 6, 60, and 180 RPM were investigated and 60 RPM was selected as a rate for the final experiment. However, this rate proved moderately deleterious when used in combination with radiation (See Figures I-2 and I-4) instead of providing the amelioration sought.

Other possible modifications in treatment such as exposure to vibration and centrifugation g-force, individually or in combination with radiation were equally ineffective in mimicking the effects of space flight, although they afforded insight into the fundamental cytological responses. Since the period of most severe vibrations in the Biosatellite flight lasted only two seconds, one minute of vibration was conceived to cover the aspect adequately. Nevertheless, in case this might not be a long enough period, vibration for 15 times longer was applied to the wasps of Group 1. The close resemblance between the performance of Groups 1 and 2 indicate that vibration beyond one minute was superfluous. Vibration alone used before and after radiation provides the modest radioprotection seen for Group 8 in Figure I-1 and Figure I-4. This confirms the phenomenon reported earlier in Status Reports for NsG 678.

On the other hand, the acceleration profile spanned nearly 10 minutes in the Biosatellite flight, although peak acceleration occurred within 2.5 minutes. However, prolongation of the centrifugation used for high g stress tended to be deleterious to the reproductive performance of female wasps. (Status Report January - June 1970 NGR-002-023.)

Data on the stages of death provide details in the patterns of response not met in other types of experiments. Reciprocation between the proportion of Stage 1 deaths and those in later embryonic development was observed for the ground controls from the unrecovered first Biosatellite (Grosch, 1968) but a persistent predominance of Stage 1 deaths among the progeny of aged wasps seems peculiar to the present type of multi-stress experiment. In Biosatellite II the highest proportion of Stage 1 deaths was found for eggs from wasps behind the radiation shield (Grosch, 1970). Thus flight factors more than radiations were implicated. The predominance of Stage 3 rather than Stage 4 deaths in data from orbited females differs from results reported here.

Stage 1 deaths constitute those embryos in which death occurs during karyokinesis and before blastoderm formation (Von Borstel and Reckemeyer

1959). Instead of the normal clearing of the cortical zone, a white cumulus mass becomes evident within 3 hours after oviposition. A critical re-evaluation by Grosch (1969) identified the Stage 1 aspect to be an abnormal moribund condition which upon further incubation deteriorates into the peculiar zoned and retracted aspect termed Stage 2 death in the original classification (Von Borstel and Reckemeyer 1959). Since they have been shown to be of identical origin, Stages 1 and 2 have been pooled for the present report. Indeed, any traumatic influence during early embryonic development including heat shock or chemical fixation can produce the Stage 1 to Stage 2 deterioration.

In explaining the consequences for the eggs of wasps laid during several weeks following a 2-day gamut of treatments, influences upon genetic mechanisms are of prime interest. The two most prominent possibilities are (a) chromosome aberrations and (b) defects influencing DNA synthesis. The induction of chromosomal aberrations seems easily applicable to the eggs laid during the first five days because they are derived from differentiated oocytes present during the 2-days of stress and irradiation. Not only gametic nuclei but also the other structures of the follicle-enclosed complex oocyte/trophocyte units comprise much of the ovariole's contents during the ordeal experienced by treated females.

Again, chromosome aberrations provide an explanation of the 6th to 10th day trough in egg production. Potential eggs will be eliminated from the developing sequence if either cystocytes or follicular epithelia fail to complete their necessary mitotic divisions. At the same time it is difficult to conceive of gross chromosomal defects getting past the same mitotic screening to interfere with the early development of embryos in the eggs laid during that period. Selection against them would account for the demonstrated low proportion of Stage 1 deaths during the 6-10 day period. Nevertheless, the Stage 1 deaths were higher than the control level and in subsequent weeks, Stage 1 deaths increased impressively among the eggs laid later in life. Those appearing on the 11th day and later are derived from cells which were oogonia when stresses were applied to the female carrying them. As such they had to face the selective cytological crisis of the mitotic processes. Either some kind of metastable state leading to chromosome lesions activated by the constriction experienced during oviposition must be postulated, or else a quite different defect is at fault. If so, DNA synthesis or assembly might be abnormal.

The difficulty in interpretation arises from a dearth of information concerning the effects of physical influences such as centrifugation and vibration on the structure and function of insect tissues. Plant experiments provided the generalization that any factor which interferes with the restitution of chromosome lesions is expected to increase the

yield of radiation-induced aberrations (Giles, 1954). While the supportive structure of the cell walls is an essential feature of plant morphology, only membranes form the boundary of insect cells. Furthermore, the insect ovariole lies in a padding provided by an extensive fat body. Positioned partly by a terminal ligament, each braconid ovariole is loosely held in place by a flexible network of branching tubules which forms the respiratory system peculiar to insects. Even if the insect is under severe restraint during treatment its tissues cannot be considered coupled to a mechanical device to the degree possible with plants.

Much of the classic research on modifying agents was performed with Tradescantia plants. Undoubtedly this influenced the inclusion of Tradescantia microspores in the payloads of Vostok 5 and 6, as well as the interpretation that chromosome aberrations were due to dynamic factors accompanying the launch and recovery of the space craft. Kidwell and Kidwell (1968) reviewed the Russian literature on vibration experiments which also included observations on Drosophila, the fly of fermenting fruit, but presented their own evidence against mutagenic effects in their own fly experiments. As the Kidwells themselves recognized, a single frequency of 70 cps and 0.4 mm amplitude does not settle the question. Oster (1968) criticized the culture conditions of the U.S.S.R. "piggyback" spaceflight experiments and also pointed out that variation in sampling was not ruled out by the design of the experiments. More recently, diametrically opposed and non-repeatable changes in fecundity were reported for vibrated rotifers at the Leningrad Institute of Cytology (Kiro, 1970).

The vibrations forming part of the gamut of treatments for simulating space flight add a debated but poorly understood influence. Combinations of stresses need not result in increased damage. A possible balancing out is suggested when they are used pre- and post-irradiation to simulate launch and recovery. A decrease in the aberration yield occurred when Tradescantia microspores were centrifuged before irradiation, but an increase resulted from the same procedure used after irradiation (Wolff and Von Borstel 1954).

Part I Summary and Conclusions

Base line studies of the reproductive performance of braconid females indicate that no combinations of treatments employed in ground-based experiments have succeeded in eliminating the vulnerability to radiation damage of the series of cells transitional between the oogonia and the follicle enclosed oocyte-trophocyte units. Characteristically this damage is revealed during the second week after exposure as a trough or valley in the pattern of oviposition. Any eggs laid during the period tend to have poor hatchability. During the Biosatellite flight there was

protection from or antagonism to these kinds of radiation damage. By a process of elimination weightlessness has been implicated. No evidence has been obtained that rotation of the insect mimicks the effects of weightlessness on the most vulnerable internal tissues, the ovariole contents. At least in insects the clinostat approach does not promise to serve as an adequate substitute to experiments run in free fall.

Combination experiments were performed using vibrators, centrifuges, clinostats, and sources of gamma radiation. The sequence of treatments simulated launching, flight, re-entry, and recovery of an experiments vehicle. A portable power source assured immediate transitions between sequential treatments. Results were:

1. Average life spans equal to or slightly higher than that of controls.

2. Oviposition patterns similar to that of radiation alone delivered to a stational capsule of wasps. In the absence of other treatments, rotation enhanced radiation damage.

3. The trend of effects seen in oviposition data was repeated in hatchability data.

4. When the embryos dying in each of the major developmental steps were classified, a persistent high proportion were found unable to complete cleavage. The predominance of this type of death throughout all periods of oviposition seems to be characteristic of multi-stress experiments. The implication is for chromosomal aberrations or DNA defects.

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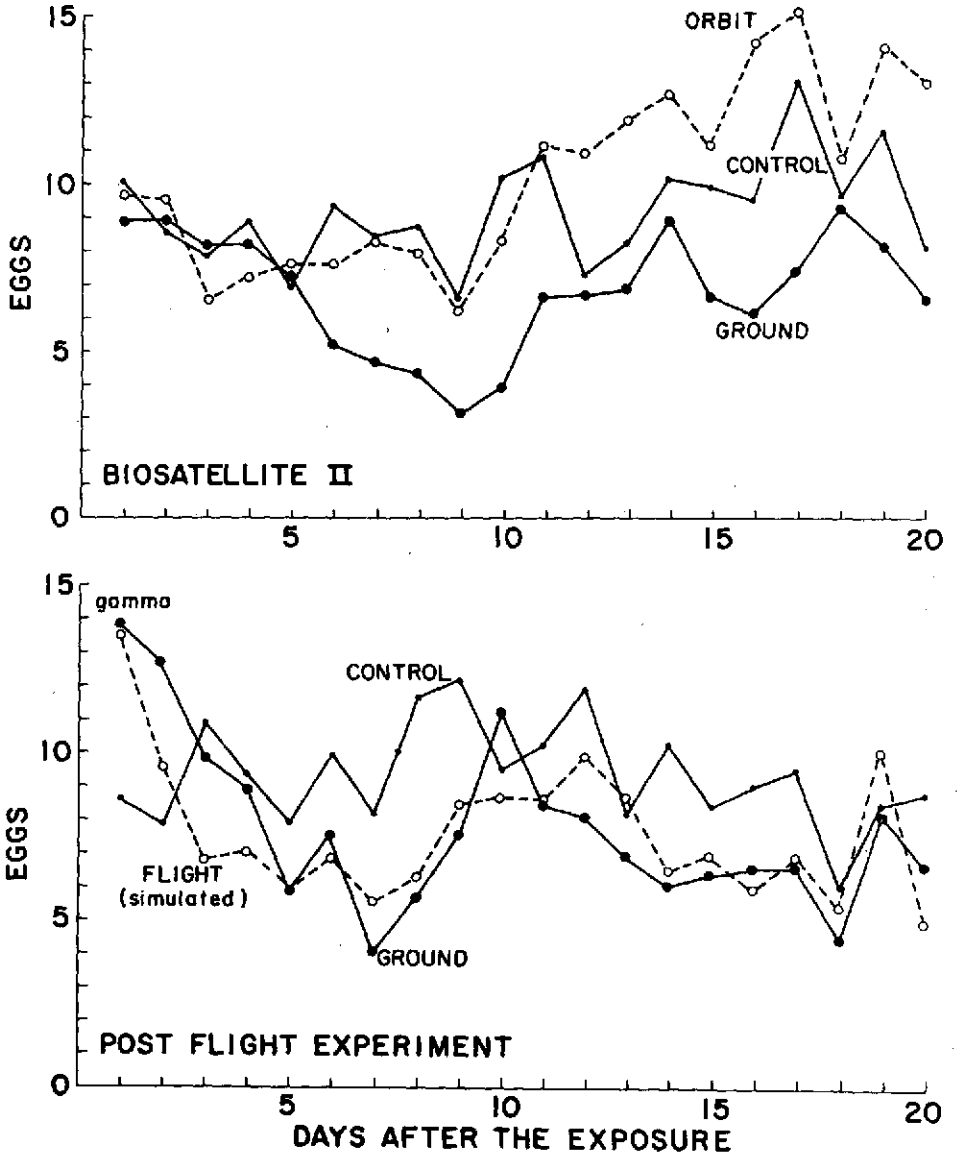
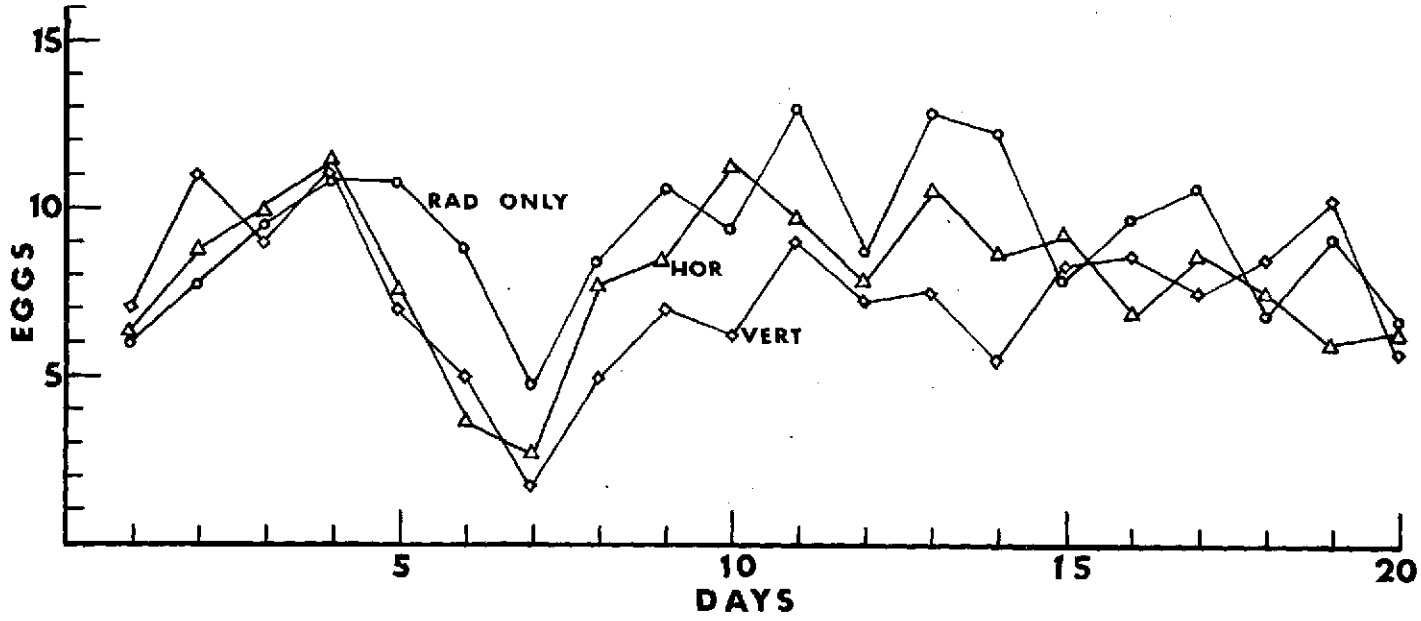


Figure 1. (Reproduced from p. 95 v. 9 Mutation Research, Grosch, 1970).

The mean daily egg production for samples of Habrobracon females maintained at 30°C in standard incubators following a 2-day period in a biosatellite experiments capsule. Daily oviposition after the highest gamma ray doses (2561 R and 2625 R respectively) is compared with that of the unirradiated "ground" control of the particular experiment. The characteristic radiation-induced decrease was effectively canceled for females irradiated while the spacecraft was in orbit (broken line designated Orbit).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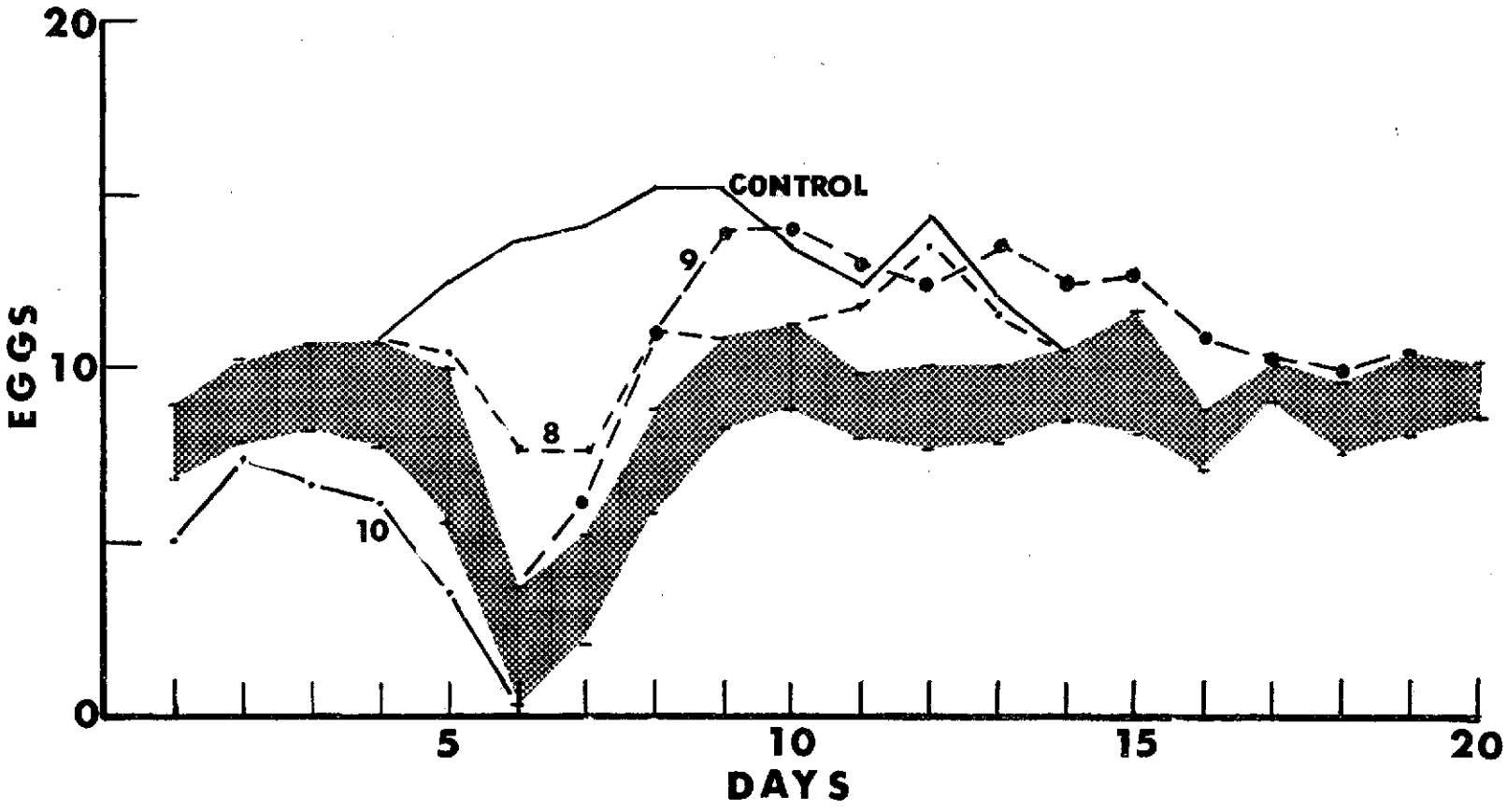


Figure I-2. The mean daily egg production per braconid female plotted for 11 samples of wasps treated as described in the text. The intertwined cluster of curves for data from Groups 1,2,3,4,5,7, and 11 are represented by the shaded area. The patterns of oviposition deviated from the cluster of curves for three Groups, 8, 9, and 10.

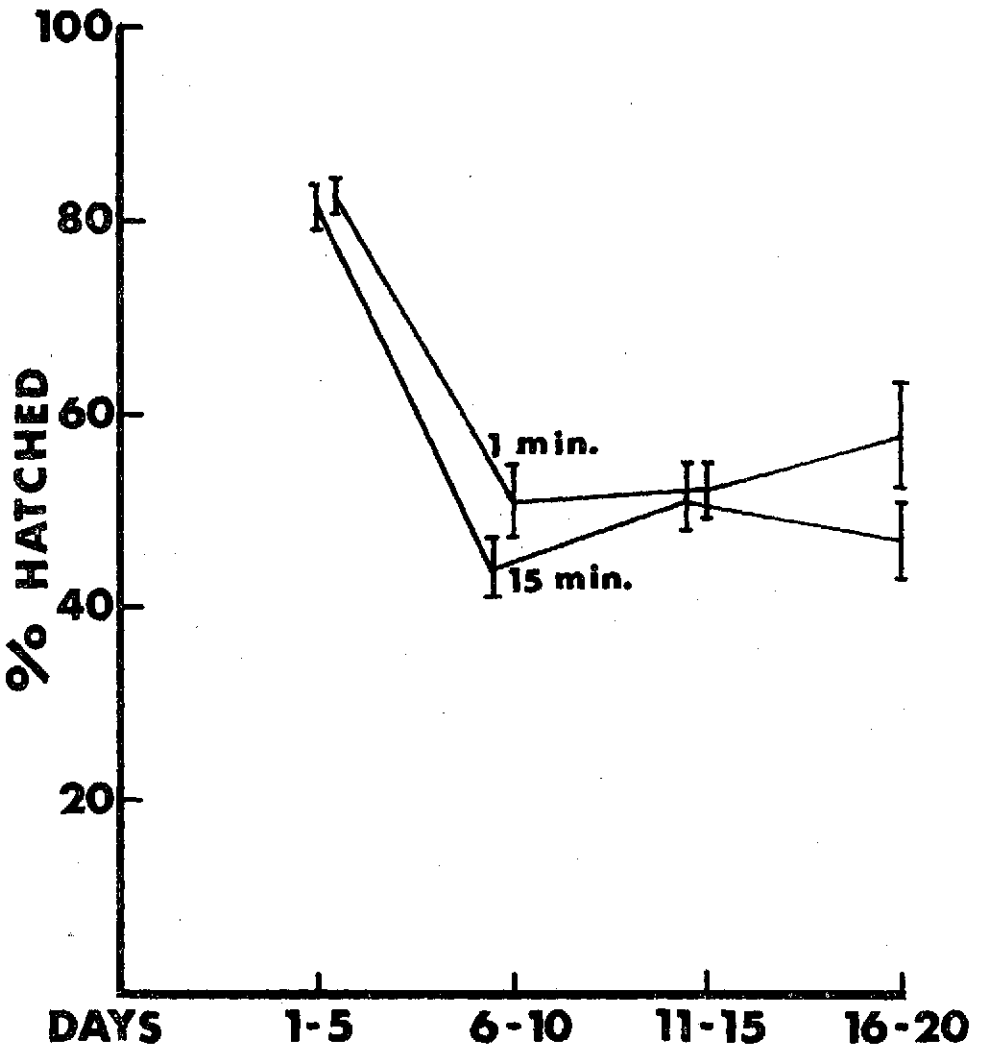


Figure I-3. Means and standard errors representing the hatchability of the eggs laid during four periods of life by Groups 1 and 2. The Groups differed in the length of the period of vibration, 15 minutes and 1 minute respectively.

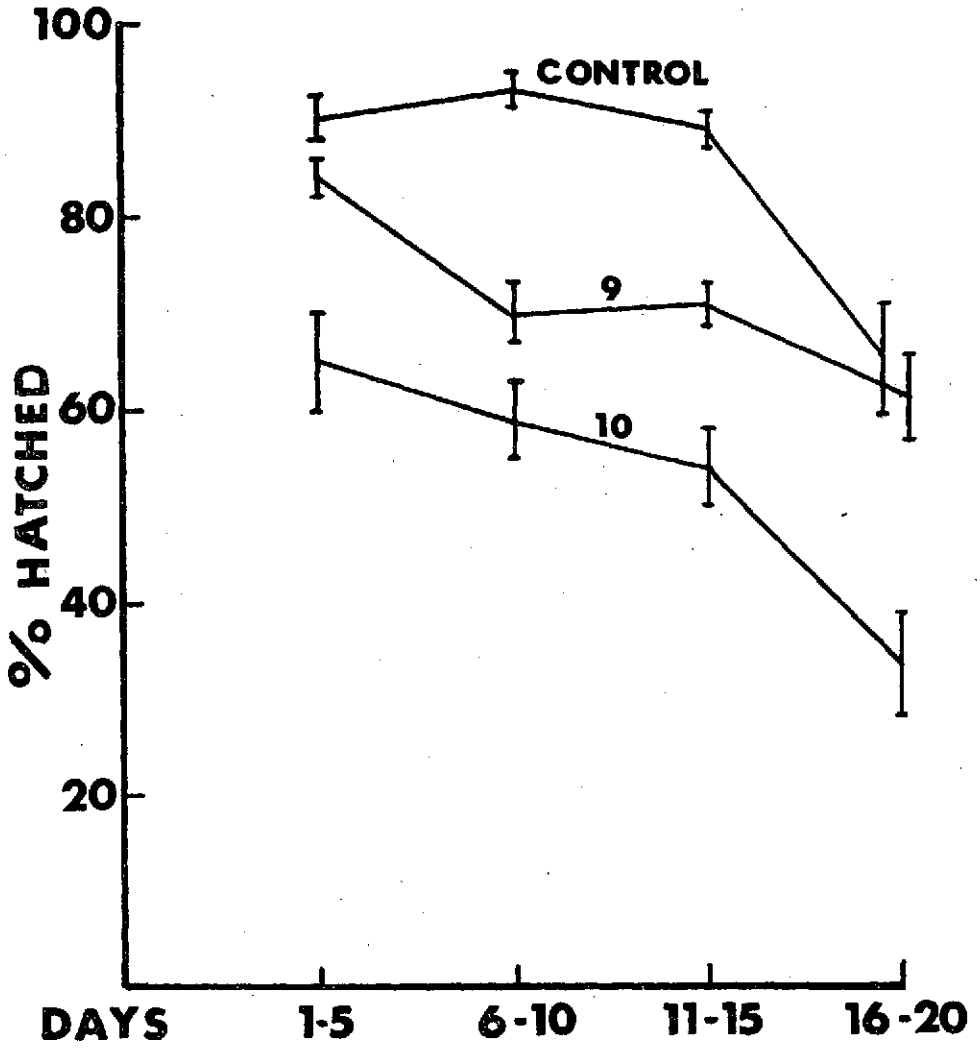


Figure I-4. The hatchability of eggs laid by the controls contrasted with that of the eggs laid by Group 9 (not rotated during irradiation) and by Group 10 (rotated during irradiation). Means and their standard errors were plotted.

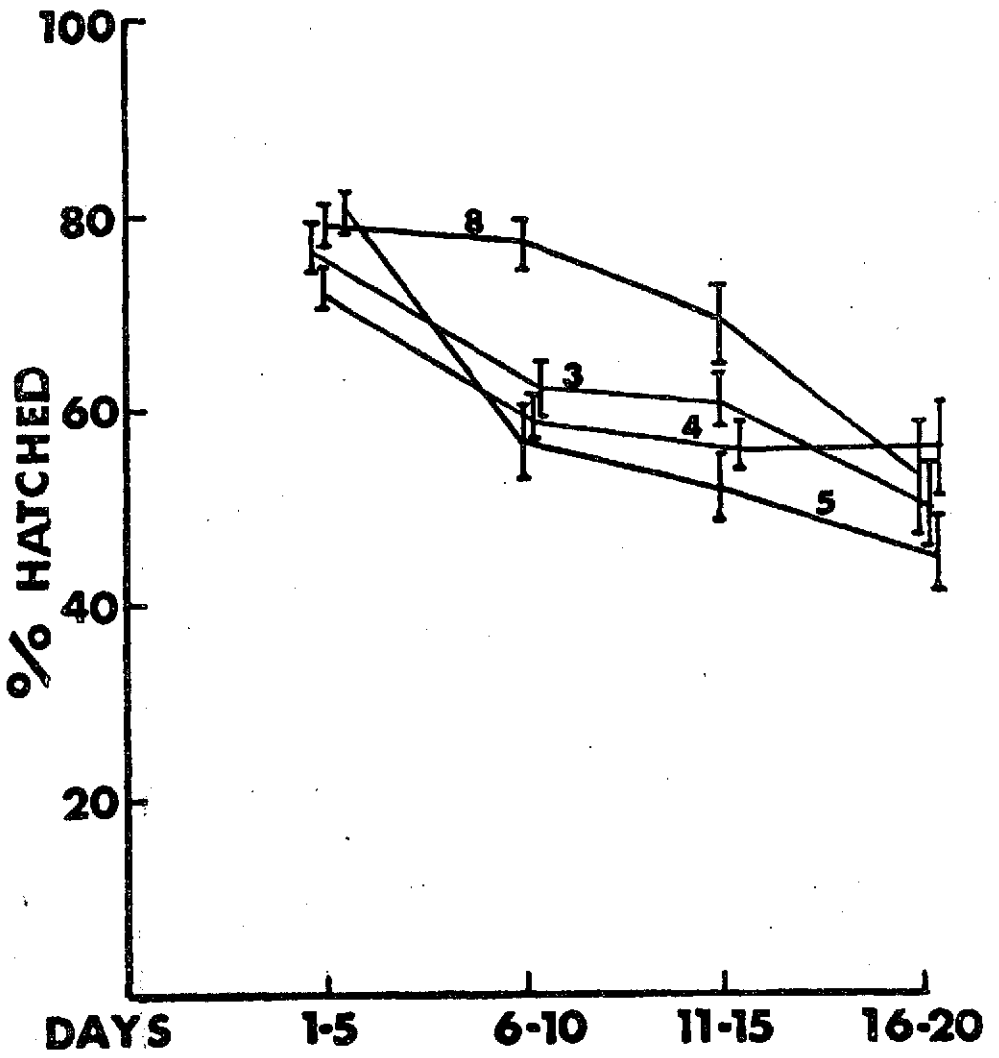
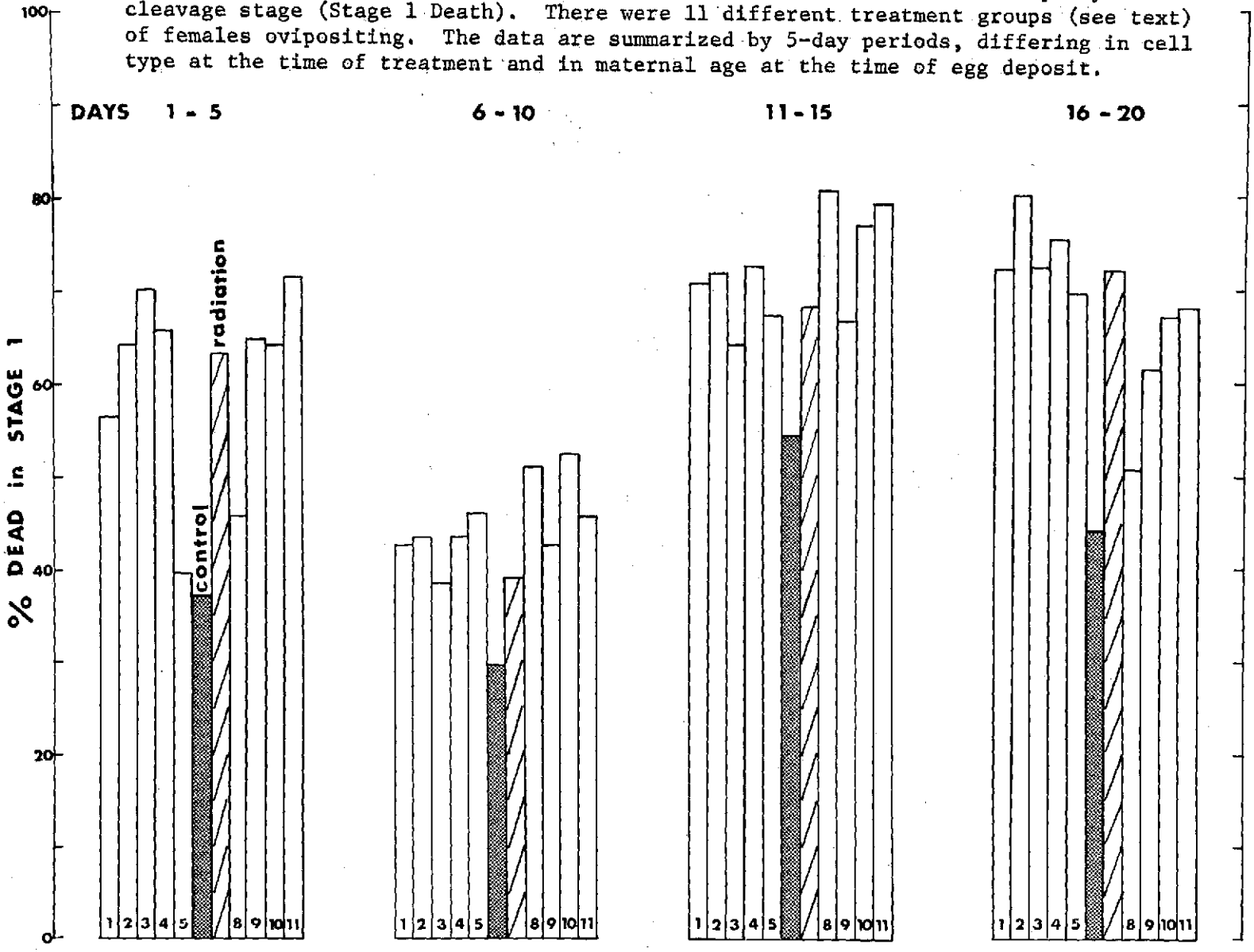


Figure I-5. Means and standard errors plotted to give a comparison of the hatchability of eggs laid by Group 8 (only vibration before and after irradiation) with those laid by three groups receiving more complex sequences of treatment: Gp. 3 (complete sequence of treatment except vibration), Gp. 4 (complete sequence except no radiation during irradiation), and Gp. 5 (complete sequence plus an 8 minute pause before and after rotation during irradiation.

Figure I-6. The % of dead Habrobracon embryos which failed to develop beyond the cleavage stage (Stage 1 Death). There were 11 different treatment groups (see text) of females ovipositing. The data are summarized by 5-day periods, differing in cell type at the time of treatment and in maternal age at the time of egg deposit.



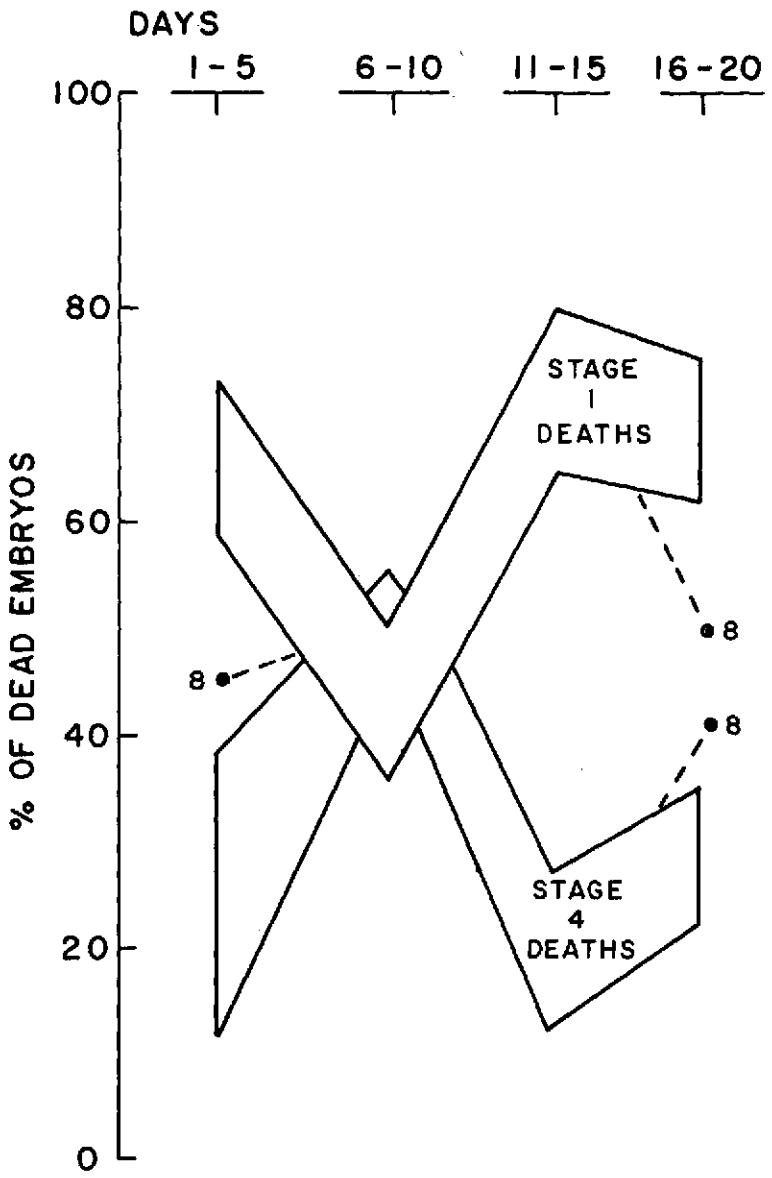


Figure 1-7. The outlines drawn for the composite plot of mean values for the two major stages of death clearly reveals the reciprocal pattern of embryonic deaths for all of the treatment groups in the final experiment on simulated flight-stress. Organizing the data by successive 5-day periods of deposit relates the eggs to the ovariole cell type present for treatment and to the physiological condition of the mothers at the time of oviposition.

PART II

HABROBRACON MALE MATING BEHAVIOR

Introduction

One of the most unexpected and exasperating aspects of the Biosatellite II Experiment was a marked difference between the mating behavior of males unpacked from the spacecraft in Hawaii and that of males from the ground-control and the back-up control incubators in Florida. In both cases the females were willing to accept a mate. The ground-control males accommodated them with dispatch but the males from the spacecraft were disoriented. The exceptional behavior characterized all males, regardless of whether they came from the aft group of shielded experiments or from the forward group of radiation experiments.

Consequently at Cape Kennedy 243 matings, one for each male, were completed in 3 hours by the manipulations of three investigators. In contrast, three experienced technicians tried for 13 hours to obtain matings of the 254 spacecraft males. Certainty of copulation was established for fewer than 1/3 of the males unpacked in Hawaii. Unfortunately, ground controls were not available at the recovery site. They should be mandatory at both launch and recovery sites in future flight experiments.

Mating is necessary for the assessment of damage induced in sperm. Occasionally females are unwilling to mate but males typically are eager and effective in sensing and finding a mate. Thirty years of experience in radiation research with braconids assured us that even much higher doses of gamma rays than received from the spacecraft's source would not interfer with male mating behavior. Furthermore, simulated periods of mechanical vibration in a number of preflight and postflight tests did not influence male behavior. In the present study, the influences of simulated weightlessness (rotation on horizontal clinostat) and time of day were investigated in an attempt to ascertain the cause(s) for the disoriented mating behavior in flown-males. The rationale for investigating these two influences are:

Rotation: Although there is no basis for expecting any braconid tissue to respond in the fashion of an orthogonic plant organ, rotation has been suggested by botanists as a universal method for counteracting the unilateral stimulation from gravity.

Time of day: A survey of mating efficiency at successive hours during the day and night is an approach to identifying circadian rhythms. The time difference between Florida and Hawaii could enter the situation.

During the course of these studies, it was observed that weather conditions were affecting the rate of mating. Accordingly, the influence of barometric pressure on the efficiency of male braconids was investigated.

Each type of experiment has contributed a piece toward solving the puzzle. A previously undescribed resting posture was obtained using certain rates of rotation, and an overriding influence of barometric pressure was identified in the 24-hour mating tests.

Materials and Methods

In the presence of a female the normal Habrobracon male from any ancestry exhibits a sequence of different elements in an elaborate pattern of behavior. The first indication of his recognition of her presence is an excited rapid flipping of the male's wings. Then he dashes to the female and attempts to mount her. At this time orientation of his body axis is important because copulatory maneuvers of the posterior abdomen accompany mounting. Often termed instinctive, the male response to the presence of a female requires a releasing stimulus which Grosch (1948) identified as an odor originating from the anterior part of the female's abdomen. Therefore an important consideration is the attractiveness of the female employed. However specific tests of stored virgins have proved they maintain their attractiveness up to a week of refrigeration, well in excess of the holding time of those females used as mates for the males from Biosatellite II. In one set of experiments run under optimum test conditions the time to accomplish mating with females stored 7 days averaged 2.3 minutes, for males stored 4 weeks, it averaged 2.7 minutes. As demonstrated in the results presented below, a very important condition is the trend in barometric pressure.

Our standard procedures for testing the time required to complete a mating is to place a male and female together in a 20 x 70 mm shell vial plugged with cotton. For the first 5 minutes the pair is merely observed. If mating has not occurred spontaneously within 5 minutes, both wasps are shaken to the bottom of the vial to bring them into proximity. Subsequently, up to 15 minutes the male is gently herded in the direction of the female by means of a soft slender artist's brush. Then if no mating has been consummated the pair is left undisturbed for an hour but observation continues. Finally the male is removed from the vial and the female is given host caterpillars. The appearance of biparental female progeny gives evidence of mating since normal males are fatherless, being produced parthenogenetically.

For subjecting wasps to rotation, plastic discs 7.5 cm in diameter were attached to the shafts of a series of electrically powered timer motors (Herbach and Rademan, Inc.) in which a system of gears determines the rate of rotation. Containers of various shapes and sizes can be attached to the disc. For wasp mating behavior five males were transferred to each No. 00 gelatin capsule which was then taped firmly at one of the marked positions on the disc. After rotation at a particular rate for a designated period of time, the males were removed and distributed individually to separate shell vials each containing a virgin female.

Rotation Experiments

An initial experiment in which males were rotated for 1 hour considered four different speeds of rotation and three different radial distances from the axis. Table II-1 presents a summary of the average time in which males of each sample completed their matings. Neither especially long nor especially short periods of time were involved. With no clear pattern of response emerging, it was decided to use the longest convenient period of time for all subsequent series of experiments. This was 6 hours of rotation which made possible the completion of an experiment within an 8-hour work day.

Figures II-1 and II-2 present a summary of results for a range of rates of rotation by plotting the earliest and latest times of mating for the males of each sample at each position from the rotating disc. The results for two different stocks show a consistent pattern for vertical clinostat experiments. The males tended to complete matings more quickly when they had been rotated at higher rates. Furthermore, positioning the capsule of males at the axis resulted in the most rapidly accomplished matings. An exception, the especially prolonged range shown for Stock 33 males rotated at the axis for 30 RPM, is due partly to deteriorating weather as a storm approached. On the other hand, when the capsules were carried on discs rotating around a horizontal axis (horizontal clinostat), no significant shortening of mating time occurred. Indeed many of the males from the 300 RPM samples took more than 25 minutes to succeed in mating. Also the sample rotated at 600 RPM at the perimeter had a greater spread in mating time than the 300 RPM sample from the perimeter position.

In addition, six 2-test comparison experiments were performed with horizontal clinostats. In each test two samples of five brothers were rotated simultaneously, one sample at 60 RPM and the second at 600 RPM. Two positions on the discs were used in different experiments, over the axis and at the 50 mm radius, respectively. Results are summarized in Table II-2. As shown, position at the axis did not necessarily result in rapid matings. To be sure, males experiencing the higher rate of rotation (600 RPM) at the axis position tended to complete matings sooner, but the reverse seemed to result for males rotated at the slower rate (60 RPM). The males carried in capsules at a 50 mm radius mated more promptly after rotation at 60 RPM.

Observations on Barometric Pressure

At this point in the investigation it became evident that good as well as poor weather was influencing the rate of mating. The 2-test comparisons were performed during a period of exceptionally good weather. The high barometric pressure seemed to stimulate braconid males, and on one day we witnessed overstimulation. The only occasion during a 6-month period in which no successful matings were obtained from the five males of an encapsuled sample occurred on a bright day with increasing barometric pressure from a high pressure air mass moving in. The males were observed and periodically herded toward females for an hour after their removal from the horizontal clinostat.

Table II-1. Time required for completion of mating by Habrobracon Stock #33 males rotated for 1 hour. Rotation rate and distance from axis of rotation were varied for vertical and horizontal clinostats.

Mean ± S.E. in Minutes.

	Vertical Clinostat			Horizontal Clinostat		
RPM	Axis 0 mm	Radius 25 mm	Radius 50 mm	Axis 0 mm	Radius 25 mm	Radius 50 mm
6	7.0 ± 0.1	7.4 ± 0.3	16.0 ± 2.4	18.4 ± 4.1	19.4 ± 4.8	10.3 ± 2.0
100	11.8 ± 1.8	15.6 ± 1.2	18.8 ± 2.6	21.6 ± 1.9	20.8 ± 1.5	22.2 ± 5.8
180	13.4 ± 1.7	13.4 ± 2.8	16.2 ± 4.4	11.2 ± 3.2	15.4 ± 7.9	10.3 ± 1.3
300	16.4 ± 3.1	8.6 ± 2.3	8.8 ± 1.1			

Table II-2. Time required for completion of mating by Habrobracon Stock #33 males as a function of rotation rate (RPM) and distance from axis of rotation on a horizontal clinostat.

		Capsule at Axis		Capsule at 50 mm	
Experiment	RPM	Range (min.)	Mean S.E. (min.)	Range (min.)	Mean S.E. (min.)
I	60	9-13	11.4 ± 0.8	0-5	2.8 ± 1.0
	600	4-7	5.6 ± 0.6	5-6	5.4 ± 0.3
II	60	6-15	12.8 ± 1.8	1-6	4.0 ± 0.1
	600	0-10	4.5 ± 1.9	12-17	14.8 ± 0.9
III	60	5-8	6.3 ± 0.8	1-6	3.4 ± 0.1
	600	2-5	3.6 ± 0.5	1-17	14.0 ± 1.0

Their recognition of the presence of a female was excellent as evidenced by their wing flippings, but in their agitated state the males would overrun the females or attempt to mount hurriedly and haphazardly. This seemed to irritate the females and no copulations resulted.

Subsequently, several additional experiments reinforced the idea that barometric pressure could be a significant factor in male mating efficiency. These were performed on successive days at 3:30 p.m. with the temperature at 75°F. On June 28, 27 minutes were required to complete the matings of five

males with a storm approaching. On June 29 in clear weather five brothers completed five matings within 6 to 7 minutes. On June 30, five other brothers took 12 to 18 minutes during deteriorating weather conditions.

Wasp Posture and Position During Rotation

At all rates of rotation on vertical clinostats which move a horizontal plane around a vertical axis, braconid males distributed themselves individually upon the walls of the capsule containing them. In contrast, when horizontal clinostats were used a previously undescribed behavioral reaction was observed. In this case when capsules of wasps were attached to a vertical plane moving around a horizontal axis, the males associated in an immobile group or clump, so oriented that their legs extended toward the interior of the group holding it together. Antennae were held tightly back along the body surface. The group presented a smooth streamlined aspect with its long axis parallel to the long axis of the capsule containing it (Figure II-3). The organisms may face in either direction. Usually half the heads are at one end and half at the other end of the group. If gently eased from the capsule and left undisturbed, the clump remained motionless for several minutes. If touched gently the clump broke up. Likewise disengagement was immediate if the clump was unceremoniously dumped from the capsule.

In the 2-test comparisons of Table II-2 clumping occurred for all 60 RPM samples but in none of the 600 RPM samples. During the rotation employed for the tests of Figures II-1 and II-2, clumps of wasps were observed in every capsule at every position on discs rotating around a horizontal axis at 6, 30, and 100 RPM. At 180 and 300 RPM clumping was seen in capsules positioned over the axis and in a majority of the capsules at the 25 mm radius.

Time of Day Experiments

The investigation of braconid mating efficiency at different hours of the day and night began during unsettled weather conditions. This turned out to be important for our understanding of the problem. Table II-3 shows only one prompt mating and a wide variation in time to accomplish mating. The 6:00 p.m. tests were attempted while a shower was approaching and the males seemed reluctant to mate. At this point we decided that it was important to record barometric pressure as well as temperature during future tests. Another series of tests was begun using males as they became available over a period of several days. The unsettled weather continued. Wide differences in the time required for mating were correlated with barometric changes, but not with time-of-day. Examples of the kind of data obtained are given in Table II-4.

Finally, a series of mating attempts was planned for which a sizable number of males were bred. The maximum age of any male used was 40 hours. The maximum age of any female used was 58 hours. The tests were performed in clear weather during relatively high atmospheric pressure and with the barometer either rising or holding steady. Tables II-5 and II-6 summarize the results.

Table II-3. The time required for completion of mating by wild type Habrobracon males at different hours of the day in unsettled weather. Temperature 28°C, barometric pressure fluctuating.

Mating No.	Time-of-Day 8 AM	10 AM	2 PM	6 PM	8 PM
	Minutes to accomplish mating				
1	5.1	5.0	2.5	60.0	12.3
2	9.7	3.5	5.0	60.0	60.0
3	10.5	47.0	5.5	6.5	6.5
4	7.4	60.0	8.5	60.0	12.0
5	8.0	8.2	7.0	60.0	5.0
Mean ± S.E.	8.1 ± 0.9	24.7 ± 11.9	5.7 ± 1.0	49.3 ± 10.7	19.2 ± 10.3

Table II-4. Examples of the wide differences in time required to complete mating by wild type Habrobracon males when changes are occurring in the barometric pressure. Temperature 24°C, relative humidity 56%.

	Time-of-Day 11 AM	3 PM	7 PM	8 PM	11 PM
Barometric Pressure	30.26	30.17	30.14	30.15	30.16
Barometric Tendency	Holding	Falling	Falling	Rising	Rising
Mating Time Mean ± S.E. (minutes)	3.5 ± 1.0	17.5 ± 3.1	21.0 ± 3.7	3.5 ± 0.9	1.8 ± 0.9

Table II-5. Time required for completion of mating by Habrobracon Stock #33 males as a function of time-of-day in clear weather with rising barometric pressure. Temperature 76°F.

Mating Time in Minutes

Mating No.	Time-of-Day: 8 PM	9 PM	10 PM	11 PM	12 PM	1 AM	2 AM	3 AM	4 AM	5 AM	6 AM	7 AM
	Barometric Pressure (inches): 30.01	30.05	30.06	30.08	30.10	30.11	30.11	30.11	30.12	30.11	30.14	30.17
1	5.0	4.67	7.0	5.0	1.17	0.1	4.83	5.0	0.42	0.42	5.0	5.0
2	2.5	5.0	5.2	8.0	0.25	5.0	5.67	5.25	7.0	2.25	1.33	3.08
3	5.4	2.0	5.4	1.5	.66	5.5	6.25	3.33	8.0	3.58	0.5	4.08
4	8.75	1.58	0.5	8.5	5.0	4.92	3.5	7.0	0.58	1.0	5.67	5.33
5	7.5	0.5	0.1	2.33	0.92	6.0	0.1	6.83	5.83	5.0	5.25	5.75
Mean	5.8	2.8	3.6	5.1	1.6	4.3	4.0	5.5	4.4	2.5	3.6	4.6
S.E.	1.0	0.9	1.4	1.4	0.9	1.1	1.1	0.7	1.6	0.8	1.1	0.5

Table II-6. Time required for completion of mating by Habrobracon Stock #33 males as a function of time-of-day in clear weather with steady barometric pressure. Temperature 76°F.

Mating Time in Minutes

	Time-of-Day												
	11 AM	12 AM	1 PM	2 PM	3 PM	4 PM	5 PM	6 PM	7 PM	8 PM	9 PM	10 PM	11 PM
Mating No.	Barometric Pressure (inches)												
	30.17	30.16	30.17	30.18	30.17	30.16	30.15	30.14	30.14	30.15	30.15	30.15	30.16
1	7.0	5.0	4.75	5.0	0.17	9.5	5.0	2.83	0.66	2.08	0.5	5.0	1.0
2	5.0	5.16	5.0	5.0	5.0	1.25	2.58	5.17	2.83	5.0	5.0	1.83	5.0
3	5.16	0.75	8.0	5.33	1.5	5.17	0.5	5.33	5.0	4.75	0.33	3.5	0.5
4	5.33	5.33	8.33	5.5	7.17	7.75	5.33	5.5	0.83	0.5	5.17	0.25	2.33
5	5.5	1.42	0.5	4.0	0.1	5.66	0.75	0.17	0.92	5.08	5.33	5.33	0.33
Mean	5.6	3.5	5.3	4.9	2.8	5.9	2.8	3.8	2.0	3.5	2.1	3.2	1.8
S.E.	0.4	1.0	1.4	0.3	1.4	1.4	1.0	1.0	0.8	0.9	1.2	1.0	0.9

Under these generally stable conditions no particularly prolonged sessions were required to accomplish any individual matings and the averages are consistently low. Most of our routine matings for stock maintenance are performed between 8:00 and 11:00 a.m. Typically, matings attempted during this period are prompt and effective. This taken together with the results shown in Tables II-4 and II-5 provide a survey of the 24-hour day. Now it is clear that under optimum conditions there is no time of the day or night at which braconids are slow to mate.

Surface Weather Observations, September 9, 1967

Both in rotation experiments and in hourly tests of mating efficiency, an overriding influence of barometric pressure was revealed. Therefore, weather station data for the day of unpacking the ground control and Biosatellite space craft took on special significance. These data were provided by the U.S. Department of Commerce Environmental Data Service in Asheville, N. C.

In Hawaii the WBAS station recorded a barometric pressure of 30.00 inches at 10:55 a.m. followed by decreasing pressure for 5 hours, reaching a low of 29.91 inches at 3:55 p.m. (15:55). During this period of falling pressure the Biosatellite experiments vehicle was unpacked and the first matings attempted. Subsequently, the barometric pressure remained low until 6:55 p.m. (18:55) but by midnight it had risen to 29.96 inches. Because of the open Lanai type of building made available for the Habrobracon laboratory, the wasps of the attempted matings were experiencing ambient atmospheric conditions. Until dissembly of the Biosatellite vehicle the wasps had been at the constant atmospheric pressure (14.5 psia) maintained throughout the 2-day flight. Meanwhile at Cape Kennedy the overall tendency was for rising barometric pressure, increasing from 29.78 inches at 6:00 a.m. to 29.85 inches by midnight. Taking into consideration the 5-hour time difference between Florida and Hawaii, the prompt matings in Florida occurred while the barometric pressure was rising from 29.81 to 29.84 inches. In meteorology the significant pressure changes per hour are of the magnitude of 1/1,000 to 1/10,000 of the bar unit, hence, the millibar is used as the standard unit of change in barometric pressure. Figure II-4 shows the divergent barometer tendencies at the two localities in a plot of the millibar changes for 10 hours following recovery of the experiments capsule. The specific barometric reading at each locality was taken as zero for the start of the 10-hour graph.

Discussion

In the mating efficiency experiments reported above, at no time of day were males reluctant to mate, provided the barometric pressure was rising or steady. Furthermore, no rate or plane of rotation applied for 6 hours succeeded in disorienting braconid males from two stocks differing in their origin and excitability. Indeed the consistent alteration of the mating response by rotation has been a decrease in average mating time, especially

with the higher rates of rotation on a vertical clinostat. The impression is that spinning tends to general excitement or irritation of males. On the other hand, a clumping and immobility response was discovered for samples rotated at moderate rates on a horizontal clinostat.

Background information on insect behavior in general, and the sexual behavior of wasps in particular will be appropriate to this discussion. In the language of the comparative psychologist, the actions characteristic of mating are termed innate consumatory behavior. As such the stereotyped pattern of response is elicited by specific stimuli under appropriate circumstances. Mating, as also found typical of other consumatory actions, is preceded by a series of orienting activities (Denny and Ratner, 1970). Although mating and other aspects of insect behavior can appear to be quite complicated, they actually involve highly developed reflex action rather than reasoning or learning (Dethier and Stellar, 1970).

Often termed instinctive, the fixed action pattern of the male's response to the presence of a female requires a releasing stimulus which Grosch (1948) identified as an odor originating from the anterior part of the female abdomen. The male's antennae are the important receptors for perception of and homing in on the female (Grosch, 1947, 1950). The mechanisms seem to be common among the Hymenoptera. In species widely separated taxonomically the searching behavior of males has been related to the olfactory sex-attractant produced by the females (Cole, 1970).

Ordinarily after mating deprivation a male shows no refractory period either in his response to the presence of a female or in his ability to find her and orient his mounting of her. All of these components of the male behavior were disrupted in the Biosatellite males. After segregation in excess of 2 days, the degree of disorientation was astonishing, especially since males of the same genetic makeup were functioning so well in the Cape Kennedy matings. With time-of-day for the matings now ruled out (see Results), along with radiation and vibration (see Introduction), the possible responses to weightlessness during the flight and the weather conditions immediately after the flight require close scrutiny. While the influence of weather conditions on mating is open to observation, the significance of a period of weightlessness followed by reentry, recovery and unpacking can only be inferred.

In insect orientation to gravity the hair plates of the body and leg joints have a gravity receptive function (Markl, 1962). To demonstrate this for wasps and other Hymenoptera the investigator used a tiltable rotating disc three times the radius of the discs employed in the present research. If the arthropoda joints are caused to be non-functioning the gravity sense is lost. Work of this type demonstrated gravity sensing organs of a type otherwise unknown in the animal kingdom. In view of the numerous body and appendage segments believed to be involved in this type of gravity sensing by the wasp it seems possible that disruption could disturb the entire behavior of the male braconid. Although mating is high in the hierarchal organization of the male braconids behavior, abrupt cessation of 2 days of akinesis by

the stresses of reentry and recovery, topped off by unpacking into unsettled weather conditions may well have presented the males with a conflict situation. An alternate behavior pattern best described as "escape" could then gain higher priority than mating. In conflict situations when the escape impulse and mating reflexes have been activated simultaneously one can be suppressed or apparently functionless actions occur (Markl and Lindauer, 1965).

An akinetic condition for orbiting male Habrobracon is postulated from their clumping at moderate rates of rotation in capsules spun on horizontal clinostats. This floating clump seen for the first time during the 1971-72 investigations constitutes a previously undescribed unlearned instinctive act for wasps. To be classified as instinctive the act should tend to safeguard the life and welfare of the individual. Although a wide range of organisms lie quiet or go limp in conflict or stress situations, the relation of such responses to the freezing postures used to evade predators is inadequately investigated (Hinde, 1970). Even less attention has been given to the responses to frustration or to the absence of appropriate stimuli.

In insects the tendency has been to study the stimulatory organs and the increased activity or kineses due to their action. Akinesis or thanatosis is the opposite condition receiving little attention. The prime examples studied have been nocturnal insects which assume a rigid, death-feigning position if subjected to bright light. The term akinesis has also been applied to the "hypnotic" state induced in the earwig by many kinds of constantly repeated stimulation (Wigglesworth, 1939). The clumping of quiescent Habrobracon during rotation adds another example caused by a completely different set of influences. It opens a new area for investigations in behavior.

Furthermore, although the influence of temperature and humidity upon the locomotor pattern of insects has been well investigated, the effect of total pressure has received little attention except for survival at the extremes. An outstanding exception was Wellington's review of the literature up to 1945 (1946a) and the publication of his results from his own fly experiments (1946b). Wellington found no general agreement on the reaction of insects to small changes in pressure, but appreciated the limitations of the field observations which predominated. A recurrent theme was an impression of irritability during barometric depression. In laboratory investigations, a change in respiration rate may be correlated with pressure changes, but information is meagre (Kiester and Buck, 1969).

Kinesis experiments with seven species of muscoid flies revealed a restless increase of activity after a sudden drop (within seconds) of 5 to 15 mb (Wellington, 1946b). On this basis the erratic prethunderstorm flights were interpreted as a "Baronegative" response to localized pressure changes. In nature such rapid changes are unusual and did not occur in the hours following dissembly of the Biosatellite. Figure 4 shows barometric pressures altering at a rate of about a millibar per hour.

A response to change in barometric pressure raises the question of identity of the receptors. The insects most sensitive to slight fluctuations in pressure may be the types with tympanal organs. In addition, Wellington (1946b) identified the antennal aristae as "external baroreceptors" for flies. Wasps lack this elaborate structure. The distal part of the Habrobracon antenna is a simple annulated flagellum. Nevertheless the possible involvement of the braconid antenna in pressure sensing arouses suspicion because the same sensory structure is concerned with perception of proximity to a female (Grosch, 1947). Neural messages about decreasing pressure might interfere with taking action upon those pertaining to presence of a female.

At the same time the possibility of an internal receptor of pressure changes should not be ignored. There is even the alternative of a non-sensory influence via physiological alteration of the most striking feature of the internal anatomy of insects, the tracheal system which pipes air to all tissues. The final ramifications end in tracheoles partially filled with a liquid column of fluid. How far the liquid column extends up the tube depends upon a balance between capillary forces, osmotic pressure, and environmental aspects. Furthermore, since pressure receptors in aquatic insects often involve adaptation of the tracheal system (Chapman, 1969) it is conceivable that terrestrial forms may possess as yet unrecognized features or abilities associated with the system.

Conclusions and Summary

A consequence of the experiments and observations reported above is that a neurophysiological basis of unknown nature need not be postulated to explain the lack of prompt matings among the males unpacked from Biosatellite II in Hawaii on September 9, 1967. Innate tendencies revealed during braconid behavior studies now provide clues to the difficulty.

While in Florida the matings occurred at an average rate of 1 every 2.2 minutes, matings in Hawaii can be estimated to have required a half hour or more. Thus, the task of observing matings by 254 males in half a day was an impossible one for only three investigators. The Florida success may be expected only when attendant conditions are optimum. The difficulties experienced in Hawaii can be encountered in routine experiments if the barometric pressure is falling and external atmospheric pressures are reflected inside the laboratory. Fortunately the standard procedure of leaving the males with the females for a number of hours usually suffices in cases of delayed matings.

However, there is an additional aspect beyond the mere records of time required to accomplish mating. This is the overall impression of male attitude and behavior. A complete duplication of the unperceptive attitude and disoriented actions of the Biosatellite males has not been accomplished with terrestrial equipment applied to braconid wasps. The most similar descriptions in the behavior literature are those of conflict situations for other species. Nevertheless, the discovery of a quiescent posture assumed

during rotation around the horizontal axis of a horizontal clinostat suggests the hypothesis that a similar attitude was assumed during the prolonged period of weightlessness in Biosatellite II. The effects of such an experience upon an insect's physiology and its subsequent behavior are unknown. Not finding the akinetic clumps of wasps in experiments with vertical clinostats, a procedure not considered to simulate weightlessness strengthens the hypothesis.

The conclusions are based on:

(1) The time required for a sample of males to complete mating is decreased by rotation around a vertical axis especially at relatively high rates of rotation.

(2) The response to rotation on a horizontal clinostat is quite different, and a new akinetic condition was discovered for wasps rotated at moderate speeds.

(3) Weather conditions, particularly barometric pressure, override the influences of stresses (including rotation) upon the time required for completion of mating. Surface weather station data for September 9, 1967 show falling barometric pressure in Hawaii and rising barometric pressure at Cape Kennedy, Florida. This was the day on which Biosatellite II and its ground controls were unpacked.

(4) Time-of-day mating experiments showed uniform promptness of coupling in stable weather conditions, and a series of variable trials during unsettled weather.

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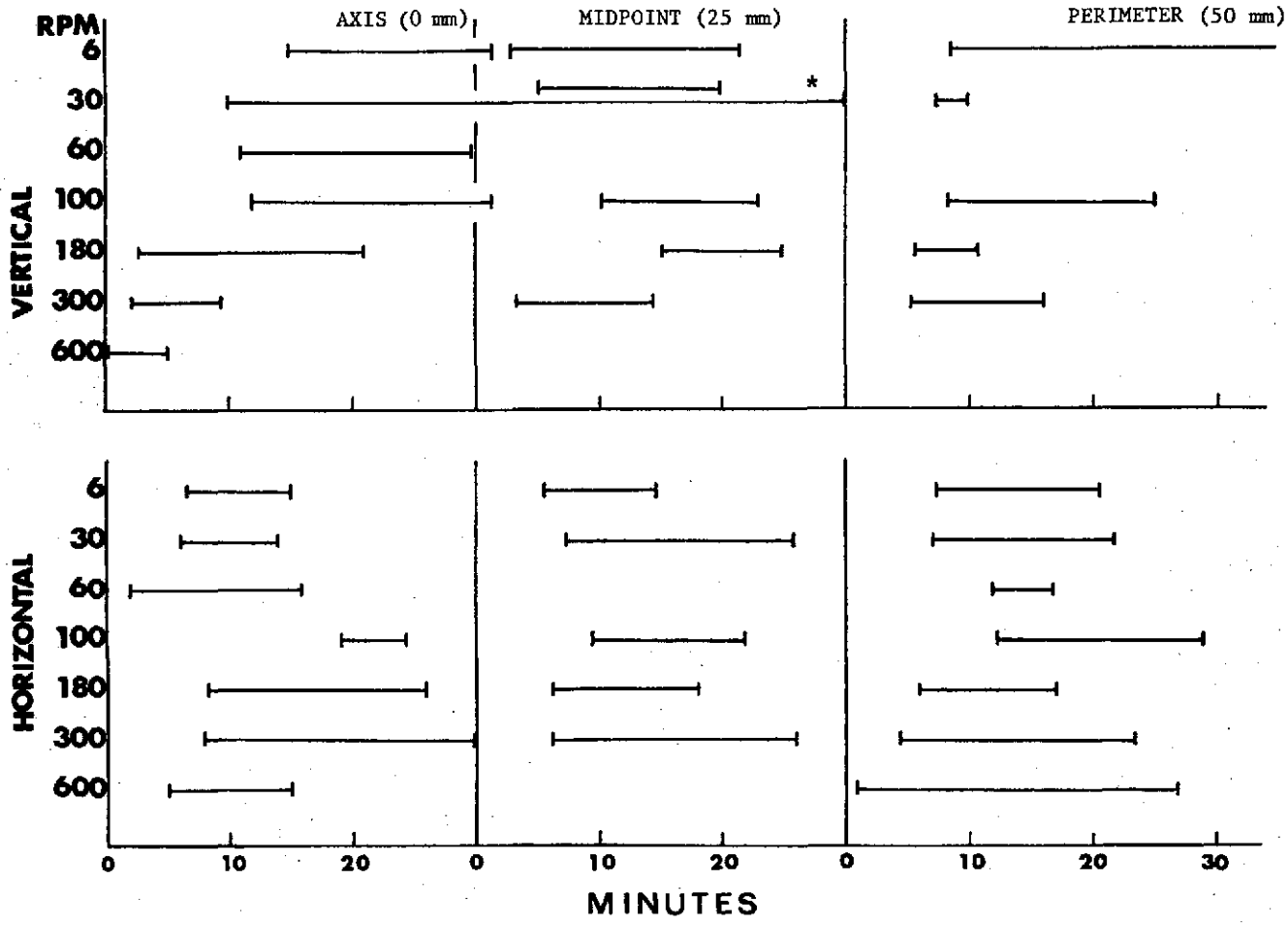
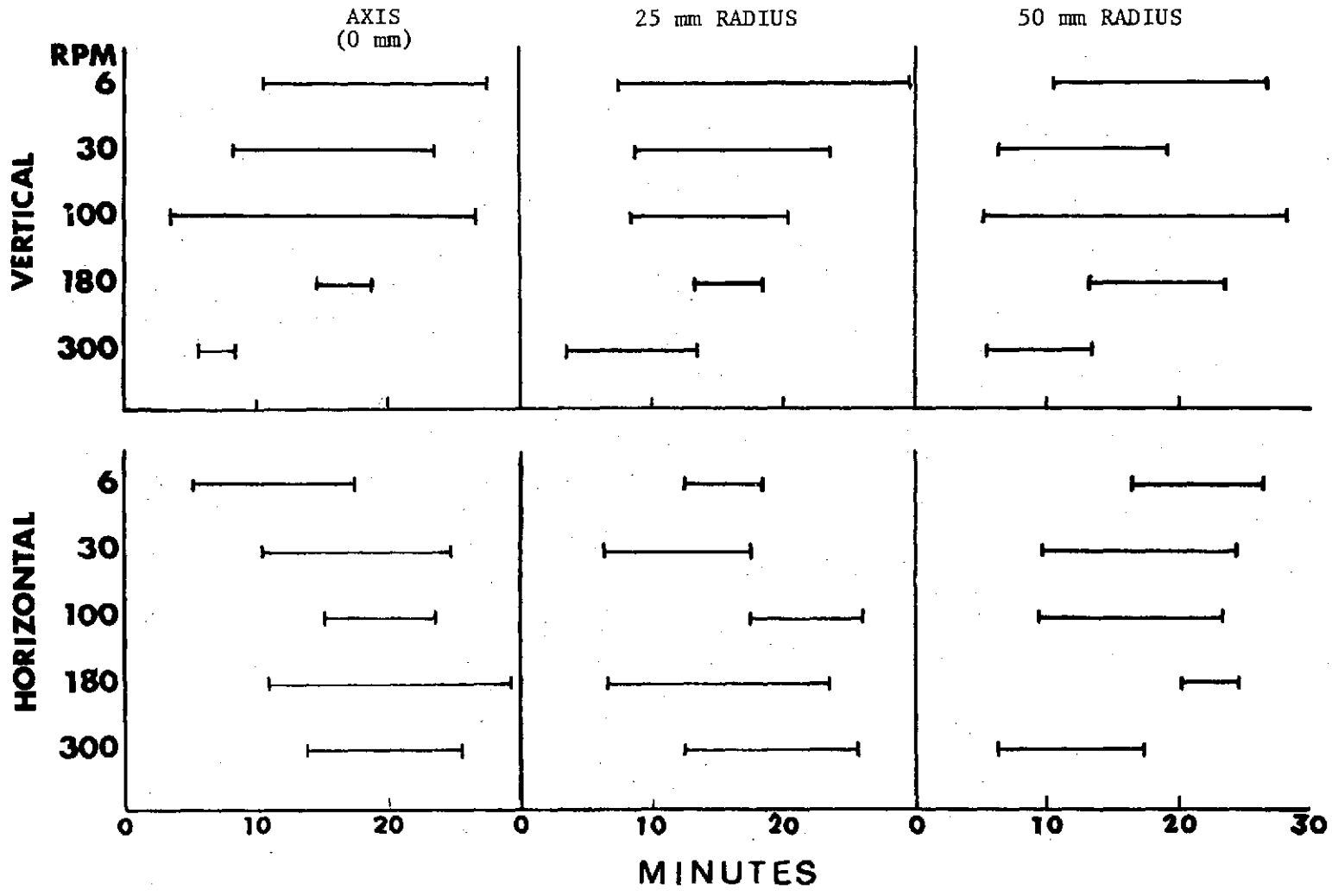
Figure II-1. Mating efficiency of Whiting Stock No. 33 Habrobracon males after 6 hours of rotation as a function of rotation rate (R.P.M.) and distance from the axis of rotation. The range in time from the earliest to the latest successful mating is plotted for both vertical and horizontal clinostats for organisms placed at the axis, midpoint and perimeter of the disc. Five males per test.



* Prolonged range influenced by deteriorating weather as a storm approached. Weather otherwise fair.

Figure II-2. Mating efficiency of Raleigh Wild Type Stock Habrobracon males after 6 hours of rotation as a function of rotation rate (R.P.M.) and distance from axis of rotation in stable weather. The range in time from earliest to the latest successful mating for vertical and horizontal clinostats for organsisms at the axis and at two radii. Five males per test.



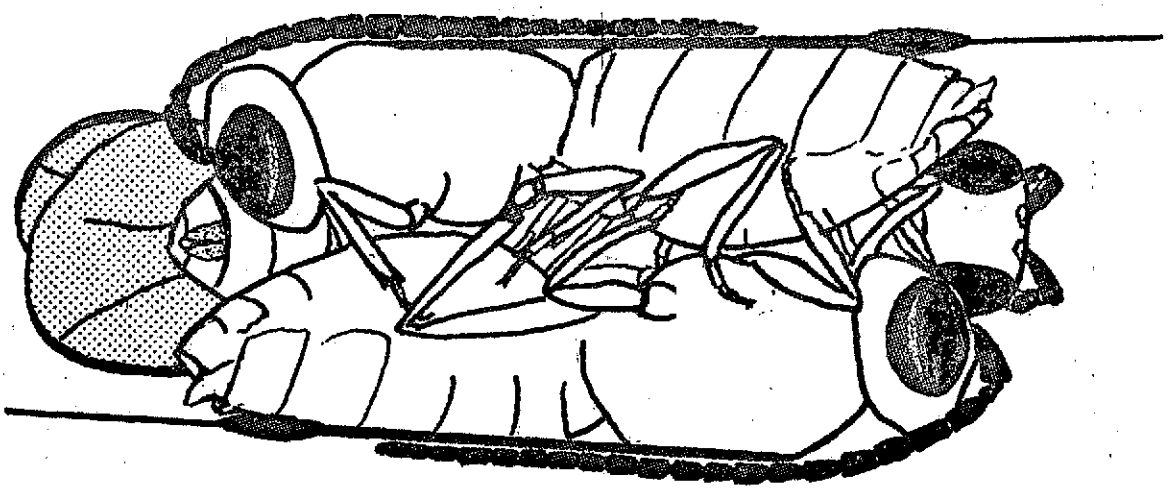
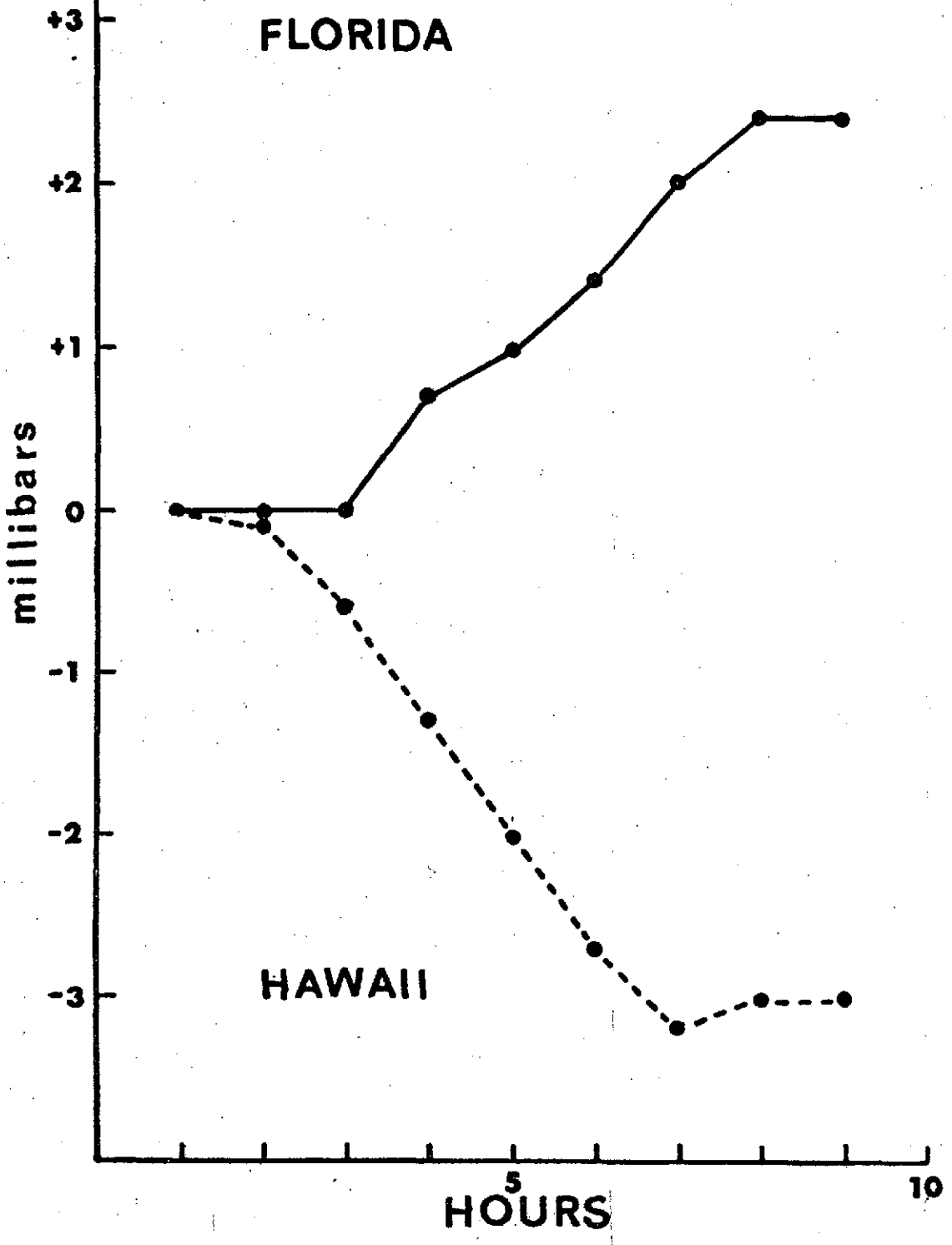


Figure II-3. A clump of male Habrobracon in the akinetic state or resting attitude assumed during moderate to high rates of rotation in a horizontal clinostat. Two to five wasps may associate in this fashion.

Figure II-4. Contrasts in the barometric tendencies at Cape Kennedy, Florida and at Honolulu, Hawaii on September 9, 1967 during unpacking and subsequent mating trials with Habrobracon males of North Carolina wild type ancestry.



PART III

EXPERIMENTS WITH ARTEMIA CYSTS

Introduction

In this part of the report two sets of experiments will be considered: (1) the tolerance of encysted shrimp embryos to Biosatellite flight factors, and (2) their tolerance of additional environmental influences which may be met in future space experiments using Artemia. Because of the variety of experiments discussed, the methods will be given immediately before the results for each type of experiment.

Biosatellite II and Related Experiments

Artemia cysts were placed in a part of each Habrobracon package originally designed for shielded dosimeters. When pre-flight tests indicated shielded dosimeters to be unnecessary, that position of the package was used for what was regarded as biological dosimeters, and monitors of other parameters.

Biosatellite II Cyst Emergence

After successful recovery of the packages in Hawaii, cysts from Biosatellite II and from the simultaneous ground experiments performed at Cape Kennedy were hydrated in the Gardner Hall laboratories of N. C. State University. Six dishes of 50 cysts each were studied from each Habrobracon package. Therefore, the average percent of emergence was based upon 300 cysts from each position.

Here by tabulation we contrast emergence results for selected packages. This has been published on page 37 of NASA SP-204, the 1971 book entitled "THE EXPERIMENTS OF BIOSATELLITE II", edited by Joseph F. Saunders.

Table III-1

Package	Radiation in flight (R)	% Emerged	t values	p values
Spaceflight	0.7	53.7		
			2.458	0.06
Earth-based Control	1.7	45.3		
Spaceflight pre-x-rayed with 16000R	2425	70.7		
			2.527	0.05
Earth-based Control pre-x-rayed with 16000R	2431	59.3		

Additional results consistent with a response to vibration rather than an effect of irradiation will follow the presentation of Biosatellite results in separate sections.

Biosatellite II Larval Survival

Each brood of larvae emerging from hydrated cysts was given its own rearing jar of standard brine and fed daily from a standard yeast suspension. As shown in Table III-2, survival to adulthood ranged between 55 and 65% for larvae derived from Biosatellite cysts and from the simultaneous ground-based controls. Survival in this range is excellent performance for mass populations reared in quart jars. Statistical analysis by "t" test comparisons provides no evidence for inequality of the mean values. Furthermore, the sex ratios of the adults obtained vary only slightly around the usual 50% males among the total number matured.

Since Artemia cysts contain dormant embryos, the study of emergence and maturation evaluates developmental damage to the treated generation. Evidently in a quantitative sense, the static encysted state is not seriously affected by sparsely ionizing gamma radiation combined with dynamic space flight stresses.

Table III-2. A summary of the proportion of larvae matured from those emerging from cysts treated as shown. The designation S indicates cysts from the flight. G indicates cysts from the ground-based control experiment. B designates cysts not irradiated before packaging, while R designates cysts receiving preflight irradiation.

Code	Gamma Radiation: Preflight 16,000R	Gamma Radiation: During Flight (R)	% Larvae Matured	Comparisons	t Values	% Male
SB-1	-	0.7	56.3	-	-	50.5
SR-1	+	0.7	56.4	SB-1 vs SR-1	.019	49.6
SR-2	+	298	64.8	SR-2	2.864*	47.3
SR-3	+	547	57.7	SR-3	.427	54.4
SR-4	+	918	55.8	SR-4	.122	48.7
SR-5	+	2425	58.6	SR-5	.568	51.6
GR-5	+	2431	55.9	GB-1	1.828	50.0
GB-1	-	1.7	63.2	GR-5	.135	56.4
				SR-1 vs SR-5	.624	
				GB-1 vs GR-5	2.000	
				SR-1 vs GR-5	.694	

* Verges on significance at the 5% level where t = 2.571, but note that the % maturing is higher than control values.

Morphological Defects

Under standard culture conditions, Artemia develop into complex arthropods with characteristic digestive, circulatory, excretory, muscular, and nervous systems. Externally the head bears five pairs of appendages. The trunk, which may grow to be an inch in length, is comprised of 11 somites, each with complex phylopod appendages. Posteriorly there are two genital somites, six simple somites and a telson (Lochhead, 1950).

Most of the adults reared from Biosatellite II cysts were normal in appearance, but scattered individuals exhibited structural malformations. The most common deviations from normal morphology were misshapen appendages and malformed tails, usually on non-survivors. In addition, abnormal types included edematous tail, incomplete tail segments, dorsal bulge, meandering digestive tract, one ovary undeveloped, penis permanently extended. Less frequent than posterior malformations were anterior abnormalities. However, several specimens with asymmetric eye pigmentation were seen and others with swollen claspers. A single example of a missing terminal segment of the claspers occurred.

The total number of abnormal shrimp comprised less than 1% of those maturing. They appeared only in larvae grown from cysts orbited in the Biosatellite and were distributed equally among "unirradiated" and gamma irradiated samples of cysts. Therefore, damage from the on-board source of gamma radiation is discounted, but "hits" by ambient HZE particles are not ruled out. Radiations of high linear ion density will be considered in greater detail in a later section.

Reproductive Performance of Adults Obtained

In order to study the reproductive capability, a series of sister by brother pairings were made using adults reared from each of eight packages of cysts representing material from the ground-based controls as well as from the Biosatellite flight. All adults used were normal in appearance. Each pair was given its own jar, and at least 20 jars were maintained from adults derived from each package of cysts. In obtaining data on the reproductive performance for the lifespan of the female, each jar was inspected daily. A dead male was replaced by a brother to provide a mate as long as the original female survived. Viviparous broods were counted during their removal from the pair-mating jar by pipette. When encysted broods appeared they were filtered out, dried on the filter paper, and resuspended to determine their hatchability. Results are summarized in Table III-3.

The encystment of 11.4% of zygotes from the unirradiated ground-based control (GB-1) was characteristic for other groups of San Francisco shrimp being studied in our laboratory during the same period of time. The higher values obtained for irradiated and Biosatellite orbited material are typical for Artemia experiencing a stress situation. However, the flight into space was made in the embryonic blastula stage and no mechanism is known for the translation of stress on the embryo to the adults obtained through development in standardized culture conditions.

The hatchability of the cysts deposited by females reared from Biosatellite II cysts typically approaches that of the unirradiated ground control. The one exception SR-1 is inconsistent with the pattern of hatchability, and comparisons with its mean gives the few significant t values on Table III-3. The t values based upon comparisons of pairs of means are otherwise non-significant. Obviously since the mean hatchability values for SR-2, -3, -4, and -5 are higher than that of SR-1, there is no evidence of radiation damage. Also given on Table III-3 are the t values for comparisons of the mean fecundity. None of the fecundity comparisons give evidence against a null hypothesis.

A lifetime average of about 1000 offspring is a characteristic total for pairs of non-mutant stocks maintained in our laboratory. In this regard, the tabulated data approach or exceed the typical value. Although the mean fecundity per pair ranged from 944.1 to 1538.5 and the mean fertility ranged from 778.0 to 1197.7, the between-pair variability reflected in the associated standard errors is so large that no statistically significant difference exists between samples. A further point not evident in the data presented in tabular form (Table III-3) is that every pair of all treatment groups continuously produced viable offspring. Such consistency in fertility is unusual except for standard wild-type strains. Infecundity and interrupted sequences of brood deposit are a striking feature of strains carrying mutations. After acute irradiation of females, brood deposit was irregular (Squire, 1970) and the fertility of males was interrupted (Squire and Grosch, 1970) after only 2 kR.

Table III-3. A summary of fitness components for the pair mating tests of adults reared from the Artemia cysts of Biosatellite II experiments. The designation S indicates cysts from the space flight, while G indicates cysts from the ground-based control experiment. B designates cysts not irradiated before packaging, while R designates cysts receiving preflight irradiation.

Calculations are on a per pair basis.

Code	Gamma Radiation: Preflight 16,000R	Gamma Radiation: During Flight (R)	% of the Zygotes Encysted	% of Cysts "Hatched"	Fecundity: No. of Cysts + Live Young	Fertility: No. "Hatched" + Live Young
SB-1	-	0.7	18.9	24.3 ± 4.3	944.1 ± 226.9	784.7 ± 201.7
SR-1	+	0.7	22.5	19.4 ± 2.2	972.1 ± 173.5	778.0 ± 141.8
SR-2	+	298	19.9	27.2 ± 2.6	1184.9 ± 211.2	975.3 ± 177.5
SR-3	+	547	24.0	30.3 ± 2.2	1239.9 ± 194.8	975.1 ± 196.9
SR-4	+	918	17.9	30.1 ± 2.6	1235.1 ± 153.6	1036.1 ± 144.4
SR-5	+	2425	22.0	25.4 ± 3.0	1360.6 ± 203.8	1149.5 ± 182.8
GR-5	+	2431	25.6	19.7 ± 5.2	1027.2 ± 243.1	915.8 ± 248.5
GB-1	-	1.7	11.4	31.5 ± 3.6	1538.5 ± 226.7	1197.7 ± 178.8

Comparisons	t values: Hatchability	t values: Fecundity
SB-1 vs. SR-1	1.021	0.098
SR-1 vs. SR-2	2.284?	0.779
SR-3	3.471**	1.026
SR-4	3.160*	1.134
SR-5	1.161	1.449
SR-2 vs. SR-3	0.873	0.191
SR-4	0.770	0.192
SR-5	0.964	0.597
SR-3 vs. SR-4	0.053	0.019
SR-5	1.306	0.428
SR-4 vs. SR-5	1.187	0.492
SB-1 vs. GB-1	1.290	1.852
SR-5 vs. GR-5	0.934	1.048
SB-1 vs. SR-5	0.205	1.366
CB-1 vs. GR-5	1.849	1.536

? - Borderline
* - Significant at .05 level
** - Highly significant

Cyst Experiments Relevant to Biosatellite II

Studies with Sparsely Ionizing Radiations

Despite exposures to total doses in excess of 18,400 R, the emergence from cysts of Biosatellite II shows no decreases ascribable to radiation damage (Table III-1). This is consistent with the published reports on the tolerance of cysts to sparsely ionizing radiations.

Rugh and Clugston (1955) obtained no decrease in hatchability from 100 kR delivered to dry cysts. Later, Engel and Fluke (1962) had to use doses from 150 to 450 kR to obtain points for a dose-effect curve. However, the radiotolerance was measured in terms of the presence of hatched larvae and not their subsequent fate. A dose of 50 kR, which was not demonstrably effective in preventing larval emergence, proved lethal during later stages of development (Bowen, 1963).

To provide a basic frame of reference for the use of Artemia cysts as a biological dosimeter in space flights, samples of dry cysts were exposed to a series of acute doses of Co-60 gamma rays. Three replicates of 100 cysts each were irradiated at each of 10 doses ranging from 10 kR to 300 kR. The larvae which emerged were followed until death or maturity.

The following table presents a summary from which the doses 150 kR, 200 kR, and 250 kR have been omitted. The decrease in hatchability is shown to be small for the entire range of doses. In contrast, the decline in survival to adulthood is pronounced and is dose dependent.

Table III-4

Dose (kR)	% Hatched	% Survived to Adulthood
19	42.0 ± 2.8	56.4 ± 4.4
38	42.3 ± 2.8	30.7 ± 4.4
58	40.3 ± 2.9	21.5 ± 4.1
77	45.6 ± 2.9	5.1 ± 1.9
96	43.3 ± 2.8	0
100	41.0 ± 2.8	0
300	28.3 ± 2.6	0

In unirradiated control samples, 58.8 ± 4.4% of the larvae that hatched survived to adulthood. This is in the normal range for groups of California Artemia reared in quart jars.

In a number of different laboratories, when Artemia cyst emergence has been plotted against dosage, sigmoid curves resulted. These were characterized by insensitivity below about 200 kilorads.

Vibration Experiments

Mechanical vibration is an environmental influence given minimum attention by biologists prior to successful space flights. As a prominent feature of the lift-off and recovery periods, it should not be ignored.

In the initial vibration experiments, loose samples of 100 cysts were vibrated for 4 hours at 160 cycles/second. The averages of the data pooled for four replicates showed hatchability slightly higher for the vibrated groups, 73.5% as compared to 67.0% for controls.

A second series of experiments was run to test the effects of vibrating cysts when the vibration was more directly coupled to the cysts. Again the results favored the vibrated cysts with the hatchability of those vibrated exceeding the control values by about 10%. Several embedding agents were employed, but the most feasible procedure was to sandwich cysts between layers of agar gel. However, embedding requires temperature control and recovery from a matrix is tedious. A convenient substitute proved to be plastic tape coated with adhesive on both sides, commercially available as "Double Sticky" tape. One side is applied to the vibrator while the cysts adhere firmly to the other side. After subjection to 160 cycles/second for 4 hours, 59.0% of the cysts hatched in comparison with 49.5% hatching from the unvibrated controls also held on tape.

Other experiments were performed at different rates for shorter periods, typically 15 minutes. After 70 cycles/second with acceleration of 20 g, six replicates of 100 cysts in each of six trials averaged 61.7% hatched, in comparison with 47.5% hatching in controls. A contingency chi square value calculated from the raw data was highly significant (15.12 with 1 d.f.). Subsequently, a series of 15-minute treatments on an MB electrodynamic shaker at frequencies up to peak capability of the equipment revealed a general trend for improvement of hatchability correlated with increased frequency of the vibrations:

	% Hatched
Control	30.0 $\pm$ 2.4
1000 c/s	31.0 $\pm$ 2.6
5000 c/s	39.0 $\pm$ 2.8
10000 c/s	36.7 $\pm$ 2.7
20000 c/s	40.3 $\pm$ 2.8

Three samples of 100 cysts were shaken at each frequency. The 10.3% difference between the control hatchability and that obtained after vibration at 20,000 cycles/second is significant at the 0.01 level in a contingency chi square determination computed with the raw data (7.025 with 1 d.f.). Larvae tended to emerge earlier from vibrated cysts. Within three days, 94% of the vibrated cysts which would hatch had done so, but only 75% of the control emergence was accomplished in the same period. The relatively low control hatchability reflects depreciation of the material due to the high humidity of late spring in Raleigh, N. C., unavoidable because the vibration laboratory was in the engineering complex of buildings in a different part of the campus from the air-conditioned biological laboratories.

Cyst Tolerance of Additional Environmental Influences

As the most environmentally resistant form known for a complex higher animal, Artemia cysts, available in quantity, afford a unique biological dosimeter and assay organism. Not only are they transportable in massive numbers per small mass and volume without special life support accommodations, but also evidently, they would withstand exposure in the hostile environments of other planets with a minimum of packaging. Studies of the factors in harsh environments may provide information applicable to the design of future experiments. The carefully controlled environment of Biosatellite II precluded concern about a majority of the factors limiting to the life processes of most plants and animals.

The brine shrimp Artemia salina is a branchiopod crustacean found in mineral springs, salt lakes, and the evaporation beds of commercial salt works. As such, it has evolved under unusual environmental stresses with which it copes partly by means of an exceptionally resistant encysted stage. This dormant phase of the life cycle is an embryo in blastula stage enclosed in a thick shell. Thus encysted, the organism can pass through long periods of drought successfully. When again hydrated the embryo develops into a nauplius larva without recourse to extensive mitotic division (Nakanishi et al., 1962) or demonstrable DNA synthesis (Emerson, 1964).

Subsequently, under standard culture conditions a complex arthropod is elaborated with characteristic digestive, circulatory, excretory, muscular, and nervous systems. Externally the head bears five pairs of appendages. The trunk, which may grow to be an inch in length, is comprised of 11 somites, each with complex phylopod appendages. Posteriorly there are two genital somites, six simple somites and a telson (Lochhead, 1950).

The exceptional durability of Artemia cysts have been established to vacuum by Whitaker (1940), to heat by Hinton (1954), and to extremely low temperatures by Skoultchi and Morowitz (1964). They tolerated vacuum to 10^{-6} mm of Hg for 6 months and survived oven heat up to 104° C. They remained viable even after 6 days storage at 2° above absolute zero. Accordingly, reports of their remaining viable up to 15 years in dry terrestrial deserts are not surprising.

A literature search failed to disclose experimental evidence about cyst resistance to ultraviolet rays and about the consequences of cysts becoming embedded in ice. The results of exploratory studies are reported below. Also presented is a brief consideration of the effects of heavily ionizing particulate radiations.

U. V. Radiation Experiments (Exciting Type)

In order to check the hypothesis that the pigmented cyst wall can effectively screen the Artemia embryo from ultraviolet rays, experiments were performed in which cysts were spread out at exposure so that no cyst was shielded by another (i.e., in a single cyst layer). The results of 5-, 10-, and 15-minute exposures were compared with suitable controls in one set of experiments. Two different lamps were employed to deliver energy of the shorter wave lengths, particularly those of mutagenic effectiveness at 2537 Angstroms. These lamps manufactured by Ultraviolet Products of San Gabriel, California, were: (1) Model No. R-51, a 6-bar cold quartz tube of high intensity, and (2) UVS-11 mineral light with a short wave tube of high transmitting self-filtered glass. At a distance of 6 inches, brief exposures to either of these ultraviolet sources induce visible and lethal mutations in bacteria. Indeed, a mere 90-second exposure is used routinely to obtain mutants in Staphylococcus aureus suspensions.

Emergence values were obtained for 100 cyst replicates hydrated after exposure. Results are summarized below as mean percentage emerged for four replicates of tests with Model No. R-51 and three replicates of tests with mineral light UVS-11.

	Control	5 min.	10 min.	15 min.
R-51	47.7 ± 5.4	39.5 ± 3.0	40.2 ± 2.8	40.5 ± 2.8
UVS-11	48.0 ± 2.8	47.6 ± 2.8	48.3 ± 2.9	47.0 ± 2.8

Obviously, the control values and experimental values for UVS-11 do not differ significantly. The three tests of replicates exposed to U.V. from R-51 gave essentially the same values, while the control with 47.7% hatching is somewhat higher. However, with a standard error of 5.4, a control value 7 or 8% higher is not significantly different from the experimental values for the brief exposures tested.

An exploratory series of exposures tested during January-June 1967 with inadequate air-conditioning revealed no significant effect of gradual increase in U.V. exposure time up to 4 hours, but a decrease in emergence occurred for three replicates exposed for 6 hours. Recent experiments using exposures for 7 hours have not verified U.V. damage from prolonged exposures in a well air-conditioned room. Furthermore, the samples of 200 cysts in each test were held on double-sticky tape for optimum immersion during the hydration following irradiation, a procedure not employed in 1967. The following emergence was observed:

	% Emerged
Control	40.4 ± 4.2
U.V.	44.5 ± 3.9
U.V.	41.3 ± 3.3
U.V.	40.0 ± 3.4
Control	38.0 ± 2.9
U.V.	38.6 ± 3.2

Our experience over the years has led us to expect emergence in the neighborhood of 40 to 50% from cysts collected in the wild and stored in a laboratory desiccator. Our counts are made from the larvae seen rather than by scoring empty cyst shells. The latter approach gives spuriously high emergence values from which no valid larval survival data can be computed. Evidence presented here indicates that the pigmented cyst shell successfully shields the embryo.

Experiments with Cysts in Ice

Despite experiments in which dry cysts were taken to much lower temperatures, no ice cake experiments seem to have been performed with Artemia cysts. In nature, ice layers form at the water's edge where cysts collect. Also, the encasement of cysts of other life forms in the ice of other planets is not inconceivable.

Data for two sets of experiments are given below. Prompt freezing was obtained by placing the container with cysts on a cake of dry ice within its insulated storage chamber immediately after water was layered on the cysts; 150 cysts comprised each setting.

In the first set of experiments, cysts were held at the bottom of a stender dish by double-sticky tape and the ice was frozen over them. A half inch of distilled water froze solid within 5 minutes after addition to the dish. The cake held in its dish was then stored for 24 hours in the freezing compartment of a commercial refrigerator. The next day the ice was melted by

incubation at 30°C for 5 minutes. The water was pipetted out and replaced by artificial sea water. Subsequent emergence in percent for three experimental replications of 41.5, 38.8, and 42.3 approach the control value of 42.7.

The question of the survival of embryos fully encased in ice was considered in the second set of experiments. Cysts were sandwiched on tape between two half-inch layers of ice. The procedure involves freezing the lower layer of ice before cysts adhering to the sticky surface of a length of tape are introduced. Small pieces of glass coverslips were used to weight each end of the tape. Then another half-inch of water was added to the dish and frozen on top of the cysts. A satisfactory preparation is difficult to obtain partly because of the stiffness of the tape at cold temperatures. With repeated trials, facility was gained in setting up the experiment. Of the three deemed acceptable, the third setting promised to be the best from the standpoint of technique. In percentage emerging the data bear this out:

Setting	% Emerged
I	33.5 ± 2.1
II	36.7 ± 1.1
III	42.7 ± 2.5
Control	41.6 ± 3.1

From these results it is clear that Artemia cysts can survive freezing in ice layers.

Radiations with High Linear Ion Density

In contrast to the adult shrimp, which is no more radio resistant than other arthropods (Grosch and Erdman, 1955), dry Artemia cysts tolerate massive gamma ray doses (Engel and Fluke, 1962) when the criterion of damage is decreased emergence. On the other hand, Hutchinson and Easter (1960) used the Yale heavy ion linear accelerator to demonstrate that the radio tolerance was reduced with 40 Mev helium ions and eliminated with 160 Mev oxygen ions. The long plateau of the dose-effect curves obtained in X-ray and gamma ray experiments disappeared when the densely ionizing tracks of the oxygen nuclei were used on encysted Artemia embryos.

Artemia cysts have been employed by a foremost early investigator of the effects of cosmic rays, J. Eugster. At first his experiments contrasted mountain peak exposures with tunnel shielded material (Hess and Eugster, 1949). Later, cysts were elevated to 100,000 feet in meteorological baloons where they received direct hits by densely ionized tracks (Eugster, 1953, 1956). During the preparation of the present report (1972), quarterly reports

have become available for the BIOSTACK Experiment M-211 of Frankfurt Professor Horst Bücker and his associates. This was flown on the Apollo 16 mission. In the experiment package, layers of biological material were sandwiched between nuclear track detectors in order to correlate the tracks or heavy ions with the position of individual Artemia cysts. The hatchability of cysts hit by heavy primaries was decreased by 41% as compared with a decrease of only 23% due to background radiation.

At N. C. State University, W. B. Bowman, a Ph.D. candidate advised by D. S. Grosch, has demonstrated that only 10 kRad of alpha rays decreased the hatchability of Artemia cysts to below 20% of control values. Figure III-1 presents his points to date. Obvious decreases from control levels occurred even at doses below 1 kRad.

In addition to poor emergence, some of the larvae obtained in the BIOSTACK Experiment were deformed, presumably due to the cellular damage induced by heavily ionizing rays. The total dose of radiation for Apollo 16 was 622 mRad, which included the contribution from a solar flare. In shrimp reared from the cysts of Biosatellite II, a small number of similar morphological deformities were observed (listed above in an earlier section). At the time of their discovery, they seemed inconsequential because only 2.9 mR was recorded from galactic radiation during the Biosatellite II mission. Nevertheless, a substantial part of the total dose was contributed by the galactic flux of 10.1 nuclei per square centimeter. Conceivably, we were seeing the teratogenic effects of this component of the radiation from space, since the radiobiologic significance of this particular type of radiation is not adequately expressed in terms of absorbed dose. On the other hand, no abnormal larvae have yet been observed in alpha ray experiments, but conceivably heavier particles than the helium nuclei of alpha rays may be required to produce the phenomenon.

Misconception stemming partly from a loose usage of the term egg can lead to a misinterpretation of radiation experiments. The Artemia cyst is not a single cell. This dormant phase of the Artemia life cycle is a blastula stage comprised of 370 nuclei. Furthermore, the nauplius larva develops from the blastula embryo without further DNA synthesis (Emerson, 1964) or mitotic divisions (Nakanishi et al., 1962). The combination of circumstances offers a basis for explaining a tolerance to sparsely ionizing radiations, while suggesting an expected effectiveness of heavily ionizing particles. It seems reasonable that an appreciable proportion of the 370 nuclei have to be injured to modify the embryo's morphology.

Conclusions and Summary

The ultimate test of the feasibility of using an organism in bio-astronautic studies is its successful survival of an actual space flight. Artemia cysts have accomplished much more. Those from Biosatellite II not only showed excellent emergence of larvae, but in turn a high proportion of larvae matured. Furthermore, the reproductive capacity of the adults was

statistically equivalent to control levels. Further corroboration of Artemia cyst utility is appearing from the BIOSTACK experiments of Apollo missions.

Literature citations affirmed that Artemia cysts are exceptionally tolerant of temperature extremes, desiccation, and vacuum. Experiments reported here indicate that the Artemia cyst wall (shell) is an effective screen for ultraviolet rays and that encysted embryos withstand freezing under and between layers of ice. Furthermore, embryos hatch from cysts after receiving impressively high doses of sparsely ionizing gamma radiations. A brief period of vibration increases the proportion of cysts hatching. Consistent with this was the finding that cysts from the Biosatellite II flight appeared to respond to the vibrations experienced rather than to the moderate radiation doses received. Survival to maturity is more radiosensitive than emergence from cysts, but by this criterion, too, the gamma radiation received during orbital flight had little effect on the dry cysts. On the other hand, recent alpha ray experiments indicate that ionizing tracks of high linear energy transfer (L.E.T.) are damaging to cyst emergence even at low delivered doses. Along related lines, a West German team analyzing the BIOSTACK results correlated low hatchability of cysts with hits by heavy cosmic particles and observed misshapen shrimp among the survivors. This leads to a reassessment of the significance of the small fraction of morphological defects seen among shrimp reared from Biosatellite II cysts.

From this background, the picture emerges of (1) the static phase of an organism, (2) resistant to extremes of temperature, humidity, and atmosphere, (3) tolerant of radiations of low linear energy transfer (L.E.T.), yet (4) damaged by heavy nuclei (high L.E.T.). Furthermore, the cysts (5) require no life support equipment, (6) but develop into organisms of considerable adult complexity.

In the future, Artemia cysts could prove useful in investigations transcending the controlled environments of man-made spacecraft. Exposure to the surface conditions on the Moon or Mars is well within the realm of biological possibility.

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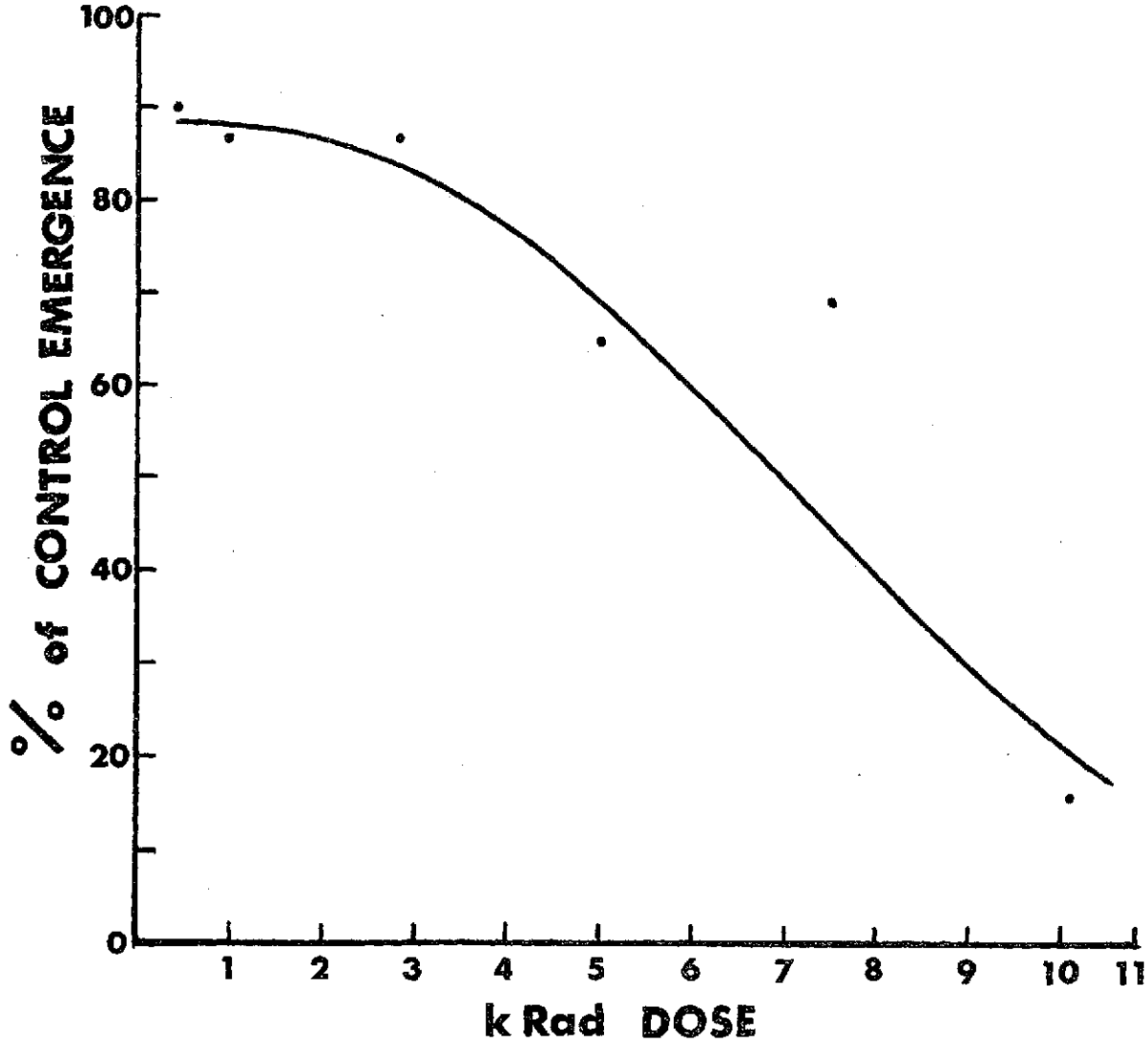

Figure III-1. Artemia larval emergence from cysts after irradiation with a series of doses of alpha rays from Plutonium 238.