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BIOLOGY DIVISION QUARTERLY PROGRESS REPORT TO THE NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

APRIL 1 - JUNE 30, 1967





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## **BIOLOGY DIVISION**

# QUARTERLY PROGRESS REPORT

#### TO THE

## NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

April 1 - June 30, 1967

# MAY 1968

## OAK RIDGE NATIONAL LABORATORY

Oak Ridge, Tennessee

operated by

# UNION CARBIDE CORPORATION

for the

### U. S. ATOMIC ENERGY COMMISSION

#### QUARTERLY PROGRESS REPORT

#### TO THE

### NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

<u>Title of Project:</u> Mutagenic Effectiveness of Known Doses of Gamma Radiation in Combination with Weightlessness on Habrobracon

For the Period: April 1 - June 30, 1967

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#### I. INTRODUCTION

R. C. von Borstel

Roger H. Smith

The data from the second-generation males in the ground-based control of the Biosatellite are presented in detail in this report. Included are a summary of the different types of recessive lethal mutations and a listing of the actual numbers and frequencies of inherited partial sterility effects that were found. The inherited partial sterility is adjudged for the most part to comprise chromosomal translocations.

An old phenomenon discovered in Habrobracon by P. W. Whiting (1932) and independently developed by Horowitz and Leupold (1951) has been revived to provide information on the molecular nature of mutations (Edgar and Lielausis, 1964). The techniques have now been employed in Drosophila (Suzuki, <u>et al.</u>, 1967). We decided to explore this area to see if it could be used as a suitable technique to enlarge the scope of the Habrobracon experiment in the Biosatellite. For this reason these "temperature-sensitive mutations" were sought in pilot experiments with the chemical mutagen mitomycin C. They were found, and the method of their detection can be easily used as an additional analytical procedure in the Biosatellite

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# II. RECESSIVE LETHALITY AND INHERITED PARTIAL STERILITY INDUCED IN SPERM IN THE GROUND-BASED CONTROL FOR BIOSATELLITE I

R. C. von Borstel Roger H. Smith Anna R. Whiting Katherine T. Cain

Joan W. Reel Martha S. Jones Margaret J. Lane

Habrobracon males were irradiated in the ground-based control set-up of the Biosatellite I experiment of 14-17 December 1966. These were mated to females of different sex allele constitution and the dominant lethal mutation frequencies were determined (von Borstel, Smith, Whiting, Valcovic, Baird, Cain, Reel, Jones, and Lane, 1967). Virgin females were collected from among the survivors of the dominant lethality experiment. From these, the recessive lethality and inherited partial sterility frequencies could be determined.

#### Materials and Methods

The males used in the ground-based control part of the experiment were from the <u>lemon</u> stock. These were mated to females of the Raleigh wild-type strain. Surviving virgins of this cross were bred unmated. Those with low hatchability were mated to males of the lum G wild-type strain, and hatchability and adult survival again were determined. The sex alleles of these strains have been described previously (von Borstel, Smith, and Whiting, 1967), as has the rationale for these matings (von Borstel and Smith, 1967).

The males were placed in modules in the 4000-R, 2000-R, 1000-R, 500-R, and 0-R nominal exposure positions. The exposures received by the males do not correspond to these exposures (von Borstel, Hewitt, Cain, Badger, and Smith, 1967), and since the actual exposures have not yet been determined with accuracy, they are omitted from the tables.

Some of the males in the O-R nominal exposure position had been X-irradiated with an acute exposure of 2000-R before being placed in the ground-based control set-up. A second control, designated as Control III, followed the temperature pattern of the spacecraft.

#### Results

The reduced data for each of the packages are presented in Tables 1-8. The inherited partial sterility data are listed as translocations since it is believed that all or most of them belong to this class of chromosomal arrangement. The data for the recessive lethal mutation frequencies are computed from the class of females lacking translocations (Table 9). The frequencies of the embryo recessive lethal mutations associated with translocations are computed separately since there appears to be a disproportionately high frequency of these at every dose.

#### Discussion

As expected, recessive lethal mutations and translocations increase in frequency with the intensity of the exposure to protracted radiation over a 65-hr period. Several features stand out from the data: (1) with protracted exposures of radiation, larval recessive lethal mutations are more frequent than embryo recessive lethals which in turn are more frequent than the pupal recessive lethals; (2) this does not appear to be the case after single, brief exposures to radiation, either in this experiment or in an earlier, more extensive experiment (Smith, <u>et al</u>., 1967) where embryo recessive lethals are more frequent than the larval recessive lethals; (3) certain of the translocations appear to be associated with embryo recessive lethals; presumably, these are comparable to the "position effect" lethals described in Drosophila.

As mentioned in the Materials and Methods, the absolute dosimetry is as yet too uncertain to permit dose-action analyses of the data.

#### Summary

Translocation frequencies and recessive lethal mutation frequencies for protracted radiation have been obtained for the different nominal exposure positions in the ground-based control part of the Biosatellite I Experiment.

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#### II. INDUCTION OF MUTATIONS IN HABROBRACON WITH MITOMYCIN C

Roger H. Smith

Mitomycin C, a streptomyces-derived antibiotic, disrupts DNA metabolism, (Shika, <u>et al.</u>, 1959) but does not interfere with RNA and protein synthesis (Reich and Franklin, 1961). Biologically inactive in its natural state, this antibiotic becomes a mono- and bifunctional alkylating agent upon chemical or enzymatic reduction. Iyer and Szybalski (1964) showed that mitomycin C inhibits synthesis by cross-linking the complementary strands of DNA, and that a high content of guanine and cytosine promotes cross-linking. Mitomycin C mimics the effects of X-radiation when dominant lethal mutations are used as criterion (Smith, 1965); metaphase I oocytes are approximately twenty times more sensitive than prophase I oocytes to both of these mutagenic agents.

In the present experiments Habrobracon males and females were injected with solutions of mitomycin C to study the pattern of induced dominant and recessive lethal mutations in sperm and oocytes, with special attention being given to possible temperature-sensitive lethal mutations.

#### Materials and Methods

Habrobracon females of the Raleigh (R) strain were isolated as virgins and pretested for spontaneous mutations. The procedure of Whiting (1945) was used to obtain females containing a maximum number of oocytes in first meiotic metaphase. These females were injected with 0, 0.01, 0.05, and 0.1 mg/ml of mitomycin C in insect saline. Each female was provided with an Ephestia larva 3 hr after injection. Oviposition was divided into five periods: 4, 2, and 2 hr; overnight (~ 12 hr), and 6 hr. The eggs laid during the first three periods were in first meiotic metaphase, and possibly diakinesis, at the time of injection; the eggs laid during the overnight period

were a mixture of metaphase I, diakinesis, and prophase I oocytes. The eggs laid during the last period were in first meiotic prophase at the time of injection. Approximately one-half of the females from each injected group were mated to males of the Lumberton D strain (lum D) to obtain female offspring.

Males of the lum D strain were injected with 0, 0.1, 0.5, and 1.0 mg/ml mitomycin C. They were kept at 30°C for 24 hr before each male was mated to two R females. These males were mated again on 2 successive days so that there were three broods (days 1, 2, and 3) with Habrobracon sperm. All R females used for these matings were pretested for heterozygosity for recessive lethal mutations. Egg counts, hatchability, stages of death, and adult survival were recorded for all mated females in each experimental group according to the procedure of von Borstel and Rekemeyer (1959). Virgin female progeny were collected to test for heterozygosity for recessive lethal mutations and translocations (as determined by inherited partial sterility patterns, von Borstel and Rekemeyer, 1959). Eggs from these virgin females were placed at high (35°C) and low (30°C) temperatures to check for temperature-sensitive lethal mutations which act during development.

Injections were accomplished with an 'Agla' micrometer-syringe equipped with a needle made from capillary tubing. The needle was inserted into the second or third dorsal conjunctivae to deliver the mutagen close to the gonads. Each one received approximately 0.3 µl of solution.

### Results

The data for the induction of dominant lethal mutations in first meiotic oocytes are presented in Table 10. Metaphase I oocytes were about 20 times more sensitive to treatments of mitomycin C than prophase I oocytes (Fig. 1). Recessive lethal mutations were induced in both metaphase I and prophase I oocytes (Table 11). Samples were small or missing for the different dose-levels so it is difficult to compare recessive lethal mutation frequencies between metaphase I and prophase I. Nevertheless, the results suggest that the mutational responses were not very different between these two stages.

The data collected from females mated to males which were injected with mitomycin C are presented in Table 12. Dominant lethal mutations were induced in sperm, and the effect was greater in subsequent matings (Table 12 and Fig. 2). Most of the deaths occurred before the blastula formed. These were classified as type I deaths (von Borstel and Rekemeyer, 1959), which indicates that death occurs during the karyokinesis stages, presumably by gross inhibition of mitosis.

Brood patterns for recessive lethal mutations induced with 1.0 mg/ml dose level are shown in Table 13. There appears to be only a small increase, if any, in recessive lethal frequency from day 1 to day 3, even though dominant lethal frequency increased significantly over the same time period. One female from day 1 and one from day 2 were heterozygous for a translocation. None of the 264 control females were heterozygous for translocations. Three temperature-sensitive recessive lethal mutations were found in the day 1 brood, and two were found in the day 2 brood.

### Discussion

The pattern of dominant lethal mutations induced in oocytes with mitomycin C is similar to that induced by X-radiation (Whiting, 1945) and the two chemical mutagens, nitrogen mustard and ethyl methanesulfonate (Löbbecke and von Borstel, 1962). However, there appears to be little difference in recessive lethal mutation frequency between metaphase I and prophase I oocytes after treatment with mitomycin C. This response is similar to the effect found by Löbbecke and von Borstel (1962) for ethyl methanesulfonate, a monofunctional alkylating agent. X-radiation induces approximately 16 times as many recessive lethals in metaphase I oocytes than in prophase I oocytes, and nitrogen mustard induces about five times as many.

Injections of mitomycin C induced dominant lethal mutations in sperm, and the effect increased over the three daily broods. This effect had not been observed previously with other mutagenic agents used on Habrobracon. X-irradiated males mated daily for several days showed no change in the pattern of dominant lethality (von Borstel,

unpublished data). Therefore, it had been assumed that a homogeneous population of cells had been exposed. Also, results from an experiment in which males were exposed to nitrogen mustard suggested a similar conclusion (Whiting and von Borstel, 1954). The results of the present experiment can be interpreted in two ways; (1) the mitomycin C was affecting a non-homogeneous population of cells (sperm, spermatids, etc.); or (2) some of the mitomycin C remains reactive for as long as 72 hr. Mitomycin C must be reduced before it is active, and therefore it may have been continuously activated over a long period of time (producing an increased dominant lethal response).

The frequency of recessive lethal mutations increased, but not significantly, over the three-day period. These results suggest that the mechanisms of mutation may be different for recessive lethality and dominant lethality.

The temperature-sensitive mutations found in this experiment suggest that mitomycin C can induce base-pair substitutions. Similar results were found in studies on Drosophila by Suzuki and his collaborators (1967).

#### Summary

Metaphase I oocytes of Habrobracon are approximately 20 times as sensitive to mitomycin C as prophase I oocytes when dominant lethality is the criterion. When recessive lethality is the criterion, metaphase I and prophase I are essentially the same in sensitivity. Injected males were mated on three successive days. The dominant lethal frequency increases progressively from brood to brood, but the recessive lethal frequency remains essentially unchanged. Temperature-sensitive recessive lethal mutations are induced by mitomycin C in sperm.

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Number of translocations and recessive lethal mutations obtained in the ground-based control I of the Biosatellite I Experiment

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\* Symbols used: Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation; r<sub>1</sub>, F<sub>1</sub> female is heterozygous for larval recessive lethal mutation; r<sub>p</sub>, F<sub>1</sub> female is heterozygous for pupal recessive lethal mutation; v, F<sub>1</sub> female is heterozygous direction that the package is seen by the <sup>85</sup>Sr-radiation source; T, F<sub>1</sub> female is heterozygous for translocation; r<sub>e</sub>, F<sub>1</sub> female is heterozygous for embryo recessive lethal for a mutation visible, for altered morphology either viable (adult) or inviable (pupal).

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 $_{\rm e}^{**}$   $r_{\rm f}$  , and  $r_{\rm p}$  do not include recessive lethal mutations associated with translocations.

\*\*\* Includes 2 r<sub>e</sub> associated with one translocation which were counted as separate lethals. ;

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Number of translocations and recessive lethal mutations obtained in the ground-based control I of the Biosatellite I Experiment

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Number of translocations and recessive lethal mutations obtained in the ground-based control 1 of the Biosatellite 1 Experiment

Table 4

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:	No. of Lethals or Trans.	121	132	253	LL LL	T = 2	T = 2	or more)		
	Total Wasps Tested	140	138	278			н	) U	Ţ	Ţ
	osition	n	LR	ы						

\* Symbols used: Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation;  $r_1$ ,  $F_1$  female is heterozygous for larval recessive lethal mutation;  $r_p$ ,  $F_1$  female is heterozygous for pupal recessive lethal mutation; v,  $F_1$  female is heterozygous direction that the package is seen by the  $85_{S-radiation}$  source; T, F<sub>1</sub> female is heterozygous for translocation;  $r_e$ , F<sub>1</sub> female is heterozygous for embryo recessive lethal for a mutation visible, for altered morphology either viable (adult) or inviable (pupal).

 $_{\rm r}^{**}$  r , and r do not include recessive lethal mutations associated with translocations.

								N)	minal exp	osure O R	. *.				-				
osition	Total Wasps Tested	No. of Lethals or Trans.	<b>-</b>	E	г. В	۲. ۲	r o	E	other	TT <sup>+</sup> other	Ľ٩	 	 r rl	-	L'I''	r1 <sup>r</sup> p	يم	r d d	>
L-outer	181	177															ო		
IL-middle	150	149	.•											-					
Ц	331	326												2			e		
R-outer	162	156				-											4		
R-middle	166	165																	
LR	328	321				-								-			4		
ы П	659	647				<del></del>								ო			4		
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	-4	0=.				r = 0			T = 1		ູື	0		E E	a.			r= 0	
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	т е	0 =				ی م		F	r = 0		<u>_</u> م	4	Ē	e = 0	÷			r = 7	
	Ţ	0 = .				-			Tr = I				•	lr ≈ 1					
Symbols use	;p							×											

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Number of translocations and recessive lethal mutations obtained in the ground-based control I of the Biosatellite I Experiment

Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation; r<sub>1</sub>, F<sub>1</sub> female is heterozygous for larval recessive lethal mutation; r<sub>p</sub>, F<sub>1</sub> female is heterozygous for pupal recessive lethal mutation; v, F<sub>1</sub> female is heterozygous for a mutation visible, for altered morphology either viable (adult) or inviable (pupal). direction that the package is seen by the <sup>85</sup>Sr-radiation source; T,  $F_1$  female is heterozygous for translocation;  $r_e'$ ,  $F_1$  female is heterozygous for embryo recessive lethal

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Number of translocations and recessive lethal mutations obtained in the ground-based control I of the Biosatellite I Experiment

Table 6

(pre-irradiated with 2000 R)\*

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TT <sup>+</sup> other		1(r_e)						
T <sup>+</sup> other				LR	80 II	= 5	more <i>j</i> = 2	N 
TTT					н		T or	- <u>1</u>
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Tr					5	7	2	
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F	2	7	4					
н		-	-					
No. of Lethals or Trans.	23	28	51	nr N	T = 5	T = 3	r or more) r = 1	e Tr=1
Total Wasps Tested	33	34	67			ŝ	_ F	
Position	UL-inner	LR-inner	w					

\* Symbols used:

Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation;  $r_1$ ,  $F_1$  female is heterozygous for larval recessive lethal mutation;  $r_p$ ,  $F_1$  female is heterozygous for pupal recessive lethal mutation; v,  $F_1$  female is heterozygous for mutation visible, for altered morphology either viable (adult) or inviable (pupal). direction that the package is seen by the <sup>85</sup>Sr-radiation source; T, F<sub>1</sub> female is heterozygous for translocation; r<sub>6</sub>, F<sub>1</sub> female is heterozygous for embryo recessive lethal

 $_{\rm e}^{**}$  r1, and r do not include recessive lethal mutations associated with translocation.

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Number of translocations and recessive lethal mutations obtained in the ground-based control III of the Biosatellite I Experiment (Nominal exposure 0 R) >

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Position	Total Wasps Tested	No. of Lethals or Trans.	⊢	F	ہے ا	_ب ج	ے≏	E	T <sup>+</sup> other	TT <sup>+</sup> other	۳.	۳. ۵	r r e e e	۔ ۲	్ల ల		1,1	rr P	د_ دھ	<u>د</u>	>
LR	204	198														8			. 0	•	_
ΓL	42	42																			
ស	246	240																			
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	μ <b>-</b>	0 = .	[=]																		
	T (1 oi	r = 0 r more)	r <sub>1</sub> = 2																		
	Т <sub>е</sub>	0	r = 2																		
	Ţ	0 = 1				ά.															
																	-		-		1

\* Symbols used:

Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation; r<sub>1</sub>, F<sub>1</sub> female is heterozygous for larval recessive lethal mutation; r<sub>p</sub>, F<sub>1</sub> female is heterozygous for pupal recessive lethal mutation; v, F<sub>1</sub> female is heterozygous for anutation, visible, for altered morphology either viable (adult) or inviable (pupal). direction that the package is seen by the <sup>85</sup>Sr-radiation source; T, F<sub>1</sub> female is heterozygous for translocation; r<sub>e</sub>, F<sub>1</sub> female is heterozygous for embryo recessive lethal

Number of translocations and recessive lethal mutations obtained on the ground-based control III of the Biosatellite I Experiment

Table 8

(pre-irradiated with 2000 R)\*

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TT <sup>+</sup> other								
T <sup>+</sup> other	1(r r) ep							
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E	-		້. <sup>ຍ</sup>	** *		* *_Q		
- F	~							
No. of Lethals or Trans.	43	٦ <u>۲</u>	[ = 19	r = 16	yr more)	4 = 6	r = 7	2. 
Total Wasps Tested	69		-	- Fran	) (] c	ŗ	Ľ	
Position	٦ſ							

\* Symbols used:

Position: The module in which the Habrobracon males were placed on each bracket UL signifies upper left position of the package (bracket plus modules) as determined by the mutation;  $r_1$ ,  $F_1$  female is heterozygous for larval recessive lethal mutation;  $r_p$ ,  $F_1$  female is heterozygous for pupal recessive lethal mutation; v,  $F_1$  female is heterozygous for mutation visible, for altered morphology either viable (adult) or inviable (pupal). direction that the package is seen by the  $85_{51}$ -radiation source; T,  $F_1$  female is heterozygous for translocation;  $r_e$ ,  $F_1$  female is heterozygous for embryo recessive lethal

 $_{\rm r}^{**}$  r1, and r do not include recessive lethal mutations associated with translocations.

Table 9	sncies of translocations and recessive lethal mutations obtained in the ground-based control of the Biosatellite I experiment*
	Fred

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Control	Nominal Exposure	T/n	T'/n	r <sub>e</sub> ⁄n-T'	r1∕n-T'	r <sub>p</sub> /n-T'	r∕n−T¹	Tr <sub>e</sub> ∕n	Tr <sub>e</sub> /T'
-	4000	49/115 = 0.426	39/115 = 0.339	18/78 = 0.231	20/78 = 0.256	6/78 = 0.0762	44/78 = 0.564	24/115 = 0.209	24/37 = 0.649
I	2000	35/207 = 0.169	30/207 = 0.145	14/177 = 0.079	23/177 = 0.130	80 <u>2</u> 0*0= 2/1/2	46/177 = 0.230	12/207 = 0.0580	12/30 = 0.400
н	1000	12/244 = 0.0492	9/244 = 0.037	13/235 = 0.0553	15//235 = 0.0638	6/235 = 0.0255	34/235= 0.145	1/244 = 0.00409	111.0 = 6/1
I	500	3/278 = 0.0108	3/278 = 0.011	7/275 = 0.0255	8/275 = 0.0291	1/275 = 0.00363	16/275= 0.0582	1/278 = 0.00359	1/3 = 0.333
ī	0	1/659 = 0.0015	1/659 = 0.0015	0/658 < 0.00151	3/658 = 0.00455	7/658 = 0.0106	10/658= 0.0152	0/659 < 0.00151	0,1 < 1,0
H	** pre-2000	13/67 = 0.194	611.0 = 76/8	5/59 = 0.0847	2/59 = 0.0339	3/59 = 0.0508	10/59 = 0.169	3/67 = 0.0448	3/8 = 0.375
Ш	0	0/246 < 0.00406	0/246<0.00406	1/246 = 0.00406	2/246 = 0.00813	2/246 = 0.00813	5/246= 0.0203	0/246 < 0.00406	
Ш	** pre-2000	19/69 = 0.275	16/69 = 0.232	7/53 = 0.132	5/53 = 0.0943	4/53 = 0.0754	16/53 = 0.302	4/69 = 0.0580	4/16= 0.250
		s 						· · · · · · · · · · · · · · · · · · ·	

\*\* pre-2000 = males exposed to 2000 R of X-radiation before being placed in O-dose region of ground-based control set-up.

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мс		MI oc	ocytes			PI oo	cytes	
Conc.	Virgins	Percent	Mated	Percent	Virgins	Percenț	Mated	Percent
0	178	91.6	154	96.1	84	96.4	65	95.4
0.01	114	46.5	134	50.7	35	94.3	48	95.8
0.05	135	0.7	135	0	43	88.4	42	76.2
0.10	173	0	160	0	48	77.1	46	82.6

Table	10	· ·

# Hatchabilities after the injection of Mitomycin C into females

Recessive lethal mutations induced in oocytes

%         %         %           tested         %         Recessive lethals/oocytes tested         %           0         -         0         -         -         -           8.6         1/26         3.8         -         -         5		Metaphase I		Prophase I	
0 8.6 3.8 - 1/26 5 5	Recessive lethals/oocytes	tested %	Reces	sive lethals/oocytes tested	%
8.6 1/26 3.8 - 1/20 5	0/110				1
- 1/20 5	3/35	8.6		1/26	3.8
	I	I		1/20	5

Survival of diploid embryos after treatment of Habrobracon sperm by injections of

	٠			•	$\sim$
- M	I	TOM	vc	in.	
	۳.		/ -		

Days after injection (Brood)	Conc. mitomycin C (mg/ml)	Total no. eggs	% Survival to blastula	% Survival to larvae	% Survival to adult females
1	0	615	98.7	98.3	62.9
	0.1	709	99.4	97.8	67.1
	. 0.5	820	91.7	86.8	61.4
	1.0	838	83.5	81.8	61.0
2	0	432	97.8	93.8	62.9
	0.1	602	88.9	85.6	62.0
	0.5	774	64.2	59,7	39.8
	1.0	585	49.2	45.4	29.0
3	0	518	99.5	97.1	59.6
	0.1	790	77.8	72.6	52.8
	0.5	858	57.8	56.6	38.1
	1.0	432	41.3	39.6	19,5

	mg/ml Mitomycin C	
Days after injection of Mitomycin C	No. ♀♀carrying a recessive lethal	% Carrying recessive lethal mutations
 ]	8*/206	3.9
2	4**/ 99	4.0
3	3/ 44	6.8
Control	1/264	0.4

Brood pattern of recessive lethal mutations induced in sperm after an injection of 1.0 ma/ml Mitomycin C

Table 13

\*Three were temperature sensitive mutants.

\*\* Two were temperature sensitive mutants.



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