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QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
JULY 1 – SEPTEMBER 30, 1966

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BIOLOGY DIVISION

QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

July 1–September 30, 1966

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QUARTERLY PROGRESS REPORT
TO THE
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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

For the Period: July 1–September 30, 1966

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I. INTRODUCTION

R. C. von Borstel Roger H. Smith

The research carried out for the Biosatellite Project, and its impending launch, must of necessity fall into two categories: (1) the preparation of the biological material, with all the corollary experiments associated with constructing the genetic strains, increasing the efficiency of analysis, and studying radiation effects at a fundamental level; and (2) the preparation for the flight itself, with all the corollary experiments associated with construction of the flight vehicle. The first requires continually refining the level of sophistication where we do radiation biology experiments, and the second requires proper management of timed experiments performed by large task forces under the alien conditions outside the laboratory.

In this Quarterly Report we list the types of genetic and cellular damage that we can assay in *Habrobracon* and the method of data analysis. We describe the results of the Ames Research Center Biocompatibility Test, the radiation conditions surrounding the *Habrobracon* packages in the ARC Test, and the results of the Flight Vehicle Tests at General Electric Company. Furthermore, we report some of the supporting experiments that are required continuously to keep improving the experimental system. Last, a discussion is included that provides a theoretical foundation for the *Habrobracon* dominant lethality data that will come from the ground control for the Biosatellite Experiment. This was taken from a lecture presented at the Third International Congress of Radiation Research held in the summer of 1966 in Cortina, Italy.

II. MUTATIONS IN *HABROBRACON*

R. C. von Borstel Roger H. Smith

In the parasitic wasp *Habrobracon*, heterozygosity at the sex locus confers femaleness, and azygosity or homozygosity confers maleness. Commonly, in studies of mutagenesis of either parent, the females having one set of sex alleles are crossed with haploid males from a strain containing different sex alleles; this avoids the necessity of correcting the data for the largely inviable diploid males arising in inbred lines.

Unmated females produce normal haploid males, and females mated with males having different sex alleles produce both haploid male and diploid female offspring. Hence when males are irradiated, the survival data must be corrected for the unfertilized eggs.

Dominant Lethality

The condition of dominant lethality is fulfilled when a haploid nucleus has been altered in such a manner that upon combination with a normal haploid nucleus the resulting heterozygote dies either immediately or eventually.

Males. – Accurate methods for estimating dominant lethality in *Habrobracon* were derived by Atwood, von Borstel, and Whiting (1956) and von Borstel and Rekemeyer (1950). Their equations are given here.

The frequency of embryo dominant lethal mutations, D, that depress hatchability when sperm are irradiated can be estimated by

$$D = 1 - \frac{m - V_u (1 - f)}{f} , \tag{1}$$

where m is the hatchability frequency from the mated females, V_u is the surviving proportion of unfertilized eggs (determined from unmated controls), and f is the proportion fertilized. Adult survival data can be inserted in Eq. (1) to estimate D', the frequency of total dominant lethal mutations from egg to adult; m' and V'_u from adults correspond to m and V_u for the embryos.

The determination of the frequency of fertilized eggs, f, is taken from the experimental animal data by the equation

$$f = 1 - \frac{p}{V'_u} , \tag{2}$$

where p is the ratio of surviving adult males from the mated females to the total eggs from the mated females.

Females. – When females are irradiated, homogeneous clusters of eggs are laid which, by accurate timing, can be placed in categories of oocytes irradiated in the first meiotic metaphase (metaphase I), diakinesis, and the earlier first meiotic prophase (presumably diplotene) stages. Since diakinesis is in the same range of sensitivity as the first meiotic metaphase (Smith and Whiting, 1966), it is often convenient to compare only the sensitive metaphase I and the resistant prophase I and discard from the calculations the eggs in diakinesis and the possible mixture of metaphase I and prophase I oocytes that can come from female-to-female variation in the samples taken between oocytes known to be in metaphase I or prophase I when irradiated.

In most experiments the females are bred unmated, so the criterion is not that of dominant lethality per se but that of total dominant and recessive lethality. For dominant lethality alone, Eqs. (1) and (2) are applicable when the females are mated, or the recessive lethal frequency can be estimated in the F_2 generation and this figure subtracted from the total lethal frequency. The latter method is a simple test, since irradiated conditionally delayed dominant lethality becomes a factor when oocytes are irradiated (Atwood *et al.*, 1956). Conditionally delayed dominant lethals are embryos from unfertilized eggs that normally die before reaching the hatching stage and which die during the larval and pupal stages when the oocytes are fertilized with normal

sperm. The use of conditionally delayed dominant lethality for analysis of translocations is described in a later section.

Recessive Lethal Mutations

Half of the offspring will die if an unmated female is heterozygous for a recessive lethal mutation. If heterozygous for two unlinked lethals, 75% of the offspring will die. If heterozygous for two complementary lethals (synthetic lethals), 25% of the offspring will die.

The normal procedure is to isolate as virgins the F_1 females from a radiation experiment and set them unmated.

The hatchabilities of the ensuing embryos are scored. When a female is found that has lowered hatchability of her eggs, she is mated to a haploid male containing sex alleles different from hers. The recessive lethal mutation should be fully covered by the normal genome, and half the F_2 females will be heterozygous for the recessive lethal mutation.

If the normal genome does not cover the mutation, but the embryos still die either as embryos, larvae, or pupae, then this type of death is classified as a translocation.

Inherited Partial Sterility

There are four criteria for determining whether or not a female having low egg viability is heterozygous for a translocation or a related chromosomal aberration:

1. Most females heterozygous for a translocation lay eggs with hatchability frequencies considerably below 50% (usually between 30 and 40%, occasionally as low as 20 to 30%).
2. Most females heterozygous for a translocation lay eggs that die between the 20th and 23d hour of development (Amy and von Borstel, 1966).
3. Embryos from females heterozygous for a translocation die at later stages when the eggs are fertilized (conditionally delayed dominant lethality).
4. Half the viable male offspring from a translocation heterozygote contain the two aberrant chromosomes. When these males are mated to normal females, the female offspring again show the typical low hatchability and associated syndromes. This pattern of inheritance is called inherited partial sterility.

Criteria 1 and 2 are reasonably diagnostic for a translocation, and criterion 3 is definitive.

Inherited partial sterility was discovered by Belling (1914) and its cause explained by him a number of years later (Belling, 1925). The behavior of chromosomes during meiosis in an individual heterozygous for a translocation is shown in Fig. 1. The alternate segregation of the chromosomes yields gametes with balanced genomes, and the adjacent segregation gives gametes deficient for part of one chromosome and duplicated for part of another.

From the alternate segregation the translocated chromosomes are preserved in half the surviving offspring, and from the adjacent segregation all of the offspring die.

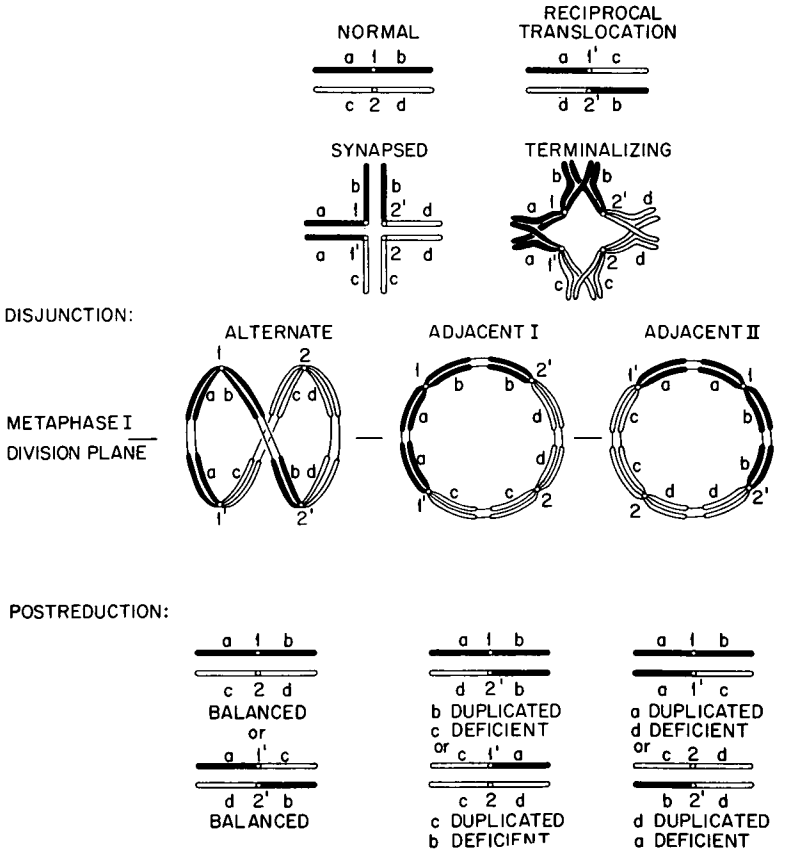

Fig. 1. Segregation Patterns of Translocated Chromosomes.

Visible Mutations

Mutations that alter the color, form, or behavior of the late pupae or adult are classed as visible mutations, even when the pupae do not eclose.

Sterility and Semisterility

Sterile females are of three classes: those that lay no eggs, those that lay eggs that have not been activated to develop, and those we call semisterile females which lay many nonactivated eggs but still have some that develop normally. These classes are easily recognized since the eggs that are not activated to develop die very early in development. A thorough study of a recessive mutant for sterility through nonactivation of eggs was made (von Borstel, 1960), and it was found that the eggs that emerge from the female anterior to the ovipositor without being squeezed by the ovipositor are not activated to develop.

Classification of Time of Death of Lethal Mutants

Embryos. – A reasonably accurate method for determining the stage of death of embryos was developed by von Borstel and Rekemeyer (1959); their description is given here. This staging method is done without fixation or staining of the embryos and is used wherever eggs are counted and hatchability is scored.

The egg chorion is 2 μ thick and is transparent. The embryos are classified in five different categories, stages 1, 2, 3, 4, and 5 (Fig. 2). The stages of death are sequential in embryogenesis, but the absolute times of death during embryogenesis have not yet been completely worked out. For simplest recognition of the stage of death, the humidity is maintained at 60% and the temperature at 30°C; the hatchability is scored between 30 and 45 hr after the laid eggs have been counted.

Stage 1 consists of embryos in which death occurs during karyokinesis before the blastoderm forms; the embryos have a mottled white appearance, and one end, or both, is filled with a clear fluid. Stage 2 is the blastula stage up to the beginnings of tissue differentiation and is characterized by two homogeneous white bands. Embryos in stage 3 are those in which the yolk mass is changing from white to yellow and the yolk is beginning to become centrally located. Stage 4 contains nearly fully developed embryos and can be readily distinguished by the presence of tracheal elements and ureate cells. Stage 5 is made up of fully developed embryos that die at any time during the hatching process. An embryo is considered fully hatched when it is no longer connected with the chorion.

Stages 3 and 4 can be further subdivided. Stage 3a is distinguished from 3b by the heterogeneous distribution of yolk in the former. Stage 4a embryos are those in which tracheal elements are just beginning to form, and 4b embryos have the fully formed tracheal elements and ureate bodies. The gut occasionally ruptures in stage 4 embryos, and the yolk in these may be homogeneously distributed throughout or be somewhat concentrated in the posterior end of the embryo.

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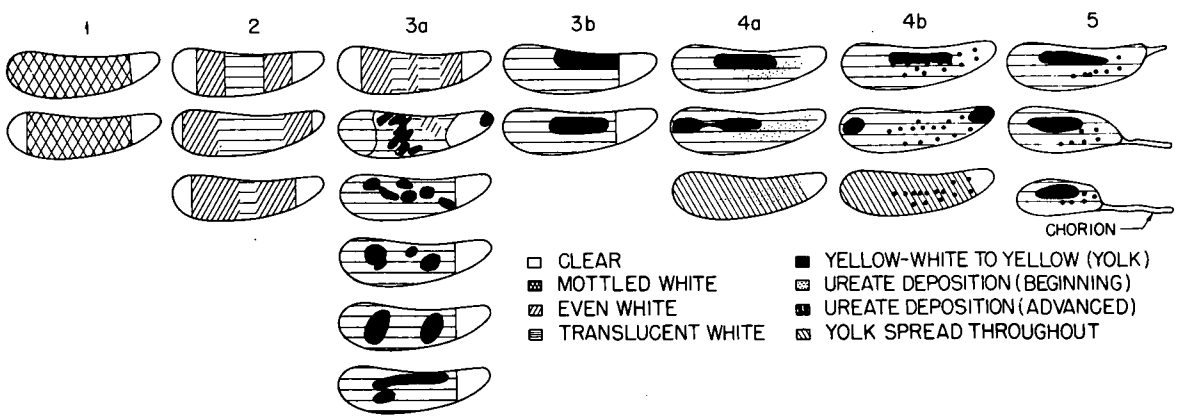

Fig. 2. Stages at Which Radiation Death Is Expressed During Development of *Habrobracon* Embryos.

Larvae. – *Habrobracon* larvae undergo five instars, and though we can comfortably categorize the times of death during larval development in five stages, these do not necessarily correspond to the instars. These stages are nonfeeding (N), early (E), mid (M), late (L), and spinning (SL) larval death. Their appearance is self-explanatory.

The nonfeeding larvae are scored when hatchability is scored. Occasionally stage E can be scored at this time also. The other stages are scored when adults are scored. If an embryo cannot be found at the time of scoring of the adult survival, it is assumed that the embryo died during stage E.

Pupae. – Pupal development begins when the meconium is ejected from the larva after the larva has spun a cocoon. This can be recognized as a black spot on the end of the cocoon.

The stages of death during pupal development are subdivided into four parts, prepupal (P), early (E), mid (M), and late (L). Sex is indistinguishable in the prepupal period. Early pupal development (E) is the time during which the pupa is nonpigmented or lightly pigmented, but sex is distinguishable. Mid-pupal development by our method of scoring occurs when the pupa is pigmented but the wings are not extended. Late pupal development is the period when the pupa appears to be a mature animal, but it has not emerged from the cocoon.

General. – All of these times of death and rules for adjustment of numbers of animals in case of egg shriveling, accidental death, or scoring error are given in the Appendix on examples of the score sheets. The score sheets are also numbered across the top for computer programming.

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Appendix. Score Sheets with Description of Times of Death and Rules for Adjusting Numbers at Separate Counts

ORNL-BIO-17016

HABROBRACON SCORE SHEET

DATE | EXPERIMENT

♀ №	EGGS	HATCH	EMBRYO 1	2	3 A	3 B	4 A	4 B	5	LARVA N	E	M	L	SL	PUPA P	E	M	L										
1 2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	24 25	26 27	28 29	30 31	32 33	34 35	36 37	38 39	40 41	42 43	44 45	46 47	48 49	50 51				

Egg Counts

(1) Record number of eggs and number of shriveled eggs (approximate shriveled eggs if large number are present)

(2) Indicate any peculiarities in eggs

Hatchability and Emergence

(1) Subtract shriveled eggs from total eggs

(2) Indicate number of shriveled eggs and stage of death of unhatched eggs

~~15~~ 12 9 1 1 1 (actually erase original number of eggs) 3 sh

(3) All larvae which appear to be sick (dying) or dead: mark in appropriate column

12 10 1 1 1 1

(4) Any accidental deaths will be subtracted from total egg count. Accidental deaths include: Eggs which have fallen from the larval Ephestia and hatched and Habrobracon larvae which have fallen from Ephestia larva and died

(5) When an Ephestia caterpillar is diseased or injured and Habrobracon larvae die, eliminate this particular count

(6) If the number of eggs is in surplus when hatchability is recorded, adjust total number of eggs to the new count

(7) If the number of eggs is deficient after a very careful survey of Ephestia larva and glass dish, then adjust data by considering these as nonfeeding Habrobracon larvae

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HABROBRACON SCORE SHEET

DATE | EXPERIMENT

♀ №	EGGS	HATCH	EMBRYO 1	EMBRYO 2	EMBRYO 3A	EMBRYO 3B	EMBRYO 4A	EMBRYO 4B	EMBRYO 5	LARVA N	LARVA E	LARVA M	LARVA L	LARVA SL	PUPA P	PUPA E	PUPA M	PUPA L	♂	
1 2 3	4 5	6 7	8 9	10 11	12 13	14 15	16 17	18 19	20 21	22 23	24 25	26 27	28 29	30 31	32 33	34 35	36 37	38 39	40 41	42 43 44 45 46 47 48 49 50 51
										Nonfeeding	Just begun to feed	Small — medium	Large	Without meconium deposit Has started to spin	Larva in a cocoon; meconium deposit present; sex indistinguishable.	Can distinguish sex Nonpigmented or lightly pigmented.	Wings not extended; pigmented	Wings extended -- ready to emerge.		
	12	10	1		1						3						1		6	Deficiency in Emergence Scoring; write in early larval stage.
													Smeared	very dark and wet	dry in cocoon or smeared and wet	red to black	dried and crisp or smeared and wet			
															disease					
	13 ~~12~~	11 ~~10~~		1		1					1		1						9	Surplus: adjust total eggs and hatchability Nos. to correspond.

(actually erase original number of eggs)

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III. COMPUTER TABULATION AND ANALYSIS OF DATA

Diana B. Smith Roger H. Smith

Because of the enormous amount of data collected from the preliminary and future Biosatellite experiments, data analysis by desk calculators is impracticable. The IBM 360 model 75 computer located at Oak Ridge National Laboratory (X-10) is now used routinely to process experimental data.

As described in Sect. II, egg counts, hatchability, stages of larval and pupal death, and adult emergence are recorded on *Habrobracon* data sheets (Sect. II). This information is then punched into cards.

A. Error Checking Program (Fig. 3)

This program produces a complete listing of data for each female in each transfer (Fig. 4). The computer indicates the errors if any discrepancies are detected. These errors are then corrected by an investigator in the *Habrobracon* laboratory and the data returned for computation.

ORNL-BIO-17018

```
**FTN,L,P,F.
      PROGRAM ERROR
      DIMENSION S(22),ILIST(22)
   99 FORMAT(1H1,11HHABROBRACON)
  100 FORMAT(I3,22F2.0,32X,I1)
  200 FORMAT(1H0,I4,2F4.0,F6.1,20F4.0,I5,9H  N  R  T)
  300 FORMAT(1H+,120X,5HERROR)
  600 FORMAT(1H-,4H NO.,2A4,6X,20A4,3X,1HT)
      ILIST(1)=4H  F
      ILIST(2)=4H  H
      ILIST(3)=4H  1
      ILIST(4)=4H  2
      ILIST(5)=4H 3A
      ILIST(6)=4H 3B
      ILIST(7)=4H 4A
      ILIST(8)=4H 4B
      ILIST(9)=4H  5
      ILIST(10)=4H  N
      ILIST(11)=4H  E
      ILIST(12)=4H  M
      ILIST(13)=4H  L
      ILIST(14)=4H SL
      ILIST(15)=4H  P
      ILIST(16)=4H  E
      ILIST(17)=4H  M
      ILIST(18)=4H  L
      ILIST(19)=4H
      ILIST(20)=4H
      ILIST(21)=4H
      ILIST(22)=4H
      PRINT 99
      PRINT 600,ILIST
    2 READ(50,100,END=2000)IX,(S(I),I=1,22),IZ
      NT=1
      IND=0.
      SCOL=0.
      DO 6 I=3,22
    6 SCOL=SCOL+S(I)
      IF(SCOL.NE.S(1))7,8
    7 IND=1
    8 SEMB=0.
      DO 9 I=3,9
    9 SEMB=SEMB+S(I)
      IF(SEMB.NE.S(1)-S(2))10,11
   10 IND=1
   11 HAT=S(2)*100.0/ S(1)
      PRINT 200,IX,S(1),S(2),HAT,(S(I),I=3,22),NT
  234 IF(IND.NE.0)18,19
   18 PRINT 300
   19 IF(IX.EQ.0)2000,2
 2000 CALL EXIT
      END
```

Fig. 3. The Error Checking Program.

HABROBRACON

NO.	E	H	%H	1	2	3A	3B	4A	4B	5	N	E	M	L	SL	P	E	M	L	♂	♀			T				
1	10.	6.	60.0	3.	0.	1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	1.	1.	0.	2.	1.	0.	0.	1	N	R	T	
1	6.	3.	50.0	2.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	3.	0.	0.	0.	1	N	R	T	
1	4.	1.	25.0	3.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	1	N	R	T	
1	8.	5.	62.5	3.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	1.	1.	0.	0.	2.	0.	0.	0.	1	N	R	T	
1	11.	4.	36.4	7.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	0.	0.	0.	2.	0.	0.	0.	1	N	R	T	
1	12.	5.	41.7	7.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	4.	0.	0.	0.	1	N	R	T	
3	10.	4.	40.0	6.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	2.	0.	0.	0.	0.	0.	1	N	R	T	
3	4.	0.	0.0	4.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	
3	4.	0.	0.0	4.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	
3	11.	3.	27.3	8.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	1.	0.	0.	0.	0.	0.	1.	0.	0.	1	N	R	T	
3	4.	1.	25.0	3.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	1	N	R	T	
3	7.	2.	28.6	5.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	1.	0.	0.	0.	1	N	R	T	
4	10.	2.	20.0	8.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	0.	0.	1	N	R	T	
4	8.	4.	50.0	7.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	1	N	R	T	ERROR
4	13.	5.	38.5	8.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	5.	0.	0.	0.	1	N	R	T	
4	5.	3.	60.0	2.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	1.	0.	0.	1	N	R	T	ERROR
5	4.	1.	25.0	3.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1	N	R	T	
5	4.	0.	0.0	4.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	
5	7.	3.	42.9	4.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	3.	0.	0.	1	N	R	T	
5	2.	0.	0.0	2.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	
6	7.	7.	100.0	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	5.	2.	0.	0.	1	N	R	T	
6	6.	0.	0.0	6.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	
6	9.	2.	22.2	7.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1	N	R	T	
6	5.	2.	40.0	3.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	0.	0.	1	N	R	T	
6	15.	6.	40.0	8.	0.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	5.	1.	0.	0.	1	N	R	T	
7	6.	2.	33.3	3.	0.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	0.	0.	0.	1	N	R	T	
7	7.	3.	42.9	4.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	3.	0.	0.	0.	1	N	R	T	
7	1.	0.	0.0	1.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1	N	R	T	

Fig. 4. A Listing of Data from the Error-Checking Program.
Headings from left to right:
NO – female number,
E – number of eggs,
H – number of hatched eggs,
%H – percent of hatched eggs,
1, 2, 3*a*, 3*b*, 4*a*, 4*b*, 5 – stages of embryonic death,
N, E, M, L, SL – stages of larval death,
P, E, M, L – stages of pupal death,
♂ and ♀ – adult emergence,
T – number of transfers,
N, R, T – refer to normal, recessive lethal, and translocation respectively. These designations are not used in this particular program.

B. Dominant Lethal Program (Fig. 5)

This program instructs the computation of the percentage of dominant lethal mutations for each stage of death (Fig. 6) using the formulas described in Sect. II. It also summarizes the percent hatchability and the number of deaths in each developmental stage for each female (Fig. 7).

```
**FTN.L.P.F.
      PROGRAM DOM
      DIMENSION VIRG(22),WENCH(22),S(22),F(22),X(22)
      DIMENSION ILIST(22)
   99 FORMAT(1H1,11HHABROBRACON//)
  100 FORMAT(I3,22F2.0)
  200 FORMAT(1H0,I4,22F5.0,I5)
  300 FORMAT(1H0,120X,5HERROR)
  400 FORMAT(1H1,15HUNMATED FEMALES,F7.0///)
  500 FORMAT(1H0,5X,A4,5X,F8.0,5X,F8.6)
  600 FORMAT(1H0,4H NO..22A5,3X,1HT)
  700 FORMAT(1H1,15H  MATED FEMALES,F7.0///)
  800 FORMAT(1H1,18HDOMINANT LETHALITY///)
 1000 FORMAT(1H0,5X,A4,5X,F9.6)
      ICON=4H  D
      ILIST(1)=4H  F
      ILIST(2)=4H  H
      ILIST(3)=4H  1
      ILIST(4)=4H  2
      ILIST(5)=4H 3A
      ILIST(6)=4H 3B
      ILIST(7)=4H 4A
      ILIST(8)=4H 4B
      ILIST(9)=4H  5
      ILIST(10)=4H  N
      ILIST(11)=4H  E
      ILIST(12)=4H  M
      ILIST(13)=4H  L
      ILIST(14)=4H SL
      ILIST(15)=4H  P
      ILIST(16)=4H  E
      ILIST(17)=4H  M
      ILIST(18)=4H  L
      ILIST(19)=4H
      ILIST(20)=4H
      ILIST(21)=4H
      ILIST(22)=4H
 3000 DO 1 I=1,22
      VIRG(I)=0.
    1 WENCH(I)=0.
      PRINT 99
      PRINT 600,ILIST
      CNT1=0.
      CNT2=0.
      READ 100,IX,(S(I),I=1,22)
    2 ID=IX
      NT=0
      DO 3 I=1,22
    3 F(I)=0.
      IND=0.
    4 DO 5 I=1,22
    5 F(I)=F(I)+S(I)
      SCOL=0.
      DO 6 I=3,22
    6 SCOL=SCOL+S(I)
      IF(SCOL.NE.S(1))7,8
    7 IND=1
    8 SEMB=0.
      NT=NT+1
      DO 9 I=3,9
    9 SEMB=SEMB+S(I)
      IF(SEMB.NE.S(1)-S(2))10,11
   10 IND=1
   11 READ(50,100,END=2000)IX,(S(I),I=1,22)
      IF(IX.EQ.ID)4,12
   12 FEM=F(21)+F(22)
      IF(FEM.EQ.0.)13,15
   13 IF(F(2)/F(1).LT.,80)15,23
   23 DO 14 I=1,22
   14 VIRG(I)=VIRG(I)+F(I)
      CNT1=CNT1+1.
      GO TO 17
   15 DO 16 I=1,22
   16 WENCH(I)=WENCH(I)+F(I)
      CNT2=CNT2+1.
   17 PRINT 200,ID,(F(I),I=1,22),NT
      IF(IND.NE.0.)18,19
   18 PRINT 300
   19 IF(IX.EQ.0.)20,2
   20 DO 21 I=1,22
      S(I)=VIRG(I)/VIRG(1)
   21 F(I)=WENCH(I)/WENCH(1)
      PRINT 400,CNT1
      PRINT 500,(ILIST(I),VIRG(I),S(I),I=1,22)
      PRINT 700,CNT2
      PRINT 500,(ILIST(I),WENCH(I),F(I),I=1,22)
      P=F(19)+F(20)
      VU=S(19)+S(20)
      FC=1.-P/VU
      CON=1.-FC
      DO 22 I=2,22
   22 X(I)=(F(I)-S(I)*CON)/FC
      DOM=1.-X(2)
      PRINT 800
      PRINT 1000,ICON,DOM
      PRINT 1000,(ILIST(I),X(I),I=2,22)
      GO TO 3000
 2000 CALL EXIT
      END
```

Fig. 5. The Dominant Lethal Program.

DOMINANT LETHALITY

D	0.720931
H	0.279069
1	0.699159
2	0.000480
3A	-0.004186
3B	-0.002049
4A	0.002041
4B	0.019674
5	0.005812
N	0.006489
E	0.011985
M	0.002675
L	-0.003448
S1	0.0
P	-0.037822
F	0.000630
M	-0.009668
L	-0.002130
	0.000000
	0.0
♀	0.310357
	0.0

Fig. 6. A Summary from the Dominant Lethal Program. Frequency of dominant lethality (D), egg hatchability (E), and frequency of dominant lethality in each developmental stage.

HABRGBRACCN

NO.	F	H	I	2	3A	3B	4A	4B	5	N	F	M	L	SL	P	E	M	L	♂		♀		T
7	110.	59.	48.	0.	0.	0.	0.	3.	0.	1.	3.	0.	1.	0.	8.	0.	1.	0.	29.	0.	16.	0.	7
10	79.	45.	29.	0.	0.	0.	0.	1.	0.	1.	1.	0.	0.	0.	6.	1.	2.	0.	14.	0.	24.	0.	7
11	95.	59.	33.	0.	0.	0.	0.	3.	0.	2.	3.	0.	3.	0.	5.	0.	0.	0.	23.	0.	23.	0.	7
12	83.	46.	38.	0.	0.	0.	0.	1.	0.	1.	0.	0.	0.	0.	10.	1.	1.	0.	19.	0.	14.	0.	7
13	97.	52.	37.	1.	0.	1.	0.	5.	1.	0.	1.	0.	2.	0.	3.	0.	0.	0.	26.	0.	20.	0.	7
14	96.	57.	37.	0.	0.	0.	1.	0.	1.	1.	2.	0.	0.	0.	6.	0.	2.	0.	20.	0.	26.	0.	7
15	84.	46.	36.	0.	0.	0.	0.	1.	1.	1.	1.	0.	0.	0.	3.	0.	0.	0.	25.	0.	16.	0.	7
16	80.	46.	32.	0.	0.	0.	0.	2.	0.	2.	2.	0.	1.	0.	3.	0.	0.	0.	22.	0.	16.	0.	7
17	76.	47.	26.	0.	1.	0.	0.	0.	2.	1.	2.	0.	2.	0.	8.	0.	0.	1.	16.	0.	17.	0.	7
18	89.	51.	37.	0.	0.	0.	0.	1.	0.	0.	0.	3.	2.	0.	14.	0.	1.	0.	8.	0.	23.	0.	7
19	93.	54.	37.	0.	0.	0.	0.	2.	0.	0.	5.	0.	0.	0.	8.	0.	3.	0.	16.	0.	22.	0.	7
20	72.	40.	32.	0.	0.	0.	0.	0.	0.	1.	0.	1.	2.	0.	2.	1.	1.	0.	18.	0.	14.	0.	7
21	74.	48.	26.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1.	1.	1.	0.	29.	0.	15.	0.	7
22	102.	58.	41.	0.	1.	0.	0.	1.	1.	1.	4.	0.	3.	0.	6.	1.	1.	0.	23.	0.	19.	0.	7
23	96.	52.	44.	0.	0.	0.	0.	0.	0.	0.	2.	2.	1.	0.	10.	1.	3.	0.	18.	0.	15.	0.	7
24	84.	46.	35.	0.	1.	0.	0.	2.	0.	1.	3.	0.	3.	0.	16.	0.	0.	0.	6.	0.	17.	0.	7
25	92.	49.	42.	0.	0.	0.	0.	1.	0.	0.	2.	0.	1.	0.	10.	0.	2.	0.	17.	0.	17.	0.	7
26	95.	53.	40.	0.	0.	0.	0.	2.	0.	1.	0.	0.	2.	0.	10.	0.	2.	0.	19.	0.	19.	0.	7
27	77.	35.	41.	0.	0.	0.	0.	1.	0.	0.	1.	0.	1.	0.	3.	1.	0.	0.	17.	0.	12.	0.	7
28	69.	38.	30.	0.	0.	0.	0.	1.	0.	0.	1.	0.	2.	0.	4.	0.	2.	0.	17.	0.	12.	0.	7
30	95.	38.	53.	0.	0.	0.	0.	4.	0.	2.	1.	2.	0.	0.	3.	1.	1.	1.	15.	0.	12.	0.	7
31	70.	34.	33.	0.	0.	0.	0.	3.	0.	0.	5.	1.	2.	0.	5.	0.	1.	0.	12.	0.	8.	0.	7
32	59.	25.	34.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1.	0.	14.	0.	9.	0.	7
33	83.	46.	36.	0.	0.	0.	0.	1.	0.	0.	2.	0.	0.	0.	7.	0.	1.	0.	20.	0.	16.	0.	7
34	105.	58.	46.	0.	0.	0.	0.	1.	0.	1.	2.	0.	0.	0.	3.	0.	0.	0.	29.	0.	23.	0.	7
35	69.	33.	35.	0.	0.	0.	0.	1.	0.	0.	2.	0.	2.	0.	4.	1.	2.	0.	11.	0.	11.	0.	7
36	108.	69.	38.	0.	1.	0.	0.	0.	0.	1.	4.	2.	1.	0.	12.	4.	3.	0.	31.	0.	11.	0.	7
37	101.	55.	42.	0.	0.	0.	0.	3.	1.	1.	1.	0.	1.	0.	11.	1.	1.	0.	24.	0.	15.	0.	7
38	89.	51.	36.	0.	0.	0.	1.	1.	0.	1.	5.	1.	1.	0.	9.	0.	1.	0.	20.	0.	13.	0.	7
39	83.	41.	34.	0.	2.	0.	0.	5.	1.	1.	0.	0.	1.	0.	4.	0.	1.	0.	21.	0.	13.	0.	7
40	92.	65.	26.	0.	0.	0.	0.	1.	0.	2.	2.	1.	3.	0.	9.	1.	0.	0.	31.	0.	16.	0.	7

Fig. 7. A Listing of Data from the Dominant Lethal Program.

C. Program for Determining Frequencies of Recessive Lethal and Inherited Partial Sterility (Fig. 8)

Data are summarized for the F_1 females as shown in Fig. 9. Information from a virgin female and information from the same female after mating are recorded adjacent to one another. This enables one to determine more efficiently whether or not the reduction in hatchability or viability is due to a recessive lethal mutation or inherited partial sterility (see Sect. II). The number of each type of mutation is then totaled and frequencies calculated.

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```
**FTN.L.F.
      PROGRAM REC
      DIMENSION S(22),F(22),ILIST(22)
   99 FORMAT(1H1,11HHABROBRACON)
  100 FORMAT(I3,22F2.0,32X,I1)
  200 FORMAT(1H0,I4,2F4.0,2F6.1,20F4.0,I5,9H  N  R  T)
  201 FORMAT(1H ,I4,2F4.0,2F6.1,20F4.0,I5)
  300 FORMAT(1H+,120X,5HERROR)
  600 FORMAT(1H-,4H NO.,2A4,12X,20A4,3X,1HT)
  700 FORMAT(1H0,I5,8H FEMALES)
      ILIST(1)=4H  E
      ILIST(2)=4H  H
      ILIST(3)=4H  1
      ILIST(4)=4H  2
      ILIST(5)=4H 3A
      ILIST(6)=4H 3B
      ILIST(7)=4H 4A
      ILIST(8)=4H 4B
      ILIST(9)=4H  5
      ILIST(10)=4H  N
      ILIST(11)=4H  E
      ILIST(12)=4H  M
      ILIST(13)=4H  L
      ILIST(14)=4H SL
      ILIST(15)=4H  P
      ILIST(16)=4H  E
      ILIST(17)=4H  M
      ILIST(18)=4H  L
      ILIST(19)=4H
      ILIST(20)=4H
      ILIST(21)=4H
      ILIST(22)=4H
 3000 PRINT 99
      NF=0
      PRINT 600,ILIST
      READ(50,100,END=2000)IX,(S(I),I=1,22),IZ
    2 ID=IX
      ID2=IZ
      NT=0
      DO 3 I=1,22
    3 F(I)=0.
      IND=0.
    4 DO 5 I=1,22
    5 F(I)=F(I)+S(I)
      SCOL=0.
      DO 6 I=3,22
    6 SCOL=SCOL+S(I)
      IF(SCOL.NE.S(1))7,8
    7 IND=1
    8 SEMB=0.
      NT=NT+1
      DO 9 I=3,9
    9 SEMB=SEMB+S(I)
      IF(SEMB.NE.S(1)-S(2))10,11
   10 IND=1
   11 READ(50,100,END=2000)IX,(S(I),I=1,22),IZ
      IF(IX.EQ.ID)144,12
  144 IF(ID2.EQ.IZ)4,12
   12 HAT=F(2)*100.0/ F(1)



      VIA=100.*(F(19)+F(20)+F(21)+F(22))/F(1)
      IF(ID2 .EQ.0)13,14
   14 PRINT 201,ID,F(1),F(2),HAT,VIA,(F(I),I=3,22),NT
      GO TO 234
   13 PRINT 200,ID,F(1),F(2),HAT,VIA,(F(I),I=3,22),NT
      NF=NF+1
      GO TO 234
  234 IF(IND.NE.0)18,19
   18 PRINT 300
   19 IF(IX.EQ.0)4000,2
 4000 PRINT 700,NF
      GO TO 3000
 2000 CALL EXIT
      END
```

Fig. 8. The Program for Determining the Frequencies of Recessive Lethal Mutations and Inherited Partial Sterility.

HABROBRACON

No.	F	H	%H	%A	1	2	3A	3B	4A	4B	5	N	E	M	L	SL	P	E	M	L	♂ +	♂ le	♀ +	♀ le	T			
80	39.	39.	100.0	79.5	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1.	0.	1.	5.	0.	14.	17.	0.	0.	4	N	R	T
81	46.	19.	41.3	32.6	2.	0.	12.	13.	0.	0.	0.	1.	1.	0.	0.	0.	0.	0.	2.	0.	8.	7.	0.	0.	4	N	R	T
81	30.	17.	56.7	16.7	0.	0.	5.	0.	1.	3.	4.	5.	4.	0.	0.	0.	2.	0.	1.	0.	1.	0.	0.	4.	3			
82	39.	47.	94.9	82.1	0.	0.	2.	0.	0.	0.	0.	0.	2.	1.	0.	0.	0.	0.	2.	0.	13.	19.	0.	0.	4	N	R	T
83	41.	40.	97.6	75.6	0.	0.	1.	0.	0.	0.	0.	0.	2.	0.	0.	2.	0.	0.	5.	0.	17.	14.	0.	0.	4	N	R	T
84	46.	24.	52.2	41.3	0.	0.	11.	10.	0.	0.	1.	0.	0.	0.	0.	0.	1.	3.	1.	0.	12.	7.	0.	0.	4	N	R	T
85	39.	13.	33.3	17.9	1.	0.	17.	5.	0.	2.	1.	0.	1.	0.	0.	0.	0.	0.	5.	0.	3.	4.	0.	0.	4	N	R	T
85	18.	11.	61.1	22.2	0.	0.	4.	0.	0.	3.	0.	3.	3.	0.	0.	0.	0.	0.	1.	0.	0.	0.	2.	2.	3			
86	29.	24.	82.8	31.0	0.	0.	1.	0.	0.	0.	4.	1.	7.	7.	0.	0.	0.	0.	0.	0.	5.	4.	0.	0.	3	N	R	T
87	32.	31.	96.9	71.9	1.	0.	0.	0.	0.	0.	0.	1.	1.	0.	0.	1.	0.	0.	5.	0.	13.	10.	0.	0.	4	N	R	T
88	47.	20.	42.6	29.8	0.	0.	17.	8.	0.	1.	1.	0.	5.	0.	0.	0.	1.	0.	0.	0.	9.	5.	0.	0.	4	N	R	T
88	26.	13.	50.0	19.2	0.	0.	6.	1.	0.	6.	0.	0.	1.	2.	1.	1.	1.	0.	2.	0.	1.	0.	1.	3.	3			
89	36.	14.	38.9	25.0	0.	0.	9.	8.	0.	4.	1.	0.	0.	0.	1.	2.	0.	0.	2.	0.	4.	5.	0.	0.	4	N	R	T
89	40.	16.	40.0	35.0	0.	0.	6.	3.	0.	9.	6.	0.	0.	0.	0.	0.	0.	0.	2.	0.	4.	5.	3.	2.	3			
90	36.	33.	91.7	88.9	0.	0.	1.	1.	0.	1.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	13.	19.	0.	0.	4	N	R	T
91	38.	38.	100.0	89.5	0.	0.	0.	0.	0.	0.	0.	0.	4.	0.	0.	0.	0.	0.	0.	0.	14.	20.	0.	0.	4	N	R	T
92	44.	43.	97.7	52.3	0.	0.	0.	0.	0.	0.	1.	1.	2.	0.	3.	1.	4.	2.	7.	0.	15.	8.	0.	0.	4	N	R	T
93	31.	30.	96.8	83.9	0.	0.	1.	0.	0.	0.	0.	0.	1.	0.	0.	0.	1.	0.	2.	0.	15.	11.	0.	0.	3	N	R	T
94	32.	14.	43.8	34.4	0.	0.	16.	2.	0.	0.	0.	1.	2.	0.	0.	0.	0.	0.	0.	0.	7.	4.	0.	0.	3	N	R	T
94	26.	8.	30.8	7.7	0.	2.	2.	0.	0.	2.	12.	1.	1.	0.	1.	2.	1.	0.	0.	0.	0.	0.	2.	0.	3			
95	45.	45.	100.0	80.0	0.	0.	0.	0.	0.	0.	0.	1.	1.	0.	0.	0.	0.	0.	7.	0.	19.	17.	0.	0.	4	N	R	T
96	47.	46.	97.9	87.2	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	1.	1.	1.	0.	2.	0.	20.	21.	0.	0.	4	N	R	T
97	37.	18.	48.6	29.7	0.	0.	14.	5.	0.	0.	0.	0.	0.	0.	0.	3.	0.	4.	0.	0.	6.	5.	0.	0.	4	N	R	T
97	27.	17.	63.0	14.8	2.	0.	3.	0.	0.	5.	0.	4.	9.	0.	0.	0.	0.	0.	0.	0.	0.	0.	2.	2.	3			
98	38.	38.	100.0	47.4	0.	0.	0.	0.	0.	0.	0.	1.	6.	2.	1.	2.	0.	8.	0.	0.	7.	11.	0.	0.	4	N	R	T
99	41.	31.	75.6	9.8	0.	0.	0.	0.	1.	0.	9.	9.	5.	9.	0.	0.	0.	4.	0.	0.	1.	3.	0.	0.	4	N	R	T
99	27.	25.	92.6	55.6	0.	0.	1.	0.	0.	1.	0.	1.	1.	0.	0.	0.	5.	0.	3.	0.	0.	0.	7.	8.	3			
100	42.	42.	100.0	88.1	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	0.	0.	1.	0.	3.	18.	19.	0.	0.	4	N	R	T
101	40.	36.	90.0	80.0	0.	0.	3.	0.	0.	1.	0.	0.	0.	1.	0.	0.	1.	0.	2.	0.	15.	17.	0.	0.	4	N	R	T
102	17.	14.	82.4	41.2	1.	0.	1.	0.	1.	0.	0.	2.	0.	0.	0.	0.	0.	4.	1.	0.	4.	3.	0.	0.	4	N	R	T
103	44.	44.	100.0	90.9	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	3.	0.	1.	0.	20.	20.	0.	0.	4	N	R	T
104	11.	10.	90.9	36.4	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.	3.	1.	0.	2.	0.	0.	3.	1.	0.	0.	4	N	R	T
105	42.	42.	100.0	95.2	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	1.	0.	0.	1.	16.	24.	0.	0.	4	N	R	T
106	39.	20.	51.3	43.6	0.	0.	5.	1.	13.	0.	0.	1.	1.	0.	0.	0.	0.	0.	1.	0.	4.	13.	0.	0.	4	N	R	T

Fig. 9. A Listing of Data from the Recessive Lethal and Inherited Partial Sterility Program.

♂ + – wild-type males,

♂ *le* – males of lemon body color,

♀ + – homozygous wild-type females,

♀ *le* – females heterozygous for lemon body color.

The N, R, or T is circled according to whether or not the female in question is normal, carrying a recessive lethal, or a translocation respectively.

IV. MEASURING FECUNDITY, FERTILITY, AND LIFE SPAN OF *HABROBRACON* FEMALES

D. S. Grosch L. R. Valcovic Roger H. Smith

Habrobracon females possess four polytrophic ovarioles, each of which contains a series of cells ranging from oocytes in the first meiotic metaphase to interphase oogonia. When females are irradiated, or are given some other kind of physiological insult, the effects usually can be detected in cells of various types by following daily changes in fecundity and egg hatchability for the life of the females.

Fecundity

Eggs are collected from each female at 24-hr intervals and placed in individual spot plate depressions filled with mineral oil. The mineral oil not only preserves the eggs in a good condition, but it allows embryo development to proceed to hatching so that embryonic stages of death and hatchability can be recorded over a period of hours, or even days, with no loss of eggs or embryos from drying or by the young larvae crawling away. Oviposition records are thus obtained for 20 days into the period of senile decline.

The differential fecundity of the females and thus the viability of the developing cells over the life-span are shown for control and irradiated females in Fig. 10. Eggs deposited at a designated time can be traced back to their cytological condition at the time of treatment. As can be seen, transitional cells between the oogonial and oocyte stages are more sensitive to x rays than either the differentiated oocytes or the oogonial cells.

Hatchability

Hatchability records of eggs deposited over the 20 days of oviposition provide additional data for measuring sensitivity of the developing germ cells. Taken together with the fecundity measurements of the transitional cells, the hatchability provides a biological amplification mechanism for observing fluctuations in an already extremely sensitive system. Most of the damage induced in the transitional cells and the oogonia is dominant (LaChance, 1959), but it is different from the dominant lethals found in eggs exposed to radiation in the first meiotic metaphase or prophase in that, although the embryos usually die later in development (stage 4), there is no delay in onset of death when the embryos are fertilized (von Borstel and St. Amand, 1963).

As the irradiated females age, more and more of their eggs die in the earliest stage (stage 1) of embryonic development (von Borstel and St. Amand, 1963). Again, the shifts of onset of these different types of death, as well as their frequencies, serve as an amplification of any type of damage seen in fecundity measurements.

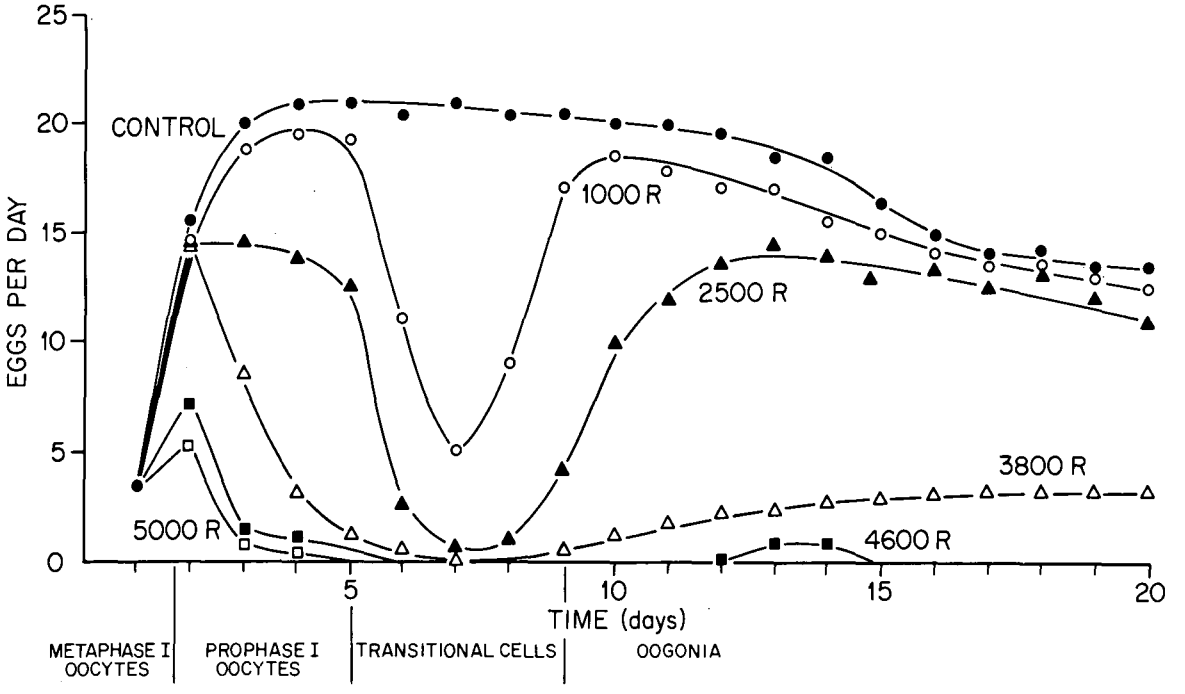

Fig. 10. The Average Number of Eggs Deposited per Day for Samples of 15 to 20 *Habrobracon* Females. The cytological condition of the ovariole for the various egg samples is given.

Life-Span

The life-span of the individual females measures gross physiological disturbances induced by the radiation.

Literature Cited

LaChance, L. E., 1959, "The Effect of Chelation and X Rays on Fecundity and Induced Dominant Lethals in *Habrobracon*," *Radiation Res.* **11**, 218–28.

von Borstel, R. C. and W. St. Amand, 1963, "Stage Sensitivity to X Radiation During Meiosis and Mitosis in the Egg of the Wasp Habrobracon," pp. 87–100 in *Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells*, ed. by F. Sobels, Pergamon, London.

V. THE SIMULATED SPACE FLIGHT TEST (BIOCOMPATIBILITY TEST) AT AMES RESEARCH CENTER, JUNE 17–20, 1966

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L. R. Valcovic
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Margaret L. Haye
Mary Lou Pardue

This test was designed as a ground-based control taking place before the actual flight, using the flight hardware under expected flight conditions insofar as they could be simulated. For the first time since the *Habrobracon* portion of the Biosatellite Project was initiated, a full complement of investigators and technical staff had been brought together. Therefore, as far as was possible with the simultaneous Flight Vehicle testing being done at General Electric Company in Philadelphia, a three-generation assay of total genetic damage to the organism was carried out at all ^{85}Sr-gamma radiation exposures.

Preparation of Materials

For this experiment we sought to use virgin *Habrobracon* of the Raleigh (*R*) wild-type strain that had been preexamined to exclude any with defective genomes. Since the time for the experiment appeared to be rigidly set, and since at that time we had the dual commitments of this test and Flight Vehicle tests at Philadelphia, we prepared only one group of females for the test.

To our chagrin, the test slipped several weeks, and there was no opportunity to prepare new females. Therefore females over a month old were used in the test.

The females were isolated from their cocoons as virgins, fed for two days, starved overnight, and placed individually in Stender dishes, each with a host *Ephestia* larva. The females were transferred once to new hosts, transferred again, and refrigerated until after they had been individually assayed for sterility or heterozygosity of genetic damage. After elimination of the defective females, the females were grouped, fed, and kept refrigerated, being removed at weekly intervals for additional feeding.

The males used in the test were in better condition than the females. Virgin females of the mutant lemon (*le*) strain were set about 15 days before the expected test and transferred daily to new host *Ephestia* larvae. Lemon males were collected on the tenth day after the initial setting. In this way it was possible to use males most of which eclosed on the day of the Ames Research Center test.

The *Habrobracon* were placed in two sets of packages, one set of which was placed in a subassembly given vibration and centrifugation before and after the irradiation. The other set of *Habrobracon* packages was placed in a subassembly given neither vibration nor centrifugation.

One group of males and females was irradiated with 2000 r (250 r/min for 8 min) of x rays (300 kev, 10 ma, HVL 2 mm Cu) prior to being placed in the zero exposure control area of the subassembly.

Results

The *Habrobracon* were irradiated for 65 hr in the flight packages. They were placed 12 to a module, 48 to a package, except for the controls, where 36 were placed in each module, 144 to a package. Survival of the *Habrobracon* is shown in Table 1.

The males survived well; many of the females died.

The numbers of gametes tested are shown in Table 2. From these data the dominant lethal frequencies were compiled using the method of von Borstel and Rekemeyer (1959). The numbers of surviving females tested are listed in Table 2.

The dominant lethal, recessive lethal, and inherited partial sterility frequencies are presented in Table 3. The inherited partial sterility frequencies are believed to be the frequencies of induced chromosomal translocations.

Table 1. Survival per Package

Exposure (kr)	Vibrated		Nonvibrated	
	♂	♀	♂	♀
0	47/48 (0.98)	20/48 (0.42)	40/48 (0.84)	18/48 (0.38)
0.5	24/24 (1.00)	6/24 (0.25)	23/24 (0.96)	5/24 (0.21)
1	23/24 (0.96)	8/24 (0.33)	21/24 (0.88)	5/24 (0.21)
2	22/24 (0.92)	7/24 (0.29)	20/24 (0.83)	8/24 (0.33)
4	20/23 (0.87)	10/24 (0.42)	23/24 (0.96)	6/24 (0.25)
pre-2	23/24 (0.96)	6/24 (0.25)	18/24 (0.75)	11/24 (0.46)

Table 2. Numbers of Gametes and Virgin Female Offspring Tested

Nominal Exposure (kr)	Gametes Tested		F_1 Females Tested	
	v[a]	n[b]	v[a]	n[b]
0	797	616	32	38
0.5	763	385	45	44
1.0	1003	651	38	42
2.0	1005	625	132	78
4.0	708	352	54	26
2.0 (pre-test)	556	872	68	97

[a]In the vibration, centrifugation, and radiation subassembly.
[b]In the control subassembly (given radiation only).

Table 3. Frequencies of Different Types of Mutational Damage Induced in the *Habrobracon* Chromosomes

Nominal Exposure (r)	Actual Exposure (r)		Survival (1 Minus Dominant Lethality)		Recessive Lethal Frequency		Inherited Partial Sterility	
	v[a]	n[b]	v[a]	n[b]	v[a]	n[b]	v[a]	n[b]
0	1.72	3.6	0.920	0.905	0.093	0	0	0
500	365	400	0.825	0.758	0.088	0.069	0.04	0.07
1000	710	740	0.717	0.779	0.210	0.191	0.03	0.07
2000	1370	1390	0.591	0.493	0.265	0.28	0.182	0.23
4000	2980	3070	0.233	0.168	0.24	0.23	0.37	0.38
2000 (pre-test)	2000	2000	0.387	0.442	0.21	0.33	0.22	0.21

[a]In the vibration, centrifugation, and radiation subassembly.
[b]In the control subassembly (given radiation only).

Discussion

A test for different types of mutational damage was carried out in two subassemblies, one given vibration and centrifugation, as well as radiation, and the other given radiation only.

Many of the females in the test died. As explained in the section on "Preparation of Materials," this was undoubtedly because the females were old; excessive deaths among females had not been found in the two previous tests at Ames Research Center. This test served to underscore the necessity for having newly emerged *Habrobracon* in subsequent tests.

The frequencies of induced genetic effects from the chronic radiation are similar throughout for the vibration and nonvibration experiments. The only places where a lack of similarity may be significant are in the recessive lethal frequencies of the group given 2000 r of x rays prior to being placed in the zero exposure control area of the subassembly and the zero exposure control. These may be caused merely by fluctuations in the data, but these points should be closely watched in future tests.

Summary

A test was performed at Ames Research Center that sought to duplicate many of the features of the proposed Biosatellite Flight and its ground-based control.

Data for estimation of dominant lethality, recessive lethality, and inherited partial sterility were obtained. These data indicated that there was slight, if any, difference between *Habrobracon* irradiated with chronic gamma radiation from an ^{85}Sr source and those irradiated with radiation from a similar source but with vibration and centrifugation being given before and after irradiation.

Literature Cited

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

VI. RADIATION CONDITIONS IN THE *HABROBRACON* BIOCOMPATIBILITY TESTS AT AMES RESEARCH CENTER, JUNE 17–20, 1966

Sohei Kondo R. C. von Borstel

Toshiba glass rods were used in the *Habrobracon* packages during dosimetry tests on June 14 and 15, 1966, and during the Biocompatibility tests of June 17–20. During the dosimetry tests, the glass rods were used under two conditions, encased in plastic and encased in gold. The rods encased in gold yield a uniformly linear response at all energies of radiation, while rods encased in plastic are approximately three times as sensitive to radiation in the integrated energy spectrum below 100 kev as rods exposed to radiation in the integrated energy spectrum above 100 kev.

Studies with these rods were expected to provide crude estimates of the scattered radiation. It is of utmost importance to find out if scattered radiation at low energies is a high fraction of the total because some organisms, particularly higher plants, will have up to 20 times as much damage to their chromosomes from radiation of energies below 100 kev as from radiation at higher energies. Thus the factors leading to scattering of the radiation would have to be present in the ground-based control of the in-flight experiment in order to ensure proper estimate of the synergistic effects of the flight dynamics of the Biosatellite.

Results and Discussion

Linearity. – Even after correcting for the positioning displacement, the readings of glass rods irradiated while inside modules deviate significantly from the expected linear curve (see Fig. 11). However, readings of rods encased in gold tubes (abbreviated "gold" readings) follow a straight line except for the 2000-r reading. Since "gold" readings are of reduced gamma photon energy dependence, the above results mean that the locations of modules are acceptable with the exception of the 2000-r position. Glass rod dosimeters have a higher sensitivity to low-energy photons than to high-energy photons. The readings of the glass rods in plastic modules ("plastic") at the 500- and 1000-r positions are higher than expected from linear extrapolation of the readings probably because of the greater response of the rods to the low-energy scattered photons. We may, therefore, conclude that the "plastic" readings at 4000 and 2000 r give the upper limit of the roentgen values at these points. Therefore, the source strength must have been lower than expected by *at least 10%* (see Tables 4 and 5 for numerical values). The deviation of the "gold" readings at 2000 r from linearity may be due to an error in positioning of the housing (and thus of the modules).

Secondary Electron Equilibrium. – This presents no problem. The 1-mm-thick shield in front of the modules is sufficient to provide adequate secondary electron equilibrium.

Low-Energy Photons. – The features of the low-energy photons whose existence has been deduced above are difficult to characterize by the glass dosimetry alone. However, a crude estimate is given below. If we assume the "gold" reading is independent of low-energy photons and if the "gold" to "plastic" ratio is 1.77 (an experimental value obtained by exposure to ^{137}Cs

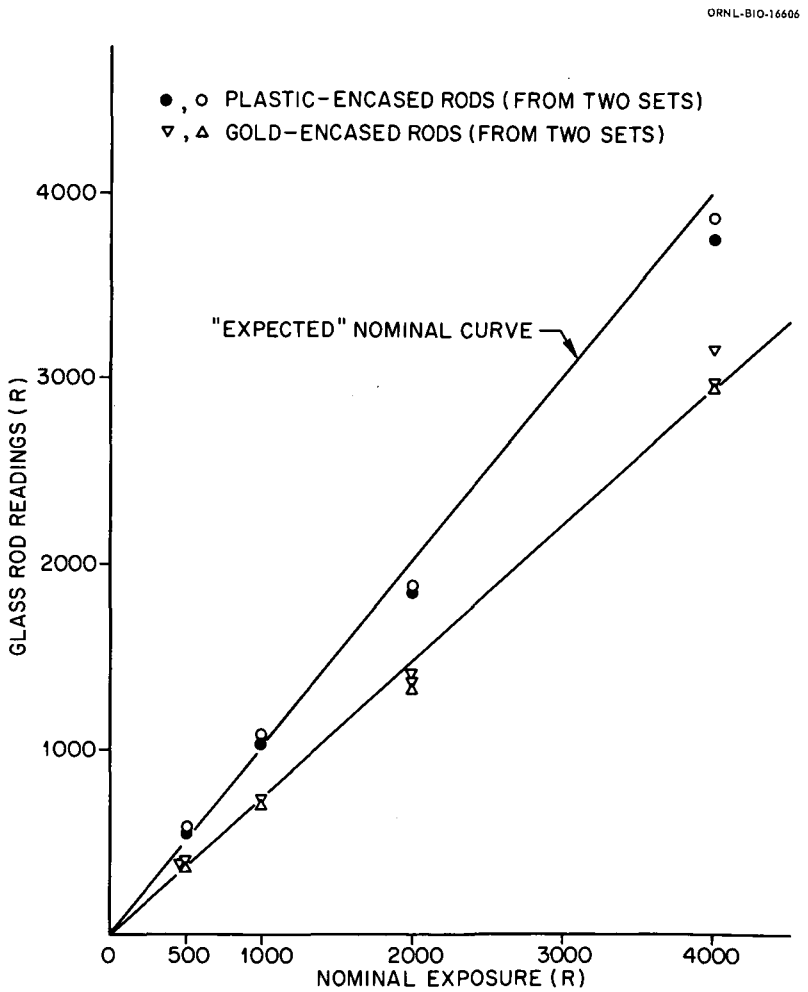

Fig. 11. Expected Nominal Exposures and Exposures Obtained from the Plastic- and Gold-Encased Glass Rod Measurements.

Table 4. Summary of Data (in Roentgens) for Tests of Linearity and Backscattering

Nominal Exposure	Rods in Modules ("Plastic")		Rods in Gold Cases ("Gold")		Average		"Plastic" / "Gold"	Backscatter Fraction	
	Vibration	Nonvibration	Pretest[a]	Nonvibration[a]	"Plastic"	"Gold"[a]		Probable	Minimum
4000	3760	3870	2940	3070	3820	3010	1.27	13.5%	6%
2000	1850	1880	1320	1390	1870	1360	1.37	18.5%	7%
1000	1030	1080	700	740	1060	720	1.47	23.5%	10%
500	545	578	360	400	562	380	1.48	24.0%	10%

[a]Converted into true roentgen units by dividing by 1.77 the original readings of the rods in the modules. The figure 1.77 came from the gold-encased-rod calibration data. The estimates came from a minimal number of four rods in the gold cases and ten rods in plastic.

Table 5. Exposures (in Roentgens) to the Wasps in the Biocompatibility Test of June 17–20, 1966

Nominal	Vibration[a]	Nonvibration
4000	2980	3070
2000	1370	1390
1000	710	740
500	365	400
0	1.72	3.6

[a]Data derived from Table 1.

gamma rays), then the "plastic" to "gold" ratio in Table 4 minus 1 divided by (3 – 1) gives the crude estimate of the fraction of dose due to low-energy photons. The "gold" readings given in this table are the estimated true roentgen values obtained by dividing the original readings by 1.77. The rationale for the above estimation is based on the fact that the readings of the control glass rods are about three times higher than those observed with ion chambers by Dr. John E. Hewitt (personal communication). Alternatively, the glass rod has a maximum relative sensitivity of 6 at 50 kev, and hence the lower limit of backscatter dose is about 10% at the 500- and 1000-r positions (see Table 4).

Source Intensity. – The source intensity differs greatly from the expected one; it may have been low by as much as 10 to 25%. We strongly recommend that the source intensity be measured before loading experimental material into the Biosatellite. This measurement must be done by ion chamber *and* some other independent method (e.g., measurement of ^{85}Sr activity).

Vertical and Intrapackage Variation of Dose. – There does not appear to be much variation from module to module within each package except at the 4000-r nominal exposure position. Here the two upper modules appear to receive a 5% higher dose than the two lower modules. Since low-energy radiations contribute approximately 10% of the dose in the 4000-r package, a much more critical study of module-to-module dose variation needs to be done.

Comparison with Previous Experiments. – The most clear-cut difference between the present and the previous experiments is that the relative dose at the 500-r position deviates in the opposite direction from that dose calculated from linear extrapolation of the doses at proximal positions. This discrepancy may be caused by dosimetry error (slightly different glass quality, a more careful calibration of glass readings done in the present experiment, etc.) and/or the different shielding material around the ^{85}Sr source. Furthermore, the control readings in "vibrational" (1.72 r, not corrected for energy dependence) rods differ from "nonvibrational" (3.6 r, not corrected for energy dependence) by a factor of 2, probably due to the difference in the control setup. This kind of difference should be minimized in the actual test.

Conclusions

Several facts emerge from the data obtained. These are important for all the radiation experiments.

1. There appears to be a large amount of scattered radiation of low energies.
2. The variation from package to package differs slightly between the vibration and non-vibration experiments. It is possible that the packages were at slightly different distances from the source in the two experiments, although they are supposed to be at identical distances.
3. The source strength was lower than expected.

Recommendations

The following suggestions for verification of reliability of the radiation experiments of the Biosatellite would appear to be mandatory:

1. Postflight dosimetric measurements in the actual flight vehicle for determination of the quality and distribution of the radiation must be done with a variety of radiation monitors.
2. There must be a ground-based control during the flight that will have the same radiation-scattering characteristics as the flight vehicle. This includes the arrangement of the instruments and packages in both the fore and aft sections.
3. Actual measurements of distances of packages from the source should be done by the experimenters' representatives during the built-in holds scheduled for the assembly period of the Biosatellite and the ground-based control. This is necessary for verification of the identity of the flight vehicle with the ground-based control.
4. Key measurements must be made by the experimenters' representatives at the termination of the flight before and during disassembly of the flight vehicle and the ground-based control vehicle.
5. A camera should be mounted at a fixed distance above the Biosatellite and ground-based control during assembly and photographs periodically taken. Such photographs would be extremely useful if an unexpected result were found in one of the experiments but not in the others.
6. The intensity of the sources used must be measured by ion chamber and some other method such as measurement of the ^{85}Sr activity.

VII. BIOCOMPATIBILITY TESTS IN FLIGHT VEHICLES

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Introduction

Three flight vehicles have been prepared by the General Electric Company for NASA. These are the qualification (201), the prime flight (301), and the backup (302) vehicles. Separate hardware and packages for the biological experiments were prepared for each. During the tests to simulate launch vibrations and the thermal and vacuum conditions, NASA officials asked the experimenters to place their organisms in the packages to ensure that the organisms could withstand the expected conditions of the flight. These tests also were used for training the investigators and the assembly teams for the actual flight itself. The tests were carried out at the General Electric Company in Philadelphia, Pennsylvania.

Results

Viability Studies. – In all of these tests, adult survival of the *Habrobracon* was the criterion. The vibration tests were short affairs, and the thermal and vacuum tests were supposed to be about 4 ½ days' duration.

It can be seen in Table 6 that the survival in the vibration tests generally was slightly higher than survival in the thermal and vacuum tests. The loss in viability can be attributed to the extended duration of the thermal and vacuum tests. These tests were conducted one following the other so closely with so many attendant delays that it was not possible to have young and vigorous *Habrobracon* on hand at all times. Young, healthy *Habrobracon* were available for the Qualification Vehicle Vibration Test, the second Qualification Thermal and Vacuum Test, and the Prime Flight Thermal and Vacuum Test. The *Habrobracon* used in the other tests were of various ages, and the mildly lowered viability in the tests can be attributed to this.

As the tests progressed, controlled experiments were carried out where more and more *Habrobracon* were placed in the modules. Originally, the modules were designed to contain 12 *Habrobracon,* but more were placed in during the testing to find out if it was possible to include larger numbers in the actual flight.

The data for the viability of *Habrobracon* under different conditions of crowding are shown in Table 7. It can be seen that though there was a difference in viability between the experiments (explained above), there was no difference between the crowded and less crowded modules.

Further tests in our laboratory have shown there is not much difference in viability among modules containing 20, 25, and 30 *Habrobracon* when held for about four days.

Suitability of the Packages. – Each package consists of the bracket, four modules, two tubes to hold lithium fluoride, and a shield to provide secondary electron equilibrium.

Table 6. Viability of *Habrobracon* at the Termination of Each Test of the Flight Vehicles

Test	Qualification Vehicle	Prime Flight Vehicle	Backup Flight Vehicle
Vibration test	$\frac{298}{303} = 0.983$	$\frac{311}{336} = 0.926$	$\frac{336}{384} = 0.875$
Thermal and vacuum test	$\frac{234}{288} = 0.818$ (5 days 6 hr)	$\frac{347}{365} = 0.951$ (4 days 11 hr)	$\frac{427}{485} = 0.880$ (4 days 19 hr)
	$\frac{331}{343} = 0.965$ (1 day 17 hr)		
	$\frac{314}{343} = 0.915$ (3 days 23 hr)		

Table 7. Viability of *Habrobracon* Under Different Conditions of Crowding During the Thermal and Vacuum Tests of the Prime Flight and Backup Flight Vehicles

Prime Flight Vehicle (4 days 11 hr)		Backup Flight Vehicle (4 days 19 hr)	
12 or 13 per Position	15 or 16 per Position	15 per Position	19 or 20 per Position
$\frac{231}{244} = 0.947$	$\frac{116}{121} = 0.959$	$\frac{184}{210} = 0.876$	$\frac{243}{275} = 0.884$

During the testing, it was found that the modules and the screens and separators were toxic unless they underwent a thorough washing regime. For all the tests, spare packages were never made available even though they were to have been. Consequently they were never tested.

For the control packages, a satisfactory system for attaching glass plate dosimeters had not been developed by the time the tests had been completed.

A great deal of difficulty was observed in having sturdy enough tubes to hold the lithium fluoride and in establishing a useful and meaningful code for the separate brackets and modules.

All the above were problems experienced with the hardware for all three flight vehicles. In addition, the separators and control caps for the control modules had to be individually fitted for the backup (302) flight vehicle packages. This problem and the testing of spare packages for the prime flight and backup flight vehicles were not resolved in the tests at General Electric, Philadelphia.

Discussion

Viability of *Habrobracon*. – It was found that the *Habrobracon* could survive easily under more crowded conditions than the modules were designed to hold. Therefore it is likely that 20(+ 0)(–5) *Habrobracon* will be placed in each assigned position in the prime flight vehicle, making a total of 560(+ 0)(–140) *Habrobracon* during the actual flight.

Suitability of the Packages. – The problems of individual fitting of some of the pieces of the hardware, testing of spare packages, attachment of glass plate dosimeters, and suitable encoding of packages remain unresolved.

Summary

Despite several problems with the hardware that have not been completely resolved, it was found that for all tests the viability of the *Habrobracon* was satisfactory enough to indicate successful recovery of the organisms in an actual flight.

VIII. SEX ALLELE STOCKS IN *HABROBRACON*

R. C. von Borstel Roger H. Smith Anna R. Whiting

For genetic testing during mutagenesis experiments with *Habrobracon*, it is useful to have a variety of different sex allele strains available. Diploid males, which arise from homozygosity of sex alleles, interfere with measurements of the effect of mutagens because they are largely inviable and die around the time of hatching. Since one-half of the fertilized eggs from an inbred line are homozygous at the sex locus, this would eliminate a substantial portion of an experiment. Also, most of our genetic tests, whether for dominant lethals, recessive lethals, or inherited partial sterility, are measured by hatchability, and the use of inbred lines would necessitate extensive correction of the data.

By intercrossing strains with different sex alleles, thus keeping them heterozygous, all fertilized eggs develop into females.

Sex Alleles

Whiting (1941) described the mechanism of sex determination in *Habrobracon*, located the sex locus on the map of chromosome I, and described nine sex alleles (x^a, x^b, x^c, . . . , x^i). A combination of any two of the nine alleles produces female offspring. Unfortunately, most of the strains were lost or discarded. The Whitings retained two of the strains, No. 33 (x^e/x^f) and No. 17o^i (x^j/x^k), for radiation studies where crosses would be involved.

With the discovery of inherited partial sterility in *Habrobracon* (von Borstel and Rekemeyer, 1959), it was recognized that availability of another sex allele stock would make genetic testing easier (the F_1 generation of a diallele cross needs to be mated, and another set of sex alleles

would rule out interference from diploid males). We examined the Raleigh (*R*) wild-type strain and found that though its sex alleles differ from No. 33 wild-type it shares a sex allele with No. $17o^i$.

A number of *Habrobracon* were collected at a feed mill in Lumberton, North Carolina, in the summer of 1965. From these, two stocks were developed which have sex alleles differing from each other and from the No. 33, No. $17o^i$, and *R* stocks. The sex allele designations of the stocks we have in our collection are shown in Table 8.

Table 8. Sex Allele Strains of *Habrobracon*

Strain	Sex Allele
No. 33	x^e/x^f
No. $17o^i$	x^j/x^k
R	x^k/x^l
lum D	x^m/x^n
lum G	x^o/x^p

Mutation Analysis

For our studies on mutagenesis we have decided to use the mutant strain of lemon body color (*le*) for the male parent; the strain has the sex allele composition of x^e/x^f. For the female parent we use the *R* wild-type, and for mating F_1 virgins heterozygous for recessive lethal mutations or translocations we use the lum G strain.

The lemon mutant is used since *le* is a marker located near the centromere of chromosome I, and it is semidominant. The *R* strain is sturdy and has a high egg productivity. The lum G strain is also a vigorous one, but its principal feature is the different sex alleles.

New stocks are being synthesized which will have more markers incorporated. These markers will be on chromosomes I and II. The lum D strain is being used to incorporate its sex alleles into some of the mutant strains.

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IX. SURVIVAL OF *HABROBRACON* LARVAE UNDER DIFFERENT DEGREES OF CROWDING

"It is better to bury a dead sheep after one week than after one day"

Sheepherders' Apothegm

Eastern Oregon

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We have often wondered what the maximum number of *Habrobracon* of the same age would be that could develop on a host *Ephestia* larva. The opportunity to find this number presented itself recently in a large experiment done for another purpose. Knowing this number is useful, since we normally permit development of 12 embryos for each host as a maximum, killing all eggs over this number. Under the conditions of our experiments, the females are starved overnight to store a large quantity of eggs in the first meiotic metaphase. After the starvation period, they oviposit about 10 to 12 eggs during a half-day period before they are transferred, and fewer than 10% of the eggs are killed in the course of an experiment.

We were taking only the hatchability on one transfer on a large number of females in the experiment under consideration; thus it was not necessary to avoid crowding conditions on the larvae. Since the laying period was slightly longer than usual, an unusually large number of eggs were laid on some of the host larvae. Therefore, we decided to keep the cultures, count the adults, and observe the effects of crowded feeding conditions.

Methods and Materials

Habrobracon females normally oviposit about 20 eggs a day, and these are usually deposited in two clutches, each being laid over several hours. Before we begin an experiment, the females are well fed for a day or two and then starved overnight to allow them to store up to 16 (no more have been observed) of their eggs in the first meiotic metaphase (metaphase I). We have used the No. 33 wild-type strain in past experiments, and 6 hr is the time that we accord for the oviposition of the first clutch of eggs.

The *Habrobracon* stock used in this experiment was the Raleigh wild-type strain, and in this strain the females lay most of their stored metaphase I oocytes during the first 4 hr after being placed with the host. The virgin females were being tested to obtain a large sterile-free and recessive-lethal-free collection for a radiation experiment.

By starving the females for 7 hr during the day, this permitted them to store their oocytes in the first meiotic metaphase. They were kept overnight for about a 16-hr period with the host larvae, and this gave them time to feed, lay their first clutch, store metaphase I oocytes, and still gave many of the *Habrobracon* the opportunity to lay their second clutches of eggs before being transferred.

Results

The host larvae used in this experiment were large, fat ones, each weighing approximately 35 mg. Six hundred ninety-seven females were tested separately, and these oviposited from 1 to 29 eggs during the 16-hr oviposition period. The hatchability was high, averaging about 95%, so that the host larvae were each holding from 1 to 28 *Habrobracon* embryos (Table 9).

It was found that the *Habrobracon* could survive to adulthood under more crowded conditions than we believed possible. The viability was up to 80% even when 25 embryos were feeding on one host. Above this number, the adult viability appears to fall off. Indeed, in one case where 27 larvae were feeding on one host, the host was devoured completely and the 27 larvae died at the same stage of midlarval development.

Table 9. Numbers of *Habrobracon* Each Providing Different Numbers of Surviving Embryos

Number of Females	Number of Embryos	Adult Survival (%)
3	1	66.67
4	2	100.00
3	3	66.67
3	4	83.33
8	5	87.50
8	6	70.83
8	7	83.93
17	8	72.06
16	9	81.94
26	10	69.23
23	11	83.40
44	12	73.11
48	13	81.41
57	14	66.92
77	15	84.16
64	16	75.49
74	17	76.39
42	18	81.08
49	19	81.53
34	20	71.76
28	21	72.79
25	22	76.00
11	23	65.22
8	24	80.21
4	25	82.00
5	26	60.77
2	27	16.67
1	28	28.57

It is possible that some of those embryos discriminated against in the heavier egg clutches were the very youngest embryos, since the embryos could have differed in age by as much as 16 hr.

Conclusion

Because a host can support larval growth and development for 25 larvae at the 80% viability level, henceforth we shall use 20 embryos per host per transfer as the maximum number of embryos allowable for our experiments.

X. CHROMOSOME BREAKAGE AND DOMINANT LETHALITY[1]

R. C. von Borstel

Many of the events leading to mutations also can induce chromosome breakage. In turn, most of the events leading to chromosome breakage can cause the death of cells. As far as one can infer, dominant lethality is cell death caused by breakage of chromosomes.

Molecular Basis of Chromosome Breakage

It has long been known that the viscosity of nucleic acid and nucleoprotein decreases after they have been x irradiated (Sparrow and Rosenfeld, 1946; Taylor, Greenstein, and Hollaender, 1947). Also it has been shown that the ultraviolet radiation action spectrum for chromosome breakage follows the nucleic acid absorption spectrum (Kirby-Smith and Craig, 1957). From these and other experiments, such as DNA strand breakage by x irradiation (Freifelder, 1966) and DNA content and radiosensitivity (Terzi, 1961), it generally has been assumed, albeit tacitly, that radiation breaks chromosomes of higher organisms by severance of the polynucleotide chains.

Nevertheless, a case has been made that x radiation breaks chromosomes by destruction of protein components of the chromosome (Kirby-Smith and Randolph, 1959; Wolff, 1961; Kaufmann and Gay, 1963). Much of this argument devolves from the experiments that indicate that more radicals are formed in proteins than in DNA (Kirby-Smith and Randolph, 1959) and a need for protein synthesis (Wolff, 1960) during the chromosomal rejoining process.

In contrast to the proposal that x radiation breaks chromosomes by destruction of their proteins, Miller, Carrier, and von Borstel (1965) have accumulated evidence that can be explained most simply by assuming the x radiation breaks chromosomes by breaking the DNA. We were able to demonstrate in vitro that x radiation can break the lampbrush chromosomes of the salamander oocyte. The frequency of the breaks is the same whether the chromosomes are isolated from the oocyte before or after being x irradiated. The action of radiation on lampbrush chromosomes in vitro is mimicked only by deoxyribonuclease, not by ribonuclease nor by various proteolytic enzymes (MacGregor and Callan, 1962; Gall, 1963). The effects of sensitization of chromosomes by

[1]Taken from a session opening lecture presented at the Third International Congress of Radiation Research, Cortina d'Ampezzo, Italy, July 1, 1966.

the presence of protein either for energy transfer or endonuclease activity are not disputed. Nevertheless, we believe that damage to protein is not the primary lesion.

A Hypothesis for the Reunion of Chromosome Fragments

After breakage, fragments of chromosomes and chromatids seem to rejoin in any way that can be imagined (cf. Evans, 1962). The only restriction seems to be that of linearity, that is, forking at the breakage and rejoining juncture is prohibited.

If it is assumed that the broken molecules are DNA molecules and that the chromosome is not composed of a high multiple of DNA strands, then the process of rejoining reduces itself to the process of how two broken ends of a DNA molecule can join. The trouble is that there are no experiments that can tell what extent of injury to the DNA can be tolerated by rejoining systems in cells where chromosomal aberrations are inducible. Nevertheless, even if we assume what might be the worst case, where, after transverse scission of the DNA, endonucleases start to depolymerize one of the single polynucleotide chains, certain observations can make a straightforward repair hypothesis credible.

First, Wolff and Atwood (1954) state: "Reunion [of broken chromosome ends] is not a passive process, but requires some metabolic activity." Second, Wolff and Luippold (1955) present convincing evidence that energy, possibly ATP production, is required for chromosome rejoining to occur. Third, enzyme systems have been found that can synthesize a new single polynucleotide strand upon a single-stranded template (Bollum, 1960; Kornberg, 1959) with triphosphate nucleosides as substrate, but neither of these enzymes can close the last gap. Fourth, an enzyme system has been found that can join an end of a double-stranded Watson-Crick helix to a single-stranded polynucleotide (Mead, 1964).

Given this mixture of the Wolff-Luippold system to generate an excess in the triphosphate nucleoside pool, the Mead system to make the first attachment, the Bollum or Kornberg system to synthesize a new single strand upon the template, and the Mead system again to close the last gap, then chromosome reunion would be effected.

The only thing lacking would be a splint to hold the broken ends together while this molecular poultice did its healing. There is evidence that the protein component of a chromosome could act as such a splint. When Miller, Carrier, and von Borstel (1965) first x irradiated lampbrush chromosomes in vitro, we found that breaks were found less and less frequently with time after isolation. This was not because of progressive repair of the breaks, but because of progressive denaturation of the chromosomal proteins. Thus the broken piece was not permitted to fall away from the rest of the chromosome. Brief exposure to pepsin removed the protein "splint" and the breaks were exposed.

It seems conceivable that the protein component of the chromosome may act as a splint in vivo as well, and the broken pieces would be held in proximity long enough for rejoining to occur. Since rejoining distances are long by molecular standards (approximately 0.2 μ, as determined by

Wolff, Atwood, Randolph and Luippold, 1958; see also Atwood, 1963), a splint may not really be a requirement. Nonetheless, we found one and would like to believe that it is useful.

One problem remains that has always been a puzzle to me. Chemical reactions don't take very long to happen, but chromosomal rejoining time seems to be extremely long and variable from cell type to cell type. It might be that time could be lost while the ends find one another. Also, while rejoining is proceeding, the endonucleases are in the process of stripping nucleotides off one of the strands. Such simultaneous activities of end-hunting and breakdown coupled with subsequent syntheses perhaps could serve to produce variable rejoining rates.

The Concept of the Rejoining Site

Lea (1947) observed that broken chromosomes could rejoin only if they were formed within a short distance of one another. This site concept was developed by Atwood and Wolff (Wolff, 1959) from rationalization of two distinct phenomena: (1) the estimation of the distances over which two broken chromosomes could rejoin (Wolff, Atwood, Randolph, and Luippold, 1958), and (2) the discovery that the distribution of broken chromosomes in a cell follows non-Poisson expectations. A rejoining site may be defined as a region in a nucleus where chromosomes lie close enough to one another so that, when both are broken, reassortment and attachment of the broken ends can take place. It is not amiss to note here that Revell (1959a, 1959b, 1963) has approached the problem of aberration production from an entirely different set of observations and assumptions. It appears that the breaks and rearrangements taking place in the "sites" of Atwood and Wolff must approach the limits of the "labile point pairing sites" of Revell, where he conceives of a process of exchange taking place without previous juxtaposition of broken ends.

When fewer cells with large numbers of aberrations were found than expected, an equation was suggested for estimating the numbers of sites from the cell fraction that contained no aberrations (von Borstel, 1960). When tested with Conger's data (Bender and Wolff, 1961), it gave the satisfactory answer of four sites per cell. I call it "satisfactory" because the dose-effect curve closely followed the theoretical curve, and an integral number was produced for the number of sites. In practice it presently became more useful to employ all the data to estimate the numbers of sites. That is, the fractions of cells containing each type of aberration, as well as the no-aberration fraction, could be used to narrow the confidence limits in any one experiment (Wolff, 1961, 1963).

In their examination of the cell life cycle, Garcia-Benitez and Wolff (1962) found that chromatid interchanges, unlike chromosomal interchanges, do indeed fit a Poisson distribution. This was interpreted to mean that there is an increase in the number of sites in the latter portion of the cell cycle.

At this time Atwood and Wolff (Atwood, 1963) were looking carefully at the underlying aspects of the site concept, and found the astonishing feature that the activated site must be defined as a member of a set of break pairs each of which will inevitably produce an interchange. This means that the fate of the broken ends in a site is predetermined. In a dialogue with D. L.

Lindsley (Atwood, 1963) the concept of multiple sets of sites as a consequence of predetermination first appeared in print. Atwood pointed out that predetermination does not preclude that within the fine structure of a site all of the potentialities for interchange do not exist.

Heddle (1965) made the important observation that chromosome-type exchanges involving a particular chromosome in *Vicia* yield symmetrical and asymmetrical rejoining with equal frequencies. He argued that this indicates that possible interchanges within a site occur at random. The alternative possibility is that there exist, in equal frequency, two sets of sites, one giving rise to symmetrical rejoining and the other to asymmetrical rejoining.

Savage (1965) presented an analysis of *Tradescantia* microspores on the basis of two different sets of sites, one for each of the two different rejoining events he was measuring. This was done because in his hands the distribution of rings and dicentrics in *Tradescantia* microspores gave a nonintegral number of sites. He found that he could interpret the data to show a site number of 2 for each event.

One of the shortcomings of the equations for estimating site number is that they demand that an exact number of sites exist and that they do not vary among cells in the population. I don't think anyone ever believed this (cf. Atwood, 1963), but Conger's data (Bender and Wolff, 1961) certainly were consistent with this notion. Savage and Papworth (1966) became concerned about it in connection with the interpretation of a paradox in the identity of the two sites found for each event (Savage, 1965) and in connection with the 1 : 3 ratio of centric rings to dicentric translocations.

To resolve this paradox, Savage and Papworth revised site theory to include the distribution of sites from cell to cell. They also found that according to the distribution parameter a low number of sites could yield a Poisson distribution of aberrations. Thus the data for chromatid interchanges (Garcia-Benitez and Wolff, 1962) may reflect a shift in distribution of sites from cell to cell rather than a large increase in number.

Taking an opposite tack, Read (1965) decided to analyze chromosome aberration production with the assumption that every broken end in a nucleus could join with every other broken end with equal probability, no matter where located in the nucleus. He assumed that, primary breaks being distributed among cells in accordance with the Poisson distribution, from the start of dicentric and ring production a warp from the Poisson distribution of aberrations would be expected. From his primary assumptions he derived equations which perfectly described production of chromosomal aberrations. For the general theory, three discrepancies should be noted: First, Catcheside (1938) demonstrated that the Poisson production of aberrations would always conform to a Poisson distribution of breaks from cell to cell. Second, at Wolff's suggestion, Diana B. Smith (pers. comm.) found that though Read's computations fit chromosome aberration production very well indeed, the equations also yield non-Poisson expectations for chromatid interchanges. This was an expected attribute of Read's equations, but the chromatid data follow a Poisson distribution (Garcia-Benitez and Wolff, 1962). Third, Read's equations do not lead to saturation of the sites.

It was recognized early that the proof of the site concept would be the saturation of the sites so that no more interchanges could be formed. Data of Parker and Hammond (1958) on detachments of attached-X chromosomes induced in stage 14 oocytes were shown by Wolff (1962, 1963) to conform to site saturation expectations. Saturation of sites has now been demonstrated in *Tradescantia* microspores (Savage, pers. comm.).

Notwithstanding the beauty of the site model, and the seemingly sturdy foundation on which it rests, it is well to keep in mind that it is just that – a model.

Let us review some of the problems: First, although Atwood (1963) has given a terse description of the possible fine structure of a site and Savage (1965) has presented a detailed model, the present equations give the site a restrictive shell. That is, exchange events can occur anywhere inside the site, and none outside. As biologists, we tend to prefer gradients through soft, fibrous plasma. This fine structure is what we eventually must seek, since this is of course the molecular structure allowing chromosomal interchange. As an example of the magnitude of the problem, I only wish to point out that the answers given by any of the present analytical procedures would be altered substantially by assuming that different sites have slightly different target sizes (cf. Steffensen, 1962), a not incredible assumption. Here I believe lies the area most accessible to attack by radiation of different LET since microscopes of any type cannot be used.

Second, R. K. Mortimer (pers. comm.) has observed that the equations demand that once a break pair has been formed no more can be formed within the site. This doesn't matter when acute radiation is used, but it raises interesting questions about the effects of chronic radiation. It suggests that with low chronic doses, the target would become nonreducing (the breaks would rejoin, and the target would be again available), and the Curie equation (Curie, 1929) for inactivation of nonreducing targets would apply. This consideration would not hold for cells such as sperm, where chronic or fractionated radiation has no effect.

Third, and related to the second, sites occasionally must have many more than one break pair contained within. An almost absurd example was found in the salivary gland cells of *Drosophila* after irradiation of the sperm: Kaufmann (1943) found a 34-break rearrangement of which 52 of the 68 broken ends were in one cyclic exchange.

Fourth, translocations occur at near-random locations on chromosomes, but the sites are limited in number. This tells us that chromatin is packed in many different ways and that the same number of sites are still a consequence of this dissimilar packing. This only means that the problem is a topological one and eventually must be approached as such.

Fifth, the Lane effect (Lane, 1951, 1952, 1953; Sax and Luippold, 1952; Haque, 1953; Evans, 1966) continues to loom like a specter in the dark outside the laboratory window. Lane (1951) used fractionated doses of x radiation on *Tradescantia* microspores and showed that as the fractions are separated by longer and longer intervals of time, two-break aberrations first decline in frequency and then rise again. Occasionally the rise is up to the frequency of a single dose equal to the sum of the two fractions. The initial decline is expected; it had been observed long

before by Sax (1939). The later rise has been difficult to repeat and even more difficult to explain. We await clear-cut, repeatable experiments demonstrating its presence. We also await clarification of whether this is a phenomenon which will require that chromosome aberration theory be rewritten, or whether it is an epiphenomenon of, say, sick cells on the way to recovery being especially sensitive to radiation at a certain time after the initial exposure to radiation.

The Theory of Radiation-Induced Dominant Lethality in Sperm

The Theory. – Dominant lethality due to chromosome breakage and bridge formation results in early death of *Drosophila* and *Habrobracon*. We assume that radiation-induced bridges can be formed in two ways: by terminal deletions and by dicentric translocations. Terminal deletions of chromosome limbs lead to attachment of the new ends together when a new chromosome is synthesized. A bridge is then formed during anaphase. Such bridges from terminal deletions arise from single events, and the proportion which escapes this fate is

$$S_1 = e^{-k_1 D} , \tag{1}$$

where k_1 is the effective sensitivity and D is the dose of radiation. Dicentric translocations require that two chromosomes be broken and rejoin in such a way that two centromeres are present in one chromosome. We assume that there are a definite number of regions ("sites") in each nucleus where such translocations can occur. With this assumption it can be shown that the proportion which escapes this fate is

$$S_2 = [1 - (1 - e^{-k_2 D})^2]^m , \tag{2}$$

where m is the number of sites. Multiplying Eqs. (1) and (2) gives the proportion which escapes both fates:

$$S = S_1 \cdot S_2 . \tag{3}$$

With the proper choice of parameters, Eq. (3) adequately describes the frequency of early deaths of embryos following fertilization of eggs by irradiated sperm. The frequency of dicentric translocations can be estimated from dominant lethal data by the use of Eq. (3).

Terminal Deletions. – The simplest explanation from studies on x irradiation of oocytes in the first prophase and first metaphase of meiosis is that terminal deletions are produced and that attachment of the chromatids leads to formation of bridges (Whiting, 1945). The breakage-fusion-bridge cycle is the consequence, and this leads to early death of the embryo through drastic decrease in the rate of mitosis (von Borstel, 1960). Further, it has been shown that the types of chromosome aberrations found after x irradiation of lampbrush chromosomes of the salamander both in vitro and in situ (Miller, Carrier, and von Borstel, 1965) are of the type expected to produce the chromosome fragments and bridges that Whiting found in *Habrobracon* (von Borstel, Miller, and Carrier, 1966).

Since there seems to be a one-hit component in the induction of damage to *Habrobracon* and *Drosophila* sperm leading to early embryonic deaths (von Borstel and Rekemeyer, 1959), we have

assumed that these can be attributed to terminal deletions. On the other hand, Lindsley (1963) has evidence that the one-hit component for dominant lethality in *Drosophila* is not from terminal deletions. He found that sperm with different numbers of chromosomes have equal frequencies of the one-hit component for dominant lethality. If terminal deletions were the primary cause of the one-hit component, then the sperm with the fewer chromosomes should have fewer terminal deletions at any one dose of x radiation.

This paradox remains unresolved.

Translocations. – The theory of dominant lethality expressed above does not take into account the problems or advances discussed in the section on the site concept. This is not only because it is a simpler expression but because sperm are a notably uniform cell population, and if an exact number of sites exists any place they ought to exist here. It is interesting to note that many of the factors leading to production of two-hit events will have lethal and viable counterparts, for example, reciprocal and dicentric translocations. Even large two-hit deletions should have a measurable and, possibly, a comparable counterpart in small two-hit deletions. If any adjustments need to be done, they can be done when the data are shown to no longer fit the simpler version of the theory.

One thing appears to be clear at this time: On the basis of the site concept there would be separate sets of sites for viable reciprocal translocations and for lethal dicentric translocations. If only one set for both existed, then it is obvious that as the sites become saturated the dominant lethal curve should flatten out. For example, if there were only one site for both, the dominant lethal curve would level out at 50% because half of the cells would have viable reciprocal translocations. Correspondingly, if there were two sites, then the curve would level out at 75%, three at 87.5%, etc.

As far as I know, no dominant lethal curve has ever been found to saturate. Therefore, the first conclusion, if Eq. (3) has any validity, is that there are at least two independent sets of sites for translocations, one for viable reciprocals and one for lethal dicentrics.

Summary

The evidence that x radiation breaks chromosomes by breaking the DNA is presented. A hypothesis for chromosomal rejoining is suggested which requires the action of at least one and probably more enzyme systems of types now known.

The development of the site concept for chromosome breakage and rejoining is reviewed. The underlying strengths and some of the less well-understood aspects of the concept are discussed. A simple expression of the site concept is used to describe dominant lethality.

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