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SUMMARY PROGRESS REPORT

TO THE

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

February 17, 1964–June 30, 1966

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ORNL-TM-1731

Contract No. W-7405-eng-26

BIOLOGY DIVISION

SUMMARY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

February 17, 1964–June 30, 1966

MARCH 1967

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Title of Project: Mutagenic Effectiveness of Known Doses of Gamma Radiation in Combination with Weightlessness on *Habrobracon*

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Interagency Agreement:
Purchase Order R-104 Task No. 8

Experiment Proposal Number:
P-1079

Supported by:
Research jointly sponsored by the National Aeronautics and Space Administration and the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

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I. INTRODUCTION

R. C. von Borstel Roger H. Smith

Our first objective in the Biosatellite Program is to use the particular qualifications of the genetic system of *Habrobracon* to assay the total measurable damage to its genome in the extraterrestrial environment. The test is to be essentially a test of the genetic effects of weightlessness in combination with known exposures of radiation. An ^{85}Sr gamma source will be activated after the satellite is in orbit. Of course, there are other aspects of the flight dynamics besides weightlessness, that is, vibrations and accelerations of launch and reentry as well as factors inside the biosatellite, that is, temperature and humidity changes that may contribute aberrations to the test system.

Our second objective is to utilize the group we have assembled and trained to obtain fundamental radiation genetic data that will help us to improve our understanding of the action of radiation on cells. Gaining this information is pertinent to the Biosatellite Program in that any refinements in interpretation of data will make the experiment more valuable.

To this end, we are conducting careful spontaneous mutation and dose-action experiments on the induction of dominant lethality, recessive lethality, and inherited partial sterility. It is from these experiments that we hope to understand better the general nature of dominant lethality in terms of the role of dicentric translocations that would confer lethality to the cell. Since for this we must also examine the genome for total recessive mutations and total chromosomal aberrations of the type conferring inherited partial sterility, the task requires the sustained effort of a group.

This report is a summary of the work that has been accomplished from the inception of the inclusion of *Habrobracon* into the Biosatellite Program on February 17, 1964, until June 30, 1966. The papers that have been included are drawn from ORNL Biology Division Semiannual Progress Reports, ORNL Space Biology Annual Reports, and Special Reports to Project Management of the Biosatellite Program at Ames Research Center. A bibliography is listed near the beginning of this report; a few items were not included in this summary because of certain overlap and duplication of material. The papers are given approximately in the order of publication.

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III. BIOSATELLITE A EXPERIMENT[1]

F. J. de Serres R. C. von Borstel
B. B. Webber Anna R. Whiting

The Office of Space Sciences and Applications of NASA has announced the details of this program as a series of flights of duration 3 to 30 days in a modified, scaled-up Discoverer satellite to determine the biological effect on plants and animals of weightlessness, radiation, and absence of diurnal cycle. The first flight is scheduled for late in 1965.

The satellite is designed to carry its own radioactive source to supply known doses of radiation and will permit this previous variable to be controlled. Natural radiation will be controlled to less than 2 r/day by the orbital altitude selected.

The temperature, excluding reentry temperature, will be controlled to 24 $\pm 2°C$. The experiments will be in individual containers and hermetically sealed. These very conditions require rather elaborate ground-based experiments in connection with the viability of the biological specimens and, moreover, the possible modification of radiation effects on these conditions that are somewhat unusual in comparison with routine laboratory-controlled experiments.

In some cases it is required to increase the vigor of stocks of organisms to ensure good viability under the potential rigors of the experiment.

These are the types of experiments under way at present, and we include in this report short summaries of work in progress for the Biosatellite experiments.

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[1]Taken from *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1964*, ORNL-3700, p. 99.

IV. PREPARATION OF THE *HABROBRACON* STOCKS FOR THE BIOSATELLITE EXPERIMENT[1]

R. C. von Borstel Anna Rachel Whiting

In the wasp *Habrobracon*, females are diploid and heterozygous for sex alleles; normal males are haploid, developing from unfertilized eggs and containing one sex allele. Within an inbred stock, one half of the fertilized eggs have the same sex alleles. The majority of these are inviable, and the few survivors are sterile diploid males. For irradiation experiments, stocks with different sex alleles are used in the crosses, so that viability of controls will be high and experimental data need not be corrected for control inviability. A constant and easily identifiable character difference is also helpful in such experiments.

In order to increase the vigor of one of the sex allele stocks to be used in the biosatellite experiment, males from stock $17o^i$, an ivory eye color, were outcrossed to females from the No. 33 stock, eye color wild-type black. Daughters from these matings were backcrossed to $17o^i$ males other than their own parents. Daughters from these crosses were selectively mated to their own male parent, and each of the lines was then maintained by brother-sister matings.

It was necessary to test these lines against the No. 33 stock to find at least one which had the same sex alleles as the original No. $17o^i$ and different from those in the No. 33 stock. Hatchability of fertilized eggs from such crosses is always high, and diploid males are never produced. Females from a number of the outcrossed, backcrossed, and inbred ivory-eyed lines were crossed with the No. 33 wild-type males and the hatchability scored. One such line was found to give high hatchability and a high female to male ratio. No diploid males were found in 20 separate crosses.

These two stocks (the rederived $17o^i$ and the No. 33) are in excellent condition for the genetic analyses for dominant and recessive lethal and visible mutations. Analyses of sex alleles in two other wild-type stocks are under way to aid in the tests for the chromosomal translocations that are anticipated in the radiation experiment.

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[1]Taken from *ORNL Space Biol. Program Ann. Rept. June 30, 1964*, ORNL-TM-924, pp. 47–48.

V. BIOSATELLITE A PREPARATORY EXPERIMENTS[1]

F. J. de Serres R. C. von Borstel
B. B. Webber Anna R. Whiting

A number of preparatory experiments are now in progress to establish the possible effects of dose rate, temperature, dehydration, anoxia, and other parameters imposed on the biological

test materials by the nature of the proposed Biosatellite experiment. The actual mockup experiments are anticipated during the reporting period.

The progress reports of the individual investigators appear in this report in the appropriate sections: (1) Cytochemistry and Cell Reproduction – R. C. von Borstel and Anna R. Whiting; (2) Cytology and Genetics – F. J. de Serres and B. B. Webber.

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[1]Taken from *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1965,* ORNL-3768, p. 110.

VI. SPONTANEOUS MUTATION FREQUENCIES IN *HABROBRACON*[1]

A. R. Whiting
R. C. von Borstel
Diane J. Goins
Martha J. Bond

Introduction

In the course of testing sex alleles of various wild-type strains of *Habrobracon,* it was found that the Raleigh wild-type (R) was especially vigorous. This stock has sex alleles different from the wild-type stock No. 33, so that crosses between them give no diploid males. Diploid males are characterized by high inviability and sterility. The lemon mutant strain (with sex alleles derived from stock No. 33), a mutant readily identifiable in the heterozygous condition as well as in the pure stock, was used as the source of males for general experimentation. The first experiment using these two strains (R and lemon) was that of determination of the spontaneous mutation frequency.

Results and Discussion

Raleigh females were isolated, tested for presence of lethals, and then mated to lemon males, after which tests for presence of lethals were continued. F_1 females from lethal-free parents were set unmated, and individual records for offspring (haploid) viability were principal criteria for the 767 females tested and their 18,785 eggs. Of these females, 6 died early in the experiment, and 10 could be listed as sterile females. Out of the 751 remaining, 6 females (0.79%) were classed as being heterozygous for "semilethals" or oogonial lethals, since the hatchability of their eggs was between 60% and 80%. Two females out of 751 (0.26%) were considered to be heterozygous for spontaneous recessive lethals, since the hatchability of their eggs was below 60% and above 0. The remainder were lethal free. One female produced 20 offspring, 2 of which were white eyed. We believe that this visible mutation originated in an oogonial cell of the mother, since the mutant and wild-type sons were not found in equal numbers.

The results of this experiment demonstrate the reliability of these stocks and serve as background information for future use in radiation synergism studies.

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[1]Taken from *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1965*, ORNL-3768, pp. 110–11.

VII. THE *HABROBRACON* EXPERIMENT IN THE SIMULATED SPACE-FLIGHT TESTS OF APRIL 13 AND 19, 1965[1]

R. C. von Borstel A. R. Whiting S. Kondo[2] D. J. Goins M. L. Pardue[3]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Introduction

The purpose of this experiment was to test the radiation subassembly and prototype packages with *Habrobracon* in conjunction with all other radiation experiments under four separate conditions. These conditions were (1) the simulated flight profile, (2) the irradiation, (3) the simulated flight profile plus the irradiation, and (4) the nonprofile-nonirradiation control. It was assumed that this would provide an adequate test of the flight hardware so that experimenters would have the opportunity to approve or disapprove of the development of the hardware.

Experimental Conditions

The tests were held on April 13 and April 19, 1965. It was expected that the biological material was to be made available for attaching the packages to the subassembly at 8:30 a.m. For the *Habrobracon* experiment this meant that distribution of the *Habrobracon* to the modules and package assembly had to begin at 6:00 a.m. in order that the deadline be met. For the April 13 simulated flight, 7 wasps were placed in each of the modules, 7 males in the upper module that is on the left side when the observer faces from the front. The 14 females were distributed into the two bottom modules. The irradiation time in this test was 66 hr 20 min. For the April 19 simulated flight, 14 males were placed in the lower left module and 14 females were distributed into two modules placed in the upper left and the lower right of the package. The irradiation time was 66 hr 40 min.

Dosimetry

Toshiba glass rods were used to measure the doses. Their readings were standardized by the rods exposed to an NBS ^{60}Co-γ field. Their energy dependence was checked by an ORNL ^{137}Cs-γ field calibrated by a Victoreen chamber which in turn was standardized by NBS. There was no energy dependence. The rods were either encased in nylon, in polyethylene tubes, or

in aluminum. They were placed in the module that is on the upper right-hand side when the observer faces the front of the package. Dosimetry was performed only on the packages receiving the gamma radiation, not on the control packages nor on the profile only packages.

The dosimetric data are listed in Table 1. The nominal exposure is listed beneath the code number of each package in column 1 of the table. In the last column are listed the ratios between the measurements of the April 19 and April 13 experiments. It appears that the drop in the dose received from April 13 to the 19 was negligible in the packages that did not receive the vibration, whereas it dropped nearly 9% in those that received the vibration. The reason for this is not clear. At first it seemed possible that the sources were switched from one week to the next; this would account for no change in the ratio in one experiment and the greater than 5% drop (expected decay of ^{85}Sr slightly <1%/day) in the other. However, the persistently higher dose

Table 1. Measurements of Doses by Toshiba Glass Rods

(May 2, 1965, Biology Division, ORNL, S. Kondo)

Code and Nominal Dose	Experiment April 13–16				Experiment April 19–22				April 19 / April 13
	Capsule				Capsule				
	Polyethylene Tube	Nylon	Aluminum	Average (kr)	Polyethylene Tube	Polyethylene Tube (or Nylon)	Aluminum (or Nylon)	Average (kr)	
					Nonprofile				
051 (4000 r)	2.56	2.50	2.72	2.59	2.39	2.45 (nylon)	3.12 (nylon)	2.65	1.02
005 (2000 r)	1.18	None	None	1.18	1.27	1.19	None	1.23	1.04
006 (1000 r)	0.75	0.75	0.81	0.77	0.73	0.79 (nylon)	0.76	0.76	0.986
007 (500 r)	0.41	None	None	0.41	0.390	0.394	None	0.392	0.955
								Average	1.000
					Profile				
052 (4000 r)	2.12	2.27	2.50	2.30	2.18	2.20	None	2.19	0.95
010 (2000 r)	1.25	1.24	1.16	1.22	1.16	1.12	None	1.14	0.93
009 (1000 r)	0.75	0.74	0.77	0.75	0.654	0.652	None	0.653	0.87
011 (500 r)	0.34	0.40	Lost	0.37	0.334	0.32	None	0.33	0.892
								Average	0.911

received in the nonprofile-irradiation experiment mitigates against this possibility. A second possibility is that the radiation exposure time was somehow shortened for the profile-irradiation experiment during the second week. Third, there may be an effect of vibration on the Toshiba glass rods. A fourth possibility is that the experiments were placed in different areas in the room where the irradiations were performed and that scattered radiation was enhanced in the nonprofile-irradiation experiment during the second week. The most plausible explanation is suggested by the large variation in three of the glass rods in the nonprofile-irradiation experiment, namely, the 005 polyethylene encased rod and the 051 aluminum encased rod of April 13 and the second 051 nylon encased rod of April 19. The effect of these could have been additive and seems to indicate that a variation larger than 5% occurred in these dosimeters.

It is possible that with the mass production now under way, the Toshiba glass rods exceed the expected tolerance limits. We believe this can be corrected either by improved quality control in the manufacture, by measuring the dose in selected rods and annealing them before reusing, or by calibrating them with known low doses before using them in the experiments.

Nevertheless, taken as a whole, the Toshiba glass rods measure doses within ±5% limits of the expected decay for the second experiment begun six days after the first experiment.

The packages may have been placed close to the appropriate isodose lines as indicated by dose-square geometry, but it is clear from Figs. 1 and 2 that the expected relation of linearity between the nominal and measured dose was not realized. If we assume that the nominal 4000-r

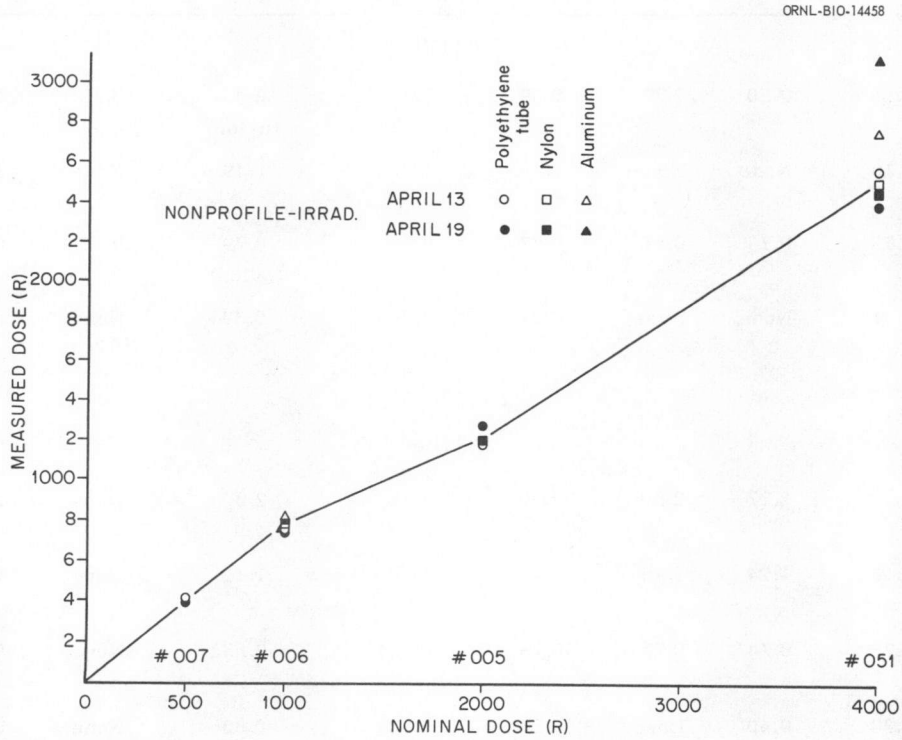

Fig. 1. Measured Dose Plotted Against Nominal Dose for Toshiba Glass Monitors Exposed in the Nonprofile-Irradiation Tests.

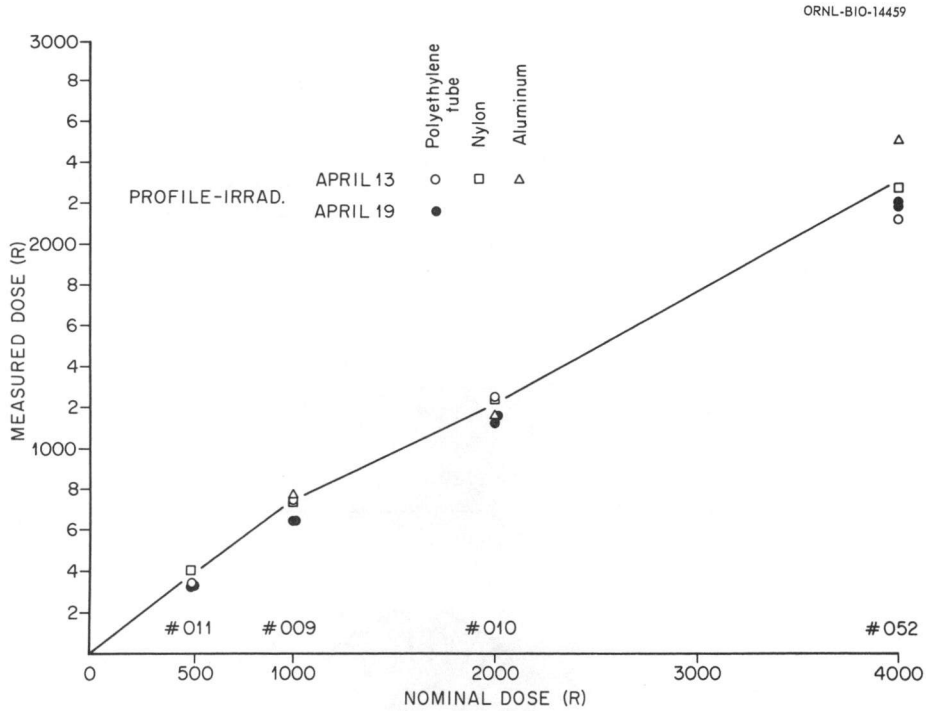

Fig. 2. Measured Dose Plotted Against Nominal Dose for Toshiba Glass Monitors Exposed in the Profile-Irradiation Tests.

dose packages were correctly placed on the backscatter shield (which they were not – see Table 2) and if a line is extrapolated to zero dose, the nominal 2000-r dose packages fall below the line and the other packages are above. This would indicate that the nominal 2000-r dose packages are partially shielded (possibly from the scattered gamma radiation) and the nominal 1000- and 500-r dose packages received more scattered radiation than is their due. That this is particularly relevant for the nominal 1000-r dose package is apparent when it is considered that the programmed dose-square isodose line is at 14.75 cm from the source, whereas the center of the irradiated volume of the package was actually placed at 15.5 cm from the source.

The occurrence of scattered gamma radiation was detected by comparison of glass rods encased in plastic, lead, and copper which were placed at different heights behind the packages (Table 3). Unfortunately, because of the structure of the packages, the lead-encased glass rods had approximately 8 mm less polystyrene in front of them than did the plastic- and copper-covered glass rods. This may have accounted for the similarity for the doses measured for all rods at any one distance. It can be seen that, except for the package closest to the source, the readings at the bottom of the package are always lower than the readings at the top. This disparity is greatest in the package farthest from the source.

From the ratios of the measurements given in the last four columns, it seems that the scattered gamma radiation does not contribute too much to the total dose. Precise determination of

Table 2. Distances of *Habrobracon* Packages from Radiation Source

Nominal Dose (r)	Programmed Distance (cm)	Distance to Center of Volume of One of Upper Modules (cm)	Distance to Center of Front Bottom Surface of Package (cm)
4000	7.4	7.7	8.15
2000	10.5	10.8	11.2
1000	14.75	15.5	15.8 (top and bottom)
500	20.9	20.95	21.7

Table 3. Comparison of Measurements of Dose by Glass Rods Encased in Plastic, Lead, and Copper at Three Different Heights in Nonprofile-Irradiation Experiment (April 13–16)

(May 2, Biology Division, ORNL, S. Kondo)

Nominal Dose (r)	Placement Behind Package	Plastic	Lead	Copper	Average (kr)	Lead/Plastic	Copper/Plastic	Plastic/Lead	Copper/Lead
4000	Top	1.64	1.55	1.57	1.59	0.945	0.965	1.06	1.014
	Middle	1.65	1.54	1.52	1.57	0.935	0.923	1.07	0.99
	Bottom	1.59	1.57	1.60	1.59	0.988	1.01	1.01	1.02
				Average	1.58	0.956	0.963	1.05	1.005
2000	Top	0.885	0.895	0.906	0.895	1.01	1.03	0.991	1.015
	Middle	1.02	0.88	0.90	0.93	0.864	0.883	1.16	1.022
	Bottom	0.87	0.82	0.83	0.84	0.942	0.954	1.06	1.014
				Average	0.888	0.906	0.917	1.07	1.017
1000	Top	0.585	0.541	0.586	0.571	0.925	1.00	1.08	1.08
	Middle	0.596	0.590	0.593	0.593	0.990	0.995	1.01	1.006
	Bottom	0.551	0.546	0.554	0.550	0.990	1.01	1.01	1.016
				Average	0.573	0.968	1.00	1.03	1.010
500	Top	0.314	0.316	0.336	0.322	1.006	1.07	0.996	1.06
	Middle	0.302	0.306	0.308	0.305	1.01	1.02	0.988	1.007
	Bottom	0.306	0.285	0.296	0.296	0.931	0.966	1.07	1.04
				Average	0.308	0.982	1.018	1.018	1.06

the contribution of scattered radiation of different wavelengths will require extensive further experimentation.

The conclusion that can be drawn about the scattered gamma radiation at this time is that it probably comes from two places: backscatter from the tungsten (which was shielded from the nominal 2000-r dose packages and gave rise to the higher-than-expected dose on the nominal 1000-r dose package that was raised on the bracket) and glancing scatter from the backscatter shield (as seen from the data in Table 3).

In order to evaluate the extent of any possible radiation synergistic effect of the simulated space-flight dynamics on the *Habrobracon*, it is necessary to make a comparison between the strengths of the sources used in the two radiation experiments. These data are shown in Table 4. The intensity for the nonprofile-irradiation experiment is calculated to have been 6% higher than that for the profile-irradiation experiment.

Table 4. Ratio of Dose for "Nonprofile" to That for "With Profile"

Nominal Dose (r)	April 13	April 19	Average
4000	1.12	$1.21 \times 0.911 = 1.10$	1.11
2000	0.97	$1.08 \times 0.911 = 0.985$	0.98
1000	1.03	$1.16 \times 0.911 = 1.06$	1.05
500	1.11	$1.19 \times 0.911 = 1.08$	1.09
		Average	1.06

Results of Biological Tests

Survival. – Although a few deaths were observed among the wasps, the only position where catastrophic results were obtained was in the nominal 1000-r isodose position, where the package was held on an inverted U-shaped bracket. The male wasps in the upper-left module were pulverized during the vibration testing in the April 13–16 experiment. For the April 19–22 experiment female wasps were placed in this module to see if the males merely happened to be more fragile. It was observed that the females were also broken to pieces and this occurred only during the reentry vibration testing to the wasps in the upper-left module at the nominal 1000-r dose position. The males in the lower module were injured but many mated. It can also be seen in Table 5 that egg production of the females decreased drastically in the nominal 1000-r dose position. This is seen first of all because half the females were pulverized. The females in the lower module were badly injured and many died. Those that did not die were slow to lay.

Induced Lethality. – The summary, unanalyzed data for hatchability and adult survival are listed in Table 5. From the data tabulated herein and using the equations of von Borstel and

Table 5. Hatchability and Adult Survival of Treated Females and Females Mated to Treated Males[a]

	April 13–16 Hatchability		April 19–22 Hatchability		April 19–22 Adult Survival	
	Eggs Laid	Hatched	Eggs Laid	Hatched	♂ ♂	♀ ♀
Control						
n p n r						
♀	630	594	1769	1700	1270	
♂ × ♀	332	260	1512	1302	411	580
Nominal 4000 r						
p n r						
♀ (1)	13	13	111	107	71	
♀ (2, 3, 4)	111	109	348	340	248	
♂ × ♀	92	68	354	323	100	164
n p r						
♀ (1)	99	40	106	69	52	
♀ (2, 3, 4)	101	89	272	178	138	
♂ × ♀	122	64	249	151	70	48
p r						
♀ (1)	60	28	124	90	66	
♀ (2, 3, 4)	77	71	320	217	164	
♂ × ♀	0	0	597	383	157	98
Nominal 2000 r						
p n r						
♀ (1)	23	18	66	57	39	
♀ (2, 3, 4)	88	84	302	267	201	
♂ × ♀	102	94	115	113	35	67
n p r						
♀ (1)	93	56	77	57	47	
♀ (2, 3, 4)	84	76	306	245	203	
♂ × ♀	145	93	655	489	189	198
p r						
♀ (1)	120	86	79	67	34	
♀ (2, 3, 4)	88	72	333	290	183	
♂ × ♀	107	60	263	206	100	62
Nominal 1000 r						
p n r						
♀ (1)	0		0	0		
♀ (2, 3, 4)	0		10	8	6	
♂ × ♀			295	281	44	130
n p r						
♀ (1)	90	62	84	65	54	
♀ (2, 3, 4)	83	63	286	240	160	
♂ × ♀	116	84	631	526	166	205
p r						
♀ (1)	28	22	14	10	8	
♀ (2, 3, 4)	39	33	90	85	48	
♂ × ♀			391	316	83	166

Table 5 (Continued)

	April 13–16 Hatchability		April 19–22 Hatchability		April 19–22 Adult Survival	
	Eggs Laid	Hatched	Eggs Laid	Hatched	♂ ♂	♀ ♀
Nominal 500 r						
p n r						
♀ (1)	14	13	82	79	60	
♀ (2, 3, 4)	84	80	301	297	203	
♂ × ♀	38	32	261	234	41	105
n p r						
♀ (1)	67	57	113	104	91	
♀ (2, 3, 4)	80	75	380	357	255	
♂ × ♀	108	68	331	286	63	127
p r						
♀ (1)	87	76	85	76	65	
♀ (2, 3, 4)	85	82	396	379	180	
♂ × ♀	42	27	157	149	54	75

[a]The figures in parentheses represent the first, or the second, third, and fourth changes of the wasps to new host caterpillars.

Rekemeyer,[4] a dose-action curve for sperm survival (1.0–dominant lethal frequency) for the April 19–22 test was constructed. This is shown in Fig. 3. It is apparent that there is no clear evidence for a synergistic effect for the flight profile and the radiation for induced dominant lethality in sperm.

The data on the exposed females in Table 5 will not be dealt with in this report. The results on recessive lethality and induced translocations are not yet recorded. Nevertheless, from the data at hand it is possible to make recommendations for package modifications (Appendix I), testing program recommendations (Appendix II), and control data program recommendations (Appendix III). A short report on the results from the group at Raleigh, North Carolina, is listed as Appendix IV.

Conclusions

It is clear that the radiation subassembly does not follow dose-square relations. No evidence for a synergistic effect of flight profile dynamics with radiation was detected. The *Habrobracon* package needs to be modified.

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[1]Special Report to Headquarters, Biosatellite Experiment, NASA, Ames Research Center, Moffett Field, Calif., submitted 15 May 1965.

[2]Department of Fundamental Radiology, Faculty of Medicine, Osaka University, Osaka, Japan.

[3]Department of Biology, Pardue University, Lafayette, Ind.

[4]R. C. von Borstel and M. L. Rekemeyer, "Radiation-Induced and Genetically Contrived Dominant Lethality in *Habrobracon* and *Drosophila*," *Genetics* **44**, 1053–74 (1959).

ORNL-BIO-14465

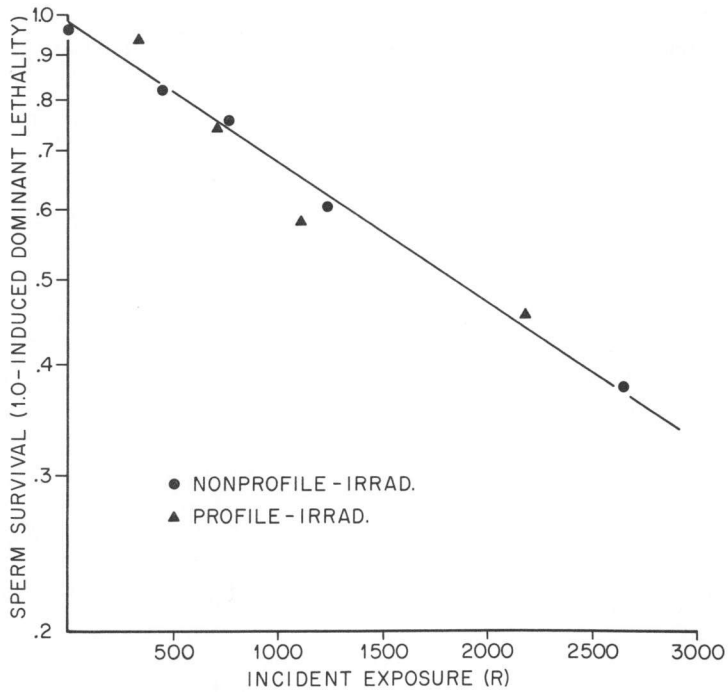


Fig. 3. Dose vs Response for Embryo Dominant Lethality Induced in Sperm During the April 19–22 Test.

Appendix I. Necessary Package Modifications

1. *Habrobracon* in the top module of the package in the nominal 1000-r dose position were pulverized during the simulated reentry vibrational testing. The bracket at the nominal 1000-r dose position needs to be strengthened to minimize amplification of the vibrations or whipping of the package.

2. When the packages were inadvertently autoclaved, some of the screens loosened and the threads for the modules warped enough to make proper fitting impossible. Although sterilization of the *Habrobracon* packages is not required, it would be best to have the package made of material that would not warp under autoclaving conditions.

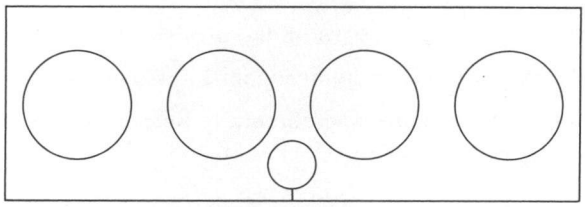
3. To obtain proper electron equilibrium, a 1-mm-thick plastic shield should be placed in front of the package.

4. The cover of the thermistor should be identical to the covers of the modules to best mimic and thus measure the temperature conditions of the wasps.

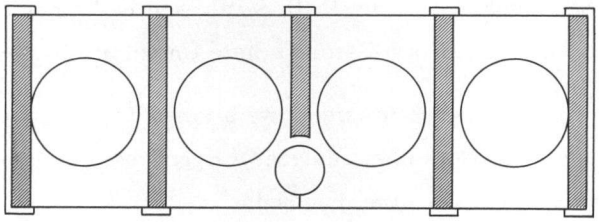
5. The position of the package on the isodose line should be alignable since the holes for the bolts holding the package were not tight fitting. Perhaps this can be done by marking lines

on the backscatter shield so that the corners of the packages can always be placed in the same position; or small wedges could be placed on the shield to serve the same purpose.

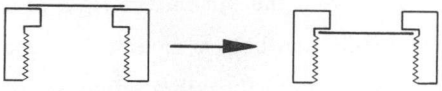
6. Vibrational effects are unequally distributed over the package (wasps in the top module of the nominal 1000-r dose package only were pulverized; see Appendix IV for other evidence). Also, as shown particularly in the nominal 500-r dose package, scattered radiation was unequally distributed from the top to the bottom of the package. Therefore it would be best if the modules in each package were aligned in a row parallel to the backscatter shield.



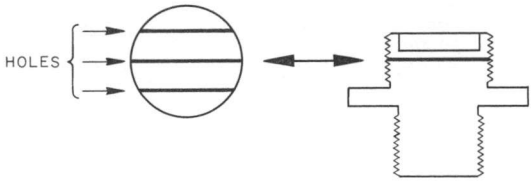
7. Holes for LiF dosimetry should be placed between the modules.



8. The module should be modified by placing the screen on the opposite side of the cover.



9. The module well should be $\frac{1}{16}$ in. deep instead of $\frac{1}{8}$ in.
10. Three holes for glass dosimeters should be drilled under the module well.



Appendix II. Testing Program Recommendations

It appears that, after modification, it will probably be necessary to test the radiation subassembly again.

In addition, it would appear to be necessary to perform three to five separate large-scale tests of wasps under known conditions of irradiation and vibration.

Appendix III. Control Data Program Recommendations

We wish to conduct dose-action experiments on the strains of wasps we are using for the biosatellite. These would entail obtaining the dominant lethal, recessive lethal, and translocation frequencies at different doses of acute and chronic radiation using exposures of 500 to 10,000 r.

Appendix IV. Egg Production and Hatchability of Eggs of Females from the Third to Fifteenth Day After the Simulated Space Flight Test

D. S. Grosch, J. C. Lin, R. H. Smith, and L. Valcovic
Department of Genetics, North Carolina State University, Raleigh, N. C.

After the data for the immediate genetic effects were taken by the group at Oak Ridge, the wasps were transferred to Raleigh for further analyses. Eggs were collected on a daily basis, and the egg production and hatchability were recorded.

A considerable variability in the results has been noted, although final recording of the data and the data analyses are not yet complete. For the purpose of this report, it is worth mentioning that there appears to be a nonuniformity of response between the irradiated and nonirradiated controls and the *Habrobracon* that underwent the simulated flight profile. This was apparent also between the wasps in the top module and bottom module in the package at the nominal 4000 r dose position where a differential effect within the vibrated package was observed even without irradiation.

Therefore we believe that more uniform results would be obtained if all modules in a package were arranged in one row parallel to the backscatter shield.

VIII. THE SIMULATED SPACE-FLIGHT TEST OF AUGUST 20, 1965, FOR *HABROBRACON*[1,2]

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Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee

Introduction

The purpose of this experiment was to test the radiation subassembly and prototype packages with *Habrobracon* under two separate conditions. These conditions were (1) the simulated flight profile with different exposures of radiation including nominal zero dose and (2) the different exposures of radiation including nominal zero dose but without the simulated flight profile.

Even though the packages in the test were the same packages used in the April 13 and 19, 1965, tests,[3] the simulated flight profile had been changed to what is believed to be a more realistic set of vibration parameters. Since the wasps had been shown to be sensitive to the effects of vibrations, it was considered mandatory to test them under the new set of conditions.

Experimental Conditions

The test was begun on August 20, 1965, and ended on August 23. A ^{137}Cs source was used for the gamma radiation. The irradiation time was 66 hr and 10 min. Ten wasps were placed in each of the modules, except for a newly designed three-layered module for the controls (designed by Nakwon Cho, Technical Division of ORGDP), where 12 wasps were placed in each layer. Facing the packages from the radiation-source position, virgin females were placed in the upper-right and lower-left modules, and males were placed in the upper-left and lower-right modules. The females tested were of the *Raleigh* wild-type strain, and the males were of the *lemon* mutant strain.

Results of Biological Tests

Survival. – In this test none of the wasps were pulverized as were some in the April test. Nevertheless, a few males died in almost all modules whether or not they had been taken through the simulated-flight profile (Table 1). On the other hand, nearly all of the females survived. Egg production of the females was unaffected. We are planning to use a hardier strain of male wasps in future tests.

Induced Lethality. – The summary data, analyzed by the method of von Borstel and Rekemeyer,[4] are shown in Table 2. The measured doses are those of J. E. Hewitt and M. G. Hershberger from Ames Research Center. The embryo survival at different exposures can be subtracted from the zero-dose controls to give the frequency of induced dominant lethality. No synergistic effect is observable for the flight profile and radiation for induced dominant lethality in sperm.

Table 1. Survival of Wasps at Termination of Three-Day Experiment

Nominal Dose (r)	Sex	Flight Profile			No Profile		
		Module Position[a]	Number of Wasps	Number of Survivors	Module Position	Number of Wasps	Number of Survivors
4000	♂	U.L.	10	8	U.L.	10	6
		L.R.	10	7	L.R.	10	5
	♀	U.R.	10	10	U.R.	10	10
		L.L.	10	10	L.L.	10	10
2000	♂	U.L.	10	9	U.L.	10	5
		L.R.	10	9	L.R.	10	9
	♀	U.R.	10	10	U.R.	10	10
		L.L.	10	10	L.L.	10	10
1000	♂	U.L.	10	7	U.L.	10	6
		L.R.	10	8	L.R.	10	4
	♀	U.R.	10	9	U.R.	10	10
		L.L.	10	10	L.L.	10	10
500	♂	U.L.	10	4	U.L.	10	5
		L.R.	10	8	L.R.	10	10
	♀	U.R.	10	10	U.R.	10	10
		L.L.	10	10	L.L.	10	10
0	♂	U.L.[b]			U.L.	10	7
		Inner	12	7	L.R.[b]		
		Middle	12	8	Inner	12	9
		Outer	12	6	Middle	12	8
		L.R.	10	8	Outer	12	6
	♀	U.R.	10	10	U.R.	10	10
		L.L.	10	10	L.L.	10	10

[a]U.L., upper left position; L.R., lower right position; U.R., upper right position; L.L., lower left position.
[b]Three-layered module.

Table 2. Dose-Survival of Embryos After Exposure of Sperm to ^{137}Cs Radiation Source

Nominal Dose (r)	Radiation Alone		Flight Profile and Radiation	
	Measured Dose (r)	Embryo Survival	Measured Dose (r)	Embryo Survival
0	0	0.99	0	1.00
500	500	0.79	505	0.73
1000	955	0.76	945	0.74
2000	1950	0.47	2110	0.46
4000	3755	0.16	3640	0.17

Conclusions

There is no evidence for a synergistic effect of flight-profile dynamics and radiation on either wasp survival or induced dominant lethality.

---

[1]Special Report to Headquarters, Biosatellite Experiment, NASA, Ames Research Center, Moffett Field, Calif., submitted 5 November 1965.

[2]Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

[3]R. C. von Borstel, A. R. Whiting, S. Kondo, D. J. Goins, and M. L. Pardue, "The *Habrobracon* Experiment in the Simulated Space-Flight Tests of April 13 and 19, 1965," Special Report to Headquarters, Biosatellite Experiment, NASA, Ames Research Center, Moffett Field, Calif., submitted 15 May 1965, 23 pp.

[4]R. C. von Borstel and M. L. Rekemeyer, "Radiation-Induced and Genetically Contrived Dominant Lethality in *Habrobracon* and *Drosophila*," *Genetics* 44, 1053–74 (1959).

IX. X-RADIATION SENSITIVITY OF *HABROBRACON* OOCYTES AT DIAKINESIS[1]

R. H. Smith Anna R. Whiting

Introduction

The complex nature of prophase I in meiosis requires that, for greatest significance of irradiation studies, the exact condition of the chromosomes at time of exposure be determined. Previously reported tests[2] of *Habrobracon* oocytes have demonstrated the high resistance of earlier stages in prophase I and the high sensitivity of oocytes in metaphase I, a ratio of 20 to 1. A similar differential radiosensitivity was later reported in other organisms, for example, *Drosophila*.[3,4] The resistant stage in *Habrobracon* and *Drosophila* is probably the diplotene

stage. The only report containing evidence contrary to this response is one on Artemia oocytes, where a decrease in sensitivity was shown during the course of meiosis.[5] Cervini and Giavelli found that prophase, mostly diakinesis, is more sensitive than metaphase. We focus our attention here on the sensitivity of diakinesis.

Results and Discussion

The *Habrobracon* female has four ovarioles, each ending posteriorly in a uterine sac. A well-fed female restrained from ovipositing for 16 hr has in each uterine sac four eggs in the first meiotic metaphase and one in diakinesis. Previous studies have demonstrated that an exposure of 2000 or 2500 r, sufficient to induce dominant lethal changes in over 99% of eggs in metaphase I, has imperceptible effects on oocytes in prediakinesis stages. In the present experiment, hatchability and times of death of embryos were followed closely over six short egg-laying intervals. By cytological examination, the maximum number of metaphase I eggs per female was found to be 16, as expected. At the exposures used (2000 and 2500 r), the number of nonhatching eggs exceeded this maximum number in 59 out of 113 females. The average number of nonhatching eggs exceeding the 16-egg maximum was 19, and the range was up to 23 eggs per female.

Therefore, the diakinesis stage in *Habrobracon* is in the same range of radiation sensitivity as the first meiotic metaphase.

---

[1]Taken from *Biol. Div. Semiann. Progr. Rept. July 31, 1966*, ORNL-3999, pp. 62–63.

[2]Anna R. Whiting, *Am. Naturalist* **79**, 193–227 (1945).

[3]A. G. Bateman and A. C. Chandley, *Intern. J. Radiation Biol.* **7**, 385–94 (1963).

[4]D. R. Parker, in *Repair from Genetic Radiation Damage*, by F. H. Sobels, pp. 11–29, Pergamon, London, 1963.

[5]A. Cervini and S. Giavelli, *Mutation Res.* **2**, 452–56 (1965).

X. TIME OF DEATH OF A TRANSLOCATION SEGREGANT IN *HABROBRACON*[1]

R. L. Amy R. C. von Borstel

Introduction

Translocations can be induced by x rays in *Habrobracon* males and detected by inherited partial sterility methods. Of the eggs laid by a translocation heterozygote, about 50% will carry a duplication for one chromosome segment and a deletion for another. Under these conditions such eggs would be deficient for some portion of the normal gene complement. An analysis of the developmental history of eggs of this type should make it possible to determine the time during embryogeny when the missing genes act.

Results and Discussion

Eggs from *Habrobracon juglandis* (T_3 stock) heterozygous for a single translocation were collected and checked for hatchability. Those that failed to hatch were examined under the dissecting microscope (30×) and classified, on the basis of their morphological appearance, according to the system described by von Borstel and Rekemeyer.[2] The results are summarized in Table 1. Of a total of 1336 eggs collected, 718 did not hatch. The majority (85%) of the latter had reached fairly advanced stages of embryonic differentiation and were classed as death types 3 or 4. Those categorized as 3a and 3b had ceased development at approximately 20 ±1 hr; those classed as type 4 had reached 23 hr (a) or at least 24 hr (b) of age before dying. It appears, therefore, that this particular translocation involves a part of the genome that exerts its influence during the last one-third of the 29-hr embryonic period.

---

[1]Taken from *Biol. Div. Semiann. Progr. Rept. July 31, 1966*, ORNL-3999, p. 63.
[2]R. C. von Borstel and M. L. Rekemeyer, *Genetics* **44**, 1053 (1959).

Table 1. Embryonic Stages at Which Death Occurred in Nonhatching T_3 *Habrobracon* Translocation Segregants

Stage of Death	Number of Embryos	Percent
1	4	0.5
2	0	
3a	160	22.3
3b	22	3.1
4a	165	23.0
4b	269	37.5
5	2	0.3
Unclassifiable	96	13.3
Total nonhatching (of 1336 laid)	718	

XI. SPONTANEOUS MUTATION FREQUENCIES IN *HABROBRACON*[1]

R. C. von Borstel
R. H. Smith
Anna R. Whiting
Diane J. Goins
Katherine T. Cain
Ellen S. Sirota
Margaret L. Haye

Introduction

We have obtained the frequencies of embryo lethals and translocations in the Raleigh wild-type strain of *Habrobracon*.

Methods

Raleigh females were isolated as virgins and tested for the presence of lethals, and females bearing lethals were discarded. Females having high hatchability were mated with *Raleigh* males, and female offspring were isolated as virgins. Large numbers of these *Raleigh* virgins were tested for lowered hatchability. When a female showed lowered hatchability, she was removed for further testing. This testing included four periods of oviposition for determining hatchability, adult survival, and time of death of haploid embryos. The females were then mated to *lemon* males containing different sex alleles and tested for hatchability, adult survival, sex ratio, and time of death of diploid embryos. If the lowered hatchability were caused by a recessive embryo lethal mutation, then the deaths during embryogenesis would be covered in the diploid. If the lowered hatchability were caused by deletion-duplication segregants from a female heterozygous for a translocation, then deaths during embryogenesis are merely delayed to a later stage of development.[2] If no change in time of death occurred, then these females were classified as semisterile or sterile, depending on hatchability.

Results

Of the 1392 *Raleigh* virgins tested, 13 were heterozygous for recessive lethal mutations, 4 were heterozygous for translocations, and 4 were semisterile.

Discussion

These tests for spontaneous mutation and chromosomal translocation frequency are for the entire genome. The recessive lethal frequency for the *Raleigh* strain is 0.0093. The spontaneous mutation frequency was obtained earlier for the *lemon/Raleigh* hybrid.[3] Two obtained from 751 females tested for a frequency of 0.0026; no translocation heterozygotes were found. The difference is significant and suggests that strain differences exist for spontaneous lethal mutation frequency in *Habrobracon*.

---

[1]Taken from *Biol. Div. Semiann. Progr. Rept. July 31, 1966*, ORNL-3999, pp. 63–64.

[2]R. C. von Borstel and M. L. Rekemeyer, *Genetics* **44**, 1053–74 (1959).

[3]A. R. Whiting *et al.*, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1965*, ORNL-3768, pp. 110–11.

XII. X-RADIATION INDUCTION OF MUTATIONS IN *HABROBRACON*[1]

R. H. Smith
Anna R. Whiting
R. C. von Borstel
Katherine T. Cain
Diane J. Goins
Ellen S. Sirota
Margaret L. Haye

Introduction

Unfertilized eggs of *Habrobracon* develop into normal haploid males; fertilized eggs develop into diploid females. Techniques have been developed for *Habrobracon* which permit analysis of the entire genome for dominant lethality, recessive lethality, and chromosomal translocations.[2,3] The purpose of this experiment is to determine these parameters for sperm irradiated with 2000 r. Three batches of F_1 virgins were tested at approximately monthly intervals to see if selection operated against mutation heterozygotes held in cold storage.

Results and Discussion

Lemon males were irradiated and crossed with *Raleigh* females pretested for high hatchability. The frequency of dominant lethality with embryo hatchability as a criterion was 0.279, with 6045 eggs being analyzed. The results of the tests for recessive lethal mutations and translocations are shown in Table 1. It can be seen that the mutation frequencies remained essentially constant in the three replicates. This fortunate circumstance makes it possible to test large numbers of individuals over a period of months, since only a few hundred can be successfully handled and tested at one time.

---

[1]Taken from *Biol. Div. Semiann. Progr. Rept. July 31, 1966*, ORNL-3999, p. 64.
[2]R. C. von Borstel and M. L. Rekemeyer, *Genetics* **44**, 1053–64 (1959).
[3]R. C. von Borstel *et al.*, sect. 4.4, this report.

Table 1. Translocations and Recessive Lethal Mutations in Female Progeny from *Lemon* Males X Irradiated with 2000 r

	Replicate 1 (Jan. 31)		Replicate 2 (Feb. 22)		Replicate 3 (Mar. 21)		Total	
	Number	Frequency	Number	Frequency	Number	Frequency	Number	Frequency
Translocations	68	0.200	80	0.253	67	0.250	215	0.232
Recessive lethal mutations								
Embryo	25	0.074	37	0.117	26	0.097	88	0.095
Larva	26	0.076	29	0.092	19	0.071	74	0.080
Pupa	13	0.038	13	0.041	13	0.045	38	0.041
Total females tested	340		316		267		923	

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