STATUS REPORT on NsG-678

UTILIZATION OF HABROBRACON AND ARTEMIA AS EXPERIMENTAL MATERIALS IN BIOASTRONAUTIC STUDIES

Prepared by D. S. Grosch
Genetics Department, N. C. State University at Raleigh

Period Covered: January through June 1965

Introduction

During the second six months of this project, the combined effects of radiation and forces higher than one gravity have been investigated on this campus using the wasp Habrobracon. Also we have participated in the April 13th and 19th simulated space flight tests at Moffet Field, California.

Our efforts with the brine shrimp Artemia have been concentrated on standardizing rearing in a chemically defined environment.

Habrobracon Experiments

The Combined Effects of Radiation and Centrifugation

In these investigations we use a motor designed for long continuous operation fitted with a head machined to hold capsules of wasps. Centrifugation for a few minutes at a speed providing a force of 1,000g was a treatment too severe for all stages of the braconid life cycle. On the other hand, the majority of adults survive 500g for as long as 24 hours. However, few eggs were laid by females subjected to the experience and hatchability was poor. Upon dissection, disturbances of abdominal contents were evident. These included posterior localizations of urate cells, distended hindguts, and enlarged egg sacs filled with amorphous material with the yellow color of degenerated eggs.

After the lower dose of 250g for 24 hours, most of the females laid eggs and the majority of the eggs hatched. Therefore, we decided to run the first full scale combination experiment at the rate of revolution providing us with 250g. Our Co^{60} gamma source delivered 30r per hour to provide a total of 720r for a 24-hour exposure.

The experiment was repeated three times. During each replication samples of 8 females were used for each of the four categories: (1) centrifuged, (2) irradiated, (3) centrifuged and irradiated, (4) control. Significant evidence of potentiated damage from the combination of treatments was obtained in only one of the three sets of experiments. Both egg production and hatchability were decreased below the records for either radiation or centrifugation. The cumulative daily evidence for the life of the female is impressive. In the other two experiments the combined effects were most obvious only on the days when radiation induced low egg deposit. This sensitive period corresponds to mitotically active cell differentiation. Differentiated oocytes and undifferentiated oogonia, respectively after and before the sensitive period were less sensitive. Values for egg deposit and hatchability after a combination of treatments were generally intermediate to results with either centrifugation or irradiation alone. An apparent superiority in performance during the last five days of life for the females in one experiment can be viewed with skepticism in the light of their being the last few females alive after the majority of their sisters making up the treated sample had died. In general we feel that death came relatively early after centrifugation and the experiments need to be performed at still lower rates of revolution and lower forces. A tendency of adult females to orient directly opposite to gravity (or to applied force in excess of gravity) renders the abdomen and the organism vulnerable. In addition to constant speed of rotation these types of experiments include acceleration to and deceleration from that level.

Simulated Flight Profile and Radiation

The Habrobracon experiments in simulated space-flight were designed to test the radiation subassembly and prototype packages under four separate conditions. These conditions were (1) the simulated flight profile, (2) the irradiation, (3) the combined effects of the profile and irradiation, and (4) the non-profile-nonirradiation control.

Dr. R. C. von Borstel of the Oak Ridge National Laboratory leads a group primarily concerned with packaging and assessment of genetic damage to metaphase and prophase oocytes and spermatozoa for the first three days after treatment (or recovery from a space satellite). Subsequently, we follow the same animals for the remainder of their lives, about 20 days. We study the patterns of fecundity and fertility and record life span.

Four positions were available in each module. Females were placed in two positions, males in the third and Toshiba glass rods in the fourth. Irradiation time for the April 13 test was 66 hrs. 20 min., and for the April 19 test 66 hrs. 40 min., with the expectation of delivering doses in the neighborhood of 4000, 2000, 1000 and 500r to the modules in their various positions. However, the measured dose fell short of the nominal dose and the radiation intensity for the profile experiment proved to differ from that of the non-profile experiment. A report dated 15 May 1965 from Dr. von Borstel to Dr. G. Dale Smith, Experiments Mgr., Biosatellite Project, NASA Ames Research Center, gives a detailed discussion of the dosimetric data, along with conclusions and recommendations.

Wasp survival was good except in the nominal 1000r position where wasps were pulverized during the simulated re-entry vibration testing. Strengthening the holding bracket is expected to minimize whipping of the package.

In both the April 13th and 19th experiments a typical radiation induced valley was obtained for fecundity at nominal doses above 500r. This valley occurring at the end of the first week reflects the radiosensitivity of differentiating oocyte-nurse-cell nests and indicates that the doses delivered were adequate for studying modifications in the pattern of egg production. The curves obtained by plotting egg deposit against days will not be presented here. They show no unusual patterns.

The cumulative effects on the sensitivity of developing eggs can be summarized as the average total eggs produced per female. These sums are shown in Table 1 for the most significant period following the April 13th experiment. The period chosen avoids (a) eggs deposited the first three days which were differentiated oocytes at the time of treatment and (b) eggs deposited late enough to be derived from undifferentiated oogonia.

The totals reinforce the impression that vibration alone has little effect upon oviposition records. On the other hand, a strikingly low total of eggs was produced in combination with nominal radiation dose 4000r. Actually by dosimeter 2.30 Kr measured for the combination treatment is less than the 2.59 Kr found for the radiation alone. However, these data are the only indications of synergism obtained. All other egg production summations appear to reflect the 6% average lower radiation dose in the combined radiation and vibration part of the experiment. Table 2 gives egg production summaries for the radiosensitive period and the subsequent period (until senility) for the April 19th experiment.

Hatchability records appear to reflect merely the radiation dose. The combined treatment in which 6% less radiation was delivered consistently shows higher hatchability than the radiation exposure alone. Variability between positions is small and for simplicity of summarization results for the two female positions are pooled. On this basis the nominal 4000r dose for the first three days of each experiment shows the following hatchability results in per cent:

	Radiation	Radiation & Vibration	Vibration
Expt. 1, April 13-16	64.50	72.26	98.30
Expt. 2, April 19-22	65.39	69.14	97.30

Control hatchability was 94.28

For the eggs of Table 1, the highest nominal dose 4000r characterizes the pattern:

Radiation	Radiation & Vibration	Vibration
52.27	55.00	42.72

These relatively low values must be viewed in the light of controls which had decreased to 57.97% hatched during this period. Nevertheless, again the combined treatment resulted in a higher value than radiation alone.

Hatchability for the eggs of Table 2 are shown in Table 3 for the highest nominal dose (4000r). With standard errors of the order of 5%, differences between treatments are not significant. Although available, hatchability data for the lower radiation doses will not be presented here. Differences from control values are even smaller for the lower doses.

Discussion of the Current Emphasis in Habrobracon Experiments

In space-flight biology problems of interpretation arise because living material is subjected to a number of factors whose action on hereditary structures is not yet adequately understood in isolation, let alone in combination with chronic radiation exposure. Extended periods of weightlessness cannot be accomplished in the laboratory but the factors of vibration and of acceleration to forces in excess of gravity can be investigated in controlled experimental fashion. Cytological investigations of the effects of centrifugation have traditionally employed germinating seeds (Kostoff, 1935; Cytologia 8:420; Sax, 1943; P.N.A.S. 29:18; Wolff and v. Borstel, 1954. P.N.A.S. 40:1138.) Attempts to produce genetical changes in insects have not been reported until recently (Reddi, 1963. Nature 198:316), possibly because results have either been inconsistent or negative.

Reports from the U.S.S.R. have been inconsistent. In crossing-over investigations with Drosophila an effect was observed in one flight but not in two others. By turning to simulated flight experiments it was possible to show that acceleration to 4000g had little effect, whereas vibration had definite consequences. Complications arose when combination experiments were set up using gamma rays. With doses of 1000r and 2000r vibration apparently had opposite effects from centrifugation. (Parfenov, 1964. Moscow Akademiya Nauk, vol. 2).

In the Vostok satellites' Tradescantia experiments, chromosomal aberrations were traced to dynamic factors accompanying launch and recovery of the space craft but not to the state of weightlessness. The smallest yield was obtained in material with the longest experience of weightlessness; that is in material fixed prior to landing. (Delone et al. 1964, NASA TT F-8896.)

Similarly, the inviability of Drosophila eggs from adults carried by the Vostok 3 and 4 spacecraft is not considered to be due to weightlessness. Nor was ionizing radiation alone a significant factor. An increase in dominant lethals seems to support the idea of effects due to vibration but also concern is expressed about lax temperature control during the recovery period. (Parfenov, 1964. NASA TT F-8898.)

Fortunately Habrobracon are not as temperature sensitive as Drosophila. Nevertheless, the absentmindedness of an Oak Ridge individual, not of Dr. von Borstel's group, delayed our procurement of the wasps from the second experiment and could have contributed to the relatively low control values of Table 3.

We feel it desirable to give some attention to vibration experiments in addition to centrifugation experiments. Much of this can be done on our campus. As already stated changes are planned in the simulated space flight fittings. In addition the modules in each package can be aligned in a row parallel to the backscatter shield rather than in a rectangular pattern. This may not only improve equality of radiation distribution but also distribution of vibrational effects. Dr. von Borstel's report goes into more detail on package modifications.

Artemia Experiments

The formulation of a superior synthetic sea water has made possible the rearing of Artemia in a chemically defined environment anywhere in the world. We have demonstrated this using the commercial product, "Instant Ocean", successfully employed by inland aquaria for the maintenance of larger marine organisms. However, we find that increasing the salinity by adding 50g of NaCl per liter is important here as well as when using natural sea water. During the Spring Semester two sets of 15 pair matings were followed in Instant Ocean solutions. Measured in days the average life span without salt was 20.47 for males and 29.86 for females; with salt 73.67 for males and 62.13 for females. Furthermore the addition of NaCl increased the average number of broods per female from 3.73 to 10.13 and the average number of offspring per pair from 169.6 to 1423.0.

We look forward to using Artemia as well as Habrobracon for studying the biological effects of space flight. In all probability Artemia holds promise as the most versatile and useful biological dosimeters.

Personnel of Project

Leader: D. S. Grosch, Professor of Genetics

Research Assistants (Half time during academic year):

James C-H Lin, Graduate Student
Roger H. Smith, Graduate Student

Table 1

The per female average braconid egg production from the third to the tenth day following simulated space flight (April 13, 1965).

Nominal Dose in r	Position	Radiation	Radiation & Vibration	Vibration	Controls
4000	B	45.6	29.0	88.0	67.6
	C	50.9	31.2	90.0	107.5
2000	B	43.6	64.7	74.3	105.4
	C	51.6	66.8	85.2	89.0
1000	B	71.4	73.2		83.3
	C	46.5*	53.1		93.0
500	B	71.0	64.6	82.6	71.0*
	C	77.3	75.3	92.4	97.2*

*Five days only.

Table 2

The per female average braconid egg production for the periods shown following simulated space flight (April 19, 1965).

Days 4 through 9

Nominal Dose in r	Position	Radiation	Radiation & Vibration	Vibration	Controls
4000	B	34.30	61.97	94.22	134.60
	C	35.80	59.97	97.86	106.90
2000	B	53.41	79.25	99.00	93.67
	C	68.20	71.87	82.80	98.45
1000	B	69.20			
	C	97.00			
500	B	78.00	89.45	88.27	104.33
	C	77.14	98.50	98.67	80.78

Days 10 through 17

Nominal Dose in r	Position	Radiation	Radiation & Vibration	Vibration	Controls
4000	B	42.75	108.83	97.83	147.42
	C	104.75	119.67	86.62	65.83
2000	B	83.25	93.50		147.42
	C	104.55	93.17	103.09	139.09
500	B	81.50		127.03	141.04
	C	108.04	117.33	109.80	91.93

Table 3

Hatchability of the eggs of Table 2 for the nominal dose of 4000r. B and C position data were pooled for summary. Also three rather than two periods are shown.

Days	Radiation	Radiation & Vibration	Vibration	Control
4-7	36.91	42.39	35.69	52.37
8-11	29.92	27.39	37.65	47.58
12-18	26.15	29.25	38.65	37.33