Response of an Oedionychina (Coleoptera) Karyotype to Acute Gamma Radiation[1]

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ABSTRACT

Acute doses of 250 and 500r produced chromosomal aberrations in practically all treated males of the fleabeetle *Omophoita cyanipennis*. Spermatogonia were unaffected. In spermatogenesis, first aberrations at M I became recognizable four to six hours after radiation. Although presumably induced in a later phase than the gaps, the translocates reached their M I peak simultaneously with the gaps, about 24 to 50 hours after radiation. This is due to a marked delay in development of the cells radiated during the diffuse stage. The latest prophase effects manifested at M I were sticky interchromosomal contacts and interchanges of lateral loops of coiled chromonema (="subchromatid exchanges").

Both gap and translocate yields increased by dose; 3000 and 12000r produced unanalyzable "pulverization" and clumping of chromosomes.

The long sex chromosomes are the most probable exchange partners for any chromosomes. Interautosomal exchanges comprised only 6.8 percent of all exchanges.

Aberrations caused irregular chromosome segregation and fragments both at A I and A II, resulting in undersized and supernumerary spermatid nuclei, and a great variation of chromatin content even in those nuclei that had a normal appearance.

INTRODUCTION

All known Oedionychina (Chrysomelidae: Alticinae) karyotypes are grossly size-asymmetrical, having small autosomes and very large sex chromosomes. The latter form a synorientated distance bivalent in the male meiosis (34), but conjugate in the female (31). The prevailing karyotype formula is 10 II + X + y (31).

For several reasons, such an asymmetrical karyotype forms an interesting radiation object. The present study aims to determine, qualitatively and semiquantitatively, the aberrations induced in the spermatogenesis, using acute doses so low (500r and less) that survival of the beetles is not affected (38), and induction of recessive lethals is kept within tolerable limits (7). The aberrations are analyzed in the treated beetles and not in the F_1 progeny, because adequate rearing methods for producing enough progeny for analysis have not yet been developed. The

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ultimate goal is to assess the genetical significance of the large sex chromosomes by establishing and comparing strains with an altered structure of the sex chromosomes.

MATERIALS AND METHODS

Of the two more common Puerto Rican Oedionychina, *Omophoita cyanipennis* F. has the advantage over *Alagoasa* (= *oedionychus*) *bicolor* (L.) in that the chromosomes are more condensed at M I, and the sex chromosomes can be easily distinguished from one another by their size and shape. Because these characteristics are of value in interpreting the aberrations, *O. cyanipennis* was chosen for the work.

Beetles were collected at Playa de Salinas on the south coast of Puerto Rico. They were transported to Río Piedras in plastic bags kept in an ice-box at about 20°C. Next morning the beetles were briefly anesthetized with CO_2 to prevent their jumping, and were separated by sex. Males were sealed in an one-inch wide plastic envelope with a tack iron. The envelope containing the males was fixed with five rubber bands on the concave front of a carton one inch high and eight inches wide. The concave front was cut to correspond to an arc whose radius is 1 m. When the box was placed 1 m from the radiation source with the concave front facing it, all the beetles were equidistant from the source.

The beetles were radiated with a ^{60}Co source in air, at the Puerto Rico Nuclear Center at Río Piedras. The distance from the source was 100 cm; dosage was adjusted by varying the radiation time. Temperature in the radiation room was about 22°C. Samples that received 250 or 500r were the largest, 16 males containing 857 M I cells, and 63 males containing 2881 M I cells, respectively. These samples are the main basis for the quantitative analysis. Three beetles were tentatively treated with 125r, and this group is usually excluded from comparisons. Some samples were radiated with comparatively high doses: 3 kr (2 samples totalling 7 males) and 12 kr (3 samples totalling 14 males).

After radiation, the males were kept in plastic containers provided with a plastic mesh cover. *Clerodendrum* (=*Volkameria*) *aculeatum* (L.) leaves were given daily for food. After 6, 12, 36, 48, 60 hours, or longer, a maximum of four specimens from each sample were killed and examined cytologically. All stages of gametogenesis were observed, however, quantitative analysis was practically limited to M I.

Testes were fixed in Kahle-Smith fixative for 1 min., and stained in Dyer's (4) stain. Adult Oedionychina males have a single testis about 2 mm in diameter, containing four follicles. Squashing one *O. cyanipennis* testis under a 22x22 mm coverslip exposes practically the entire momentary spermatogenetic status of the individual. Preparations thus made were studied and photographed with a Zeiss photomicroscope II provided with phase contrast optics.

The study started in 1969, and was continued with frequent interruptions until 1976. Progress was slow because the species has a limited ecologic and geographic range. It is practically restricted to the coastal regions of Puerto Rico, where diverse urban developments limit the demes to such an extent that only small numbers of beetles could be collected.

OBSERVATIONS

NORMAL KARYOTYPE

Omophoita cyanipennis has a characteristic Oedionychina karyotype with 20 small metacentric autosomes and giant sex chromosomes: a metacentric *X* and a submetacentric *Y* (fig. 1). The male meiotic associ-

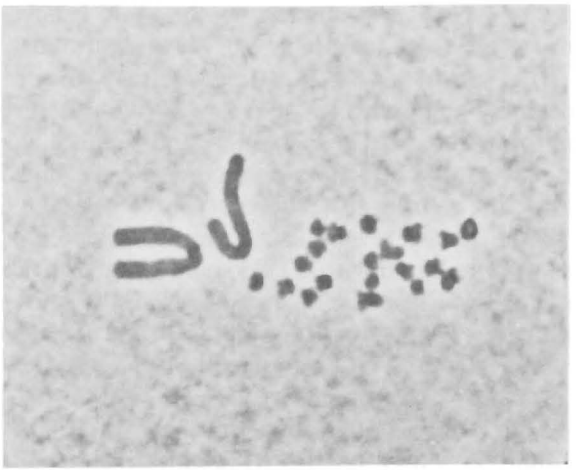

FIG. 1.—Normal karyotype of *Omophoita cyanipennis*. Spermatogonial metaphase, 20 + *X* + *Y*. The short procentric regions (including the entire second arm) of the autosomes are undercondensed. 1440×.

ation is 10 II + *X* + *Y* (fig. 2). At early A II (fig. 4), chromosome lengths are as shown in table 1.

The length of *X* and *Y*, measured in metaphases of the last gonocyst (fig. 1), was only 17.8 μm and 15.2 μm, respectively. The short arm of the autosomes tends to be undercondensed in these cells and thus it is difficult to relate to the long arm. M II and early A II therefore provide more reliable measurements. Fig. 3 shows a karyogram based on the lengths shown in table 1.

The relation of autosomes to sex chromosomes is 41.7:46.8, that of *X* to *Y*, 25.2:21.6. Autosomes assume 47.09 percent of the total karyotype length, *X*, 28.54 percent, *Y* 24.37 percent, and *X*+*Y*, 52.91 percent.

TABLE 1.—*Chromosome lengths and arm lengths (μm) in* Omophoita cyanipennis *as measured at early A II (four cells)*

Total length	Short arm	Long arm
1.38	0.69	0.69
1.52	0.69	0.83
1.93	0.76	1.17
2.08	1.04	1.04
2.08	1.04	1.04
2.08	1.04	1.04
2.12	1.01	1.11
2.22	1.11	1.11
2.35	1.04	1.31
3.06	1.29	1.77
(X) 25.24	11.79	13.45
(Y) 21.55	7.48	14.07
67.61	28.98	38.63

The course of male meiosis is characteristic for the subtribe. Fig. 4 gives an idea of features that facilitate chromosome analysis still while in the spermatocyte II, showing distinct chromatidal structure and shape, large size and peripheral location of the sex chromosomes in the spindle, as well as drum-shaped anaphase spindles (I and II) that allow plenty of interchromosomal space in squash preparations.

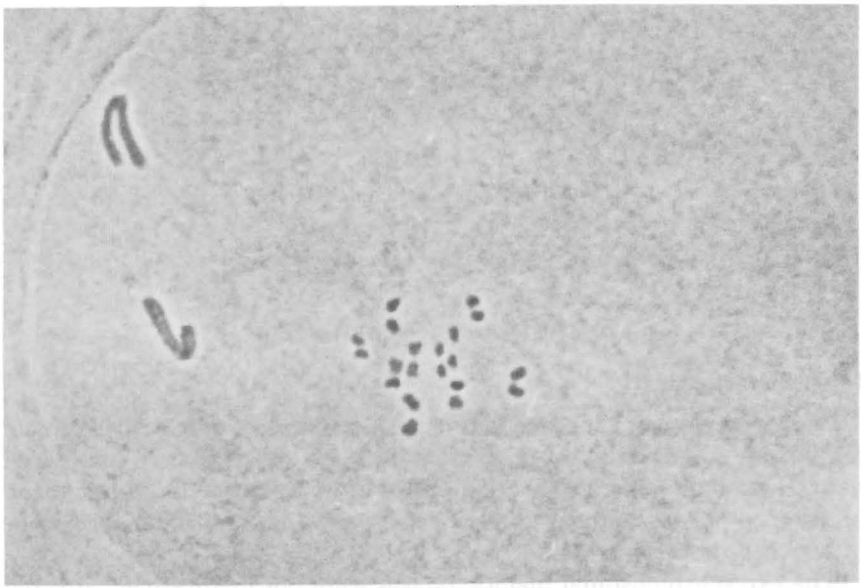

FIG. 2.—Normal karyotype of *Omophoita cyanipennis*. First metaphase of spermatogenesis. 10 II + *X* + *Y*. 1992×.

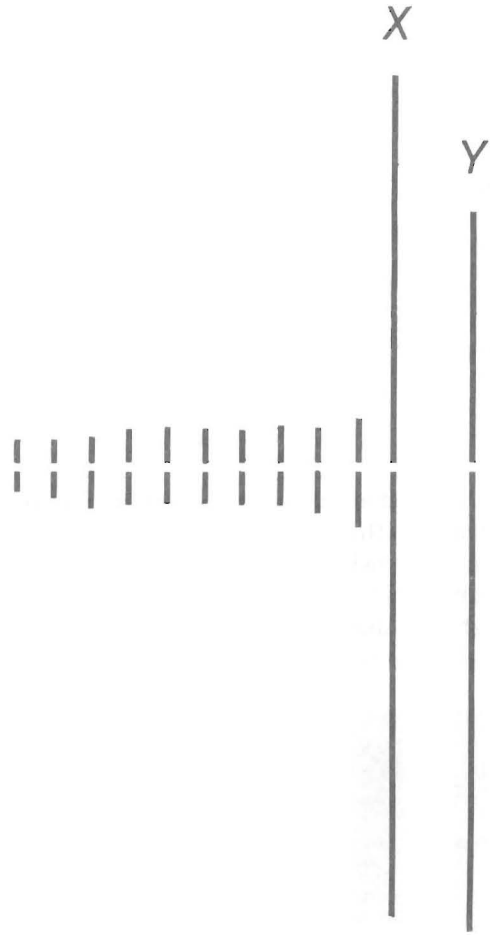

FIG. 3.—Karyogram of *Omophoita cyanipennis*, based on table 1.

RADIATED SPERMATOGENESIS

General effects

Radiation induces general effects such as cell death, mitotic cycle delay, and chromosome stickiness (3, 6, 15). Three general effects are considered here: 1) probable changes in frequencies of M I per beetle; 2) frequency of distance-pairing autosomes per beetle; and 3) the mutual involvement of sex chromosomes at M I.

The extrachromosomal meiotic phases were not qualitatively affected by the radiation. Thus prophase, contraction stage, metaphases and anaphases were recognizable even after the highest dose and despite extensive chromosomal damage (fig. 5).

FREQUENCY OF M I PER BEETLE

Although the Oedionychina of Puerto Rico are able to procreate all the year around, there are two rain correlated population density peaks. In Salinas, the first occurs in mid-May, the second in October-November. This bivoltine pattern is also reflected in oogenesis since ripe ovarial eggs and oviposition are rare except during high population peaks.

As the small testis can only accommodate a few of the unusually large spermatocytes I, the mode of preparation used permits one to count practically the total of M I per beetle. In control material of 20 males fixed in early May and November, the frequency of M I per beetle was 39.9, whereas in the radiated material, the frequency was 45.9, the September samples showing the lowest frequencies (fig. 6). Even if the

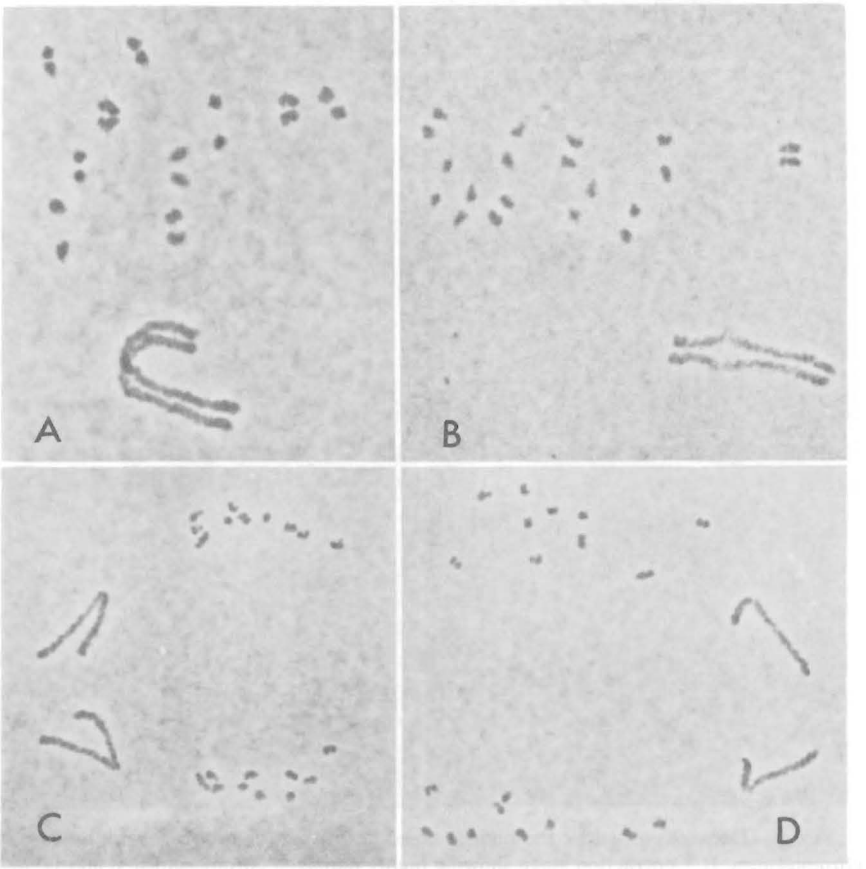

FIG. 4.—Normal karyotype of *Omophoita cyanipennis*. Second division of spermatogenesis. A and B. M II with *X* and *Y*, respectively. C and D. A II with *X* and *Y*, respectively. A 1260×, B 1050×, C and D, 895×.

spermatogenetic activity never ceased, nevertheless it was reduced during the interval between population peaks.

The relationship between dose and M I frequency is shown in fig. 7. The beetles that received either 250 or 500r show a mean M I frequency

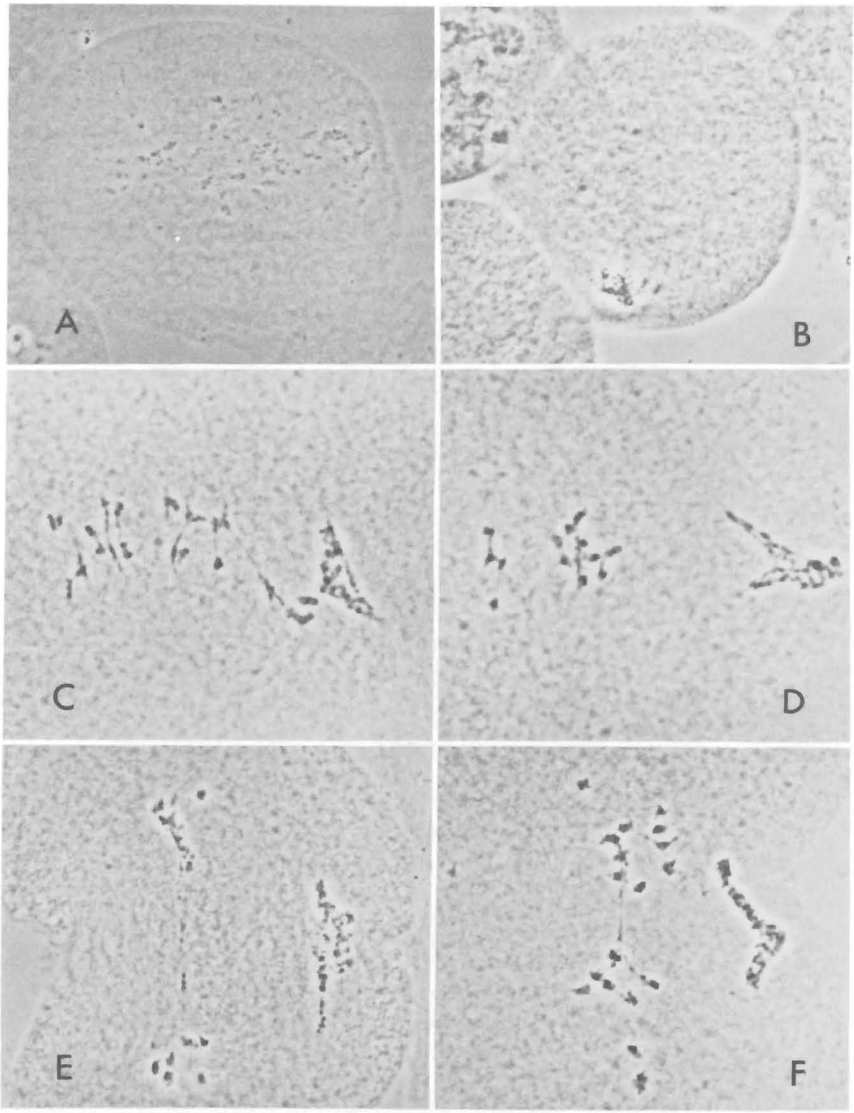

FIG. 5.—Damage caused by the highest dose, 12 kr. A. Late prophase with pulverized chromosomes. B. Contraction stage showing highly damaged chromosomes. C and D. M I showing beaded chromosome structure and nondescript involvement of the sex chromosomes (to the right). E and F. AI bridge formation by involved sex chromosomes and autosomes. A 84 h, others, 12 h after radiation.

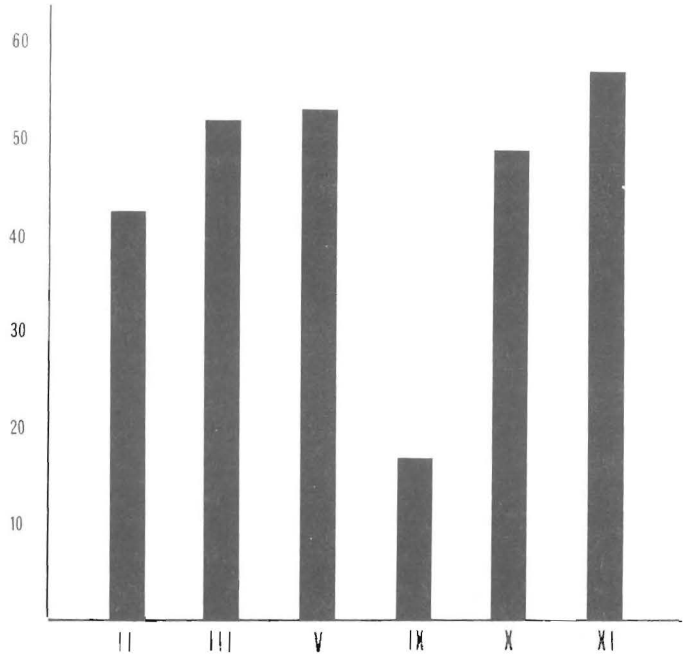

FIG. 6.—Frequency of M I per beetle per month in the radiated material.

of about 50 per beetle as expected. The seasonal distribution of these beetles is as follows: 56 (70%) represent peak population; 23 inter-peak population. At higher doses, 3 and 12 kr, the frequency was lower even though the samples were collected in October and November.

The relationship between M I frequency and time after radiation is shown in fig. 8. The frequency of M I decreases rapidly during the first 6 to 50 h, and increases slightly during the next 400 h (in grasshoppers 160r caused tapering of diplotene frequency to almost zero from 9th to 24th day after radiation 20).

Although the present statistics may not be conclusive, the data indicate that the frequency of M I per beetle 1) was positively correlated with the peak density of population, 2) decreased with an increased radiation dose, and 3) dropped during the first postradiation hours and recovered during the weeks following.

FREQUENCY OF DISTANCE-PAIRING AUTOSOMES

Although only *X* and *Y* of Oedionychina regularly segregate from a distance bivalent condition, occasionally one or more autosomal bivalents may behave in a similar manner. In the autosomal spindle-portion, such bivalents usually are located next to the "sex spindle." Observations of

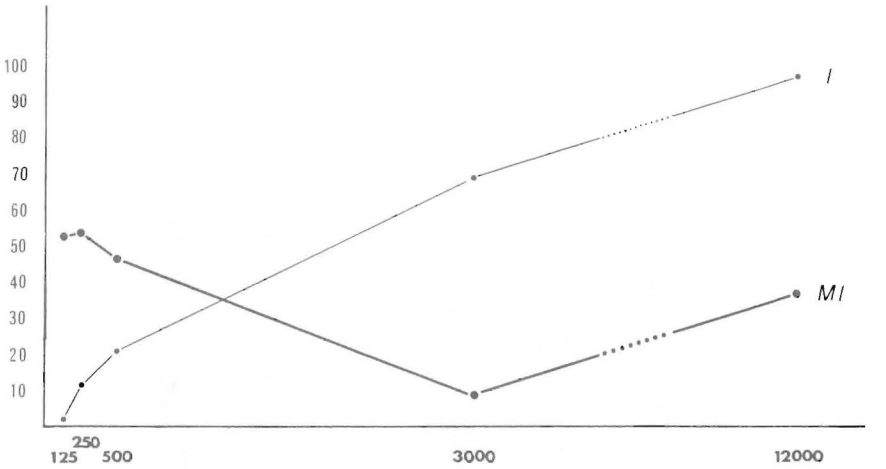

FIG. 7.—Dependency of the frequency of M I per beetle (M I), and of the percentage of mutual involvement of sex chromosomes (I), on dose.

living preparations have shown that distance-pairing autosomes remain facing one another, and then migrate to poles simultaneously with other chromosomes. It is unknown whether such bivalents are asynaptic or desynaptic, and in the latter case, originally chiasmate or achiasmate. It

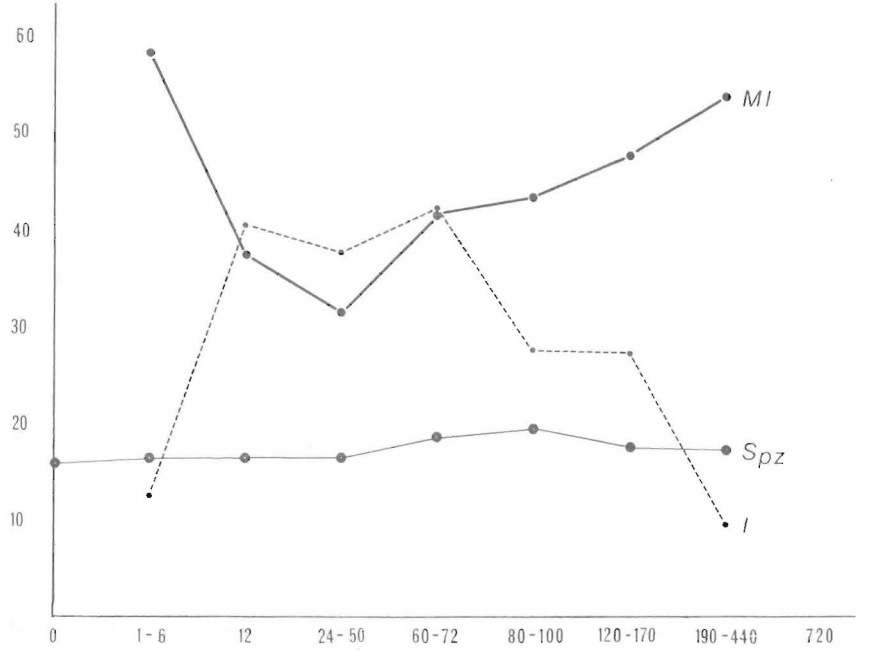

FIG. 8.—Dependency on post-radiation time (h) of M I frequency per beetle (M I), of percentage of mutually involved sex chromosomes (I), and of spermatozoa per bundle (Spz).

is difficult to distinguish between a far-stretched ordinary bivalent, and a distance-pairing one. The criteria used for the present analysis have been the complete absence of visible link between homologues, and intercentromeric distance at least twice as great as in the rest of the bivalents [see figure 17b in (34)].

Chromosome pairing is controlled by multiple factors (31) and therefore it might be influenced by radiation in a complicated way. The present analysis revealed up to 16 univalents (= 8 distance bivalents) per cell, the 250r-group having more than twice the amount the 500r-group had. The difference between these groups is significant ($\chi^2=10.209^{xx}$; $t=8.26^{xx}$); however, the control group does not differ significantly from the 500r-group. Thus the cause that is producing significant differences here might be other than the radiation.

MUTUAL INVOLVEMENT OF SEX CHROMOSOMES

A more or less intimate contact between *X* and *Y* chromosomes occurs frequently. The number of contacts increases with dose, the increase appears to be linear at low doses. Then a smaller rate of increase is observed at intermediate doses, and at high doses (12 kr), practically all sex chromosomes are joined (fig. 7). This is a heterogenic category of effects. The two principal factors causing this complex phenomenon are thought to be the sticky terminal contacts comparable with those found by others in insects (3, 18, 22) and plants (15, 26), and the more or less pulverized *XY* and *XYA* translocates (figs. 5c to f) produced by higher doses. In the former, which are ephemerous, late prophase effects disappear in A I and leave no trace. The latter enter meiosis and produce complicated bridges, and new such compounds do not reach M I, presumably due to a long-lasting blockage of PM I. Thus the number of the involvements is less after the third day (fig. 8). Occasional sticky contacts have been observed in untreated beetles also, although in this control consisting of 20 beetles there were none.

ANALYZABLE ABERRATIONS

Single (chromatid) and double (chromosome) gaps, fragments, and translocates, usually involving the large sex chromosomes, were produced in spermatocytes I at 125, 250, and 500r. No gaps were seen in autosomes, and rearrangements that involved autosomes alone were rare. No aberrations whatsoever were seen in gonial metaphases, the frequency of which is about 1/10 of the M I frequency.

It is not possible to tell true fragmentation from paled or decondensed gaps, except when the fragmented faces are clearly released from their original juxtaposition. If all gaps are taken as breaks, and the minimum number of breaks that explain the translocations are added to them, the

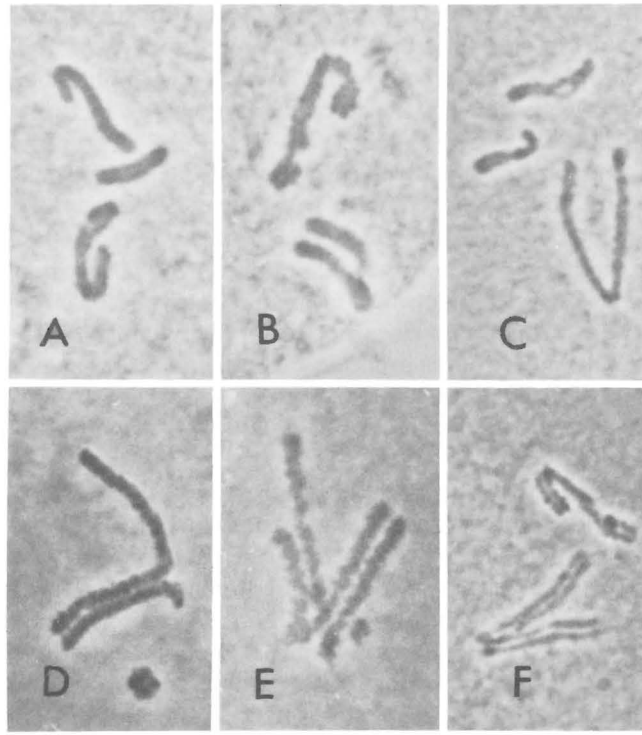

FIG. 9.—Breaks, gaps, and deletions in the sex chromosomes. A. Double break in *X*, single gap in *Y*. B. Single gap (or a short chromatid deletion) in *Y*. C. Double break in *Y*. D and E. Single break in *X*, with a (partly?) corresponding fragment. F. Single gap (or a short chromatid deletion) in *Y*. D and E, M II, others, M I. A and D 125r+72h; B and E 125r+60h, C and F 500r+60h.

following general results emerge:

	125r	250r	500r
Breaks per beetle	15.00	17.81	33.13
Percentage of beetles affected	100	93.8	82.5

The seeming tendency of more individuals escaping injury at an increasing dose is not real, since the 125r-sample comprises only three males, and the 500r-sample is the only one containing specimens checked six hours or less after radiation, at a time when the manifestation of the aberrations has only begun. It suffices to say that nearly all males treated with 125, 250, and 500r had chromosomally altered M I cells.

GAPS

Gaps in the following are interpreted as breaks that were apparent, as well as paled regions in the chromosomes. In both gap analysis and the

translocation statistics, the control material was not included since neither spontaneous aberration nor paled gaps have been seen in this species among the about 2000 specimens that had been observed earlier.

Sites of gaps in sex chromosomes vary considerably (fig. 9), their distribution being probably random. Single and double gaps had a similar frequency, 350 and 368, respectively, but if only non-juxtaposed gaps are considered, the relation is over 2.4 (119 vs. 287), not much different from the estimated number of three times (6).

It is usually assumed that double gaps arise from treatments preceding, and the single gaps from treatments following the synthetic (S) period of the interphase (6, 10). Thus the double gaps should reach M I later than the single gaps. This is not shown in the present material (fig. 10). It

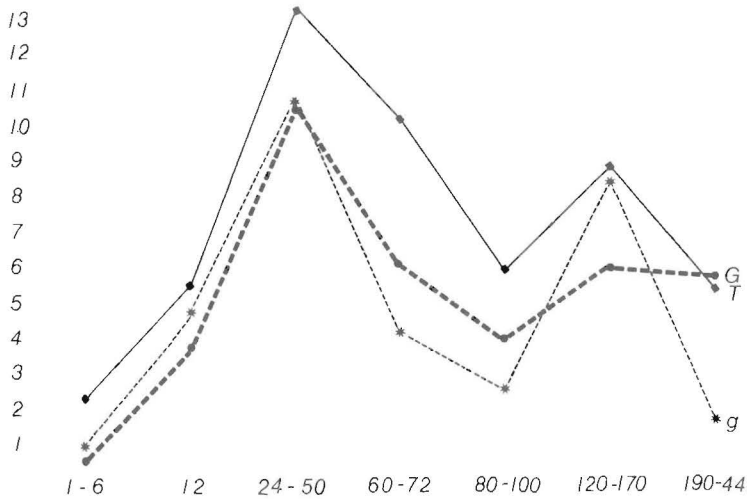

FIG. 10.—Dependency on post-radiation time of the frequency per beetle of translocates (T), and of single (g) and double (G) gaps.

seems possible that the induction of most of these gaps occurs during or close to, the chromatid synthesis. It is interesting that an affected Oedionychina spermatocyte I may delay up to one month before reaching M I (38). Manna and Mazumder (18) observed a 10-day delay in aberration appearance, and proposed that this could be explained by an asynchronous replication of the *X* chromosome. If this happens in a grasshopper that has a short *X* chromosome, it is even more probable for the long sex chromosomes of *O. cyanipennis*.

Because the normal timing of *O. cyanipennis* spermatogenesis is not known, it is not possible to evaluate the extent of the delay. Since rate of death among the treated spermatocytes did not increase, delay in cell

TABLE 2.—*Relation of all sex chromosome gaps to dose (double gaps taken as single events)*

		Number of gaps																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
250r	16 beetles: *X*	6	3	2	0	2	2					1												
	35 gaps:		3	4		8	10					10												
	16 beetles: *Y*	6	2	3	2	1					1		1											
	35 gaps:		2	6	6	4					1		11											
500r	63 beetles: *X*	18	2	7	2	9	1	3	2	4	2	3	2	2		1	2			2			1	
	322 gaps:		2	14	6	36	5	18	14	32	18	30	22	24		14	30			36			21	
	63 beetles: *Y*	16	5	6	3	3	5	7	3	5	2	1	1	1				2			1	1		1
	310 gaps:		5	12	9	12	25	42	21	40	18	10	11	12				32			19	20		22

	250r		500r	
	X	*Y*	*X*	*Y*
Gaps per beetle	2.19[1]	2.19	5.11[1]	4.92
Percentage of beetles affected	62.5	62.5	71.4	74.6

[1] $t = -3.02^{x}$

cycle rather than cell death appears to be the factor responsible for the drop of M I frequency 24 to 50 h after radiation (fig. 8).

If all gaps are considered together (double gaps taken as single events), then the number of gaps per beetle is more than twice at 500r (table 2), although the difference between the *X*s alone reaches a statistical significance ($t=3.02^{x}$). The trend remains the same (X_{250r} *vs.* X_{500r}: $t=-4.07^{xx}$) if the comparison is limited to those cases where the fragmented faces are released from juxtaposition. The number of such cases remained rather low in the *X*s treated with 250r (table 3). If single and double gaps are observed separately, the difference between the 250r and 500r groups is significant ($t=7.47^{xx}$ and $=6.51^{xx}$, respectively).

TABLE 3.—*Relation of released sex chromosome gaps to dose (double gaps taken as single events)*

	Number of gaps																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
16 beetles: *X*	8	4	2	2													
14 gaps:		4	4	6													
250r																	
16 beetles: *Y*	6	2	6	1		1											
23 gaps:		2	12	3		6											
63 beetles: *X*	24	5	6	5	7	1	5	3		3	2		1			1		
196 gaps:		5	12	15	28	5	30	21		27	20		12			16		
500r																		
63 beetles: *Y*	18	11	8	6	5	4	5	2		3		1						
167 gaps:		11	16	18	20	20	30	14		18		11						

	250r		500r	
	X	*Y*	*X*	*Y*
Gaps per beetle	0.88[1]	1.44	3.11[1]	2.65
Percentage of beetles affected	50.0	37.5	61.9	71.4

[1] $t = -4.07^{xx}$

Autosomal fragmentation is harder to observe. Only nine double (chromosomal) deletions were recorded.

TRANSLOCATIONS

Five kinds of translocations were scored:

1. Between *X* and autosome (*XA*; fig. 11, table 4).
2. Between *Y* and autosome (*YA*; fig. 12, table 4).
3. Between *X* and *Y* (*XY*; fig. 13, table 4).

4. Between both sex chromosomes and autosome (*XYA*; fig. 14, table 4).
5. Between autosomes (AA; fig. 15, table 4).

There were in addition a few more complex, but still analyzable cases placed as follows: *X*AA and *X*AAA to *X*A, *Y*AA to *Y*A, and *XY*AAA to

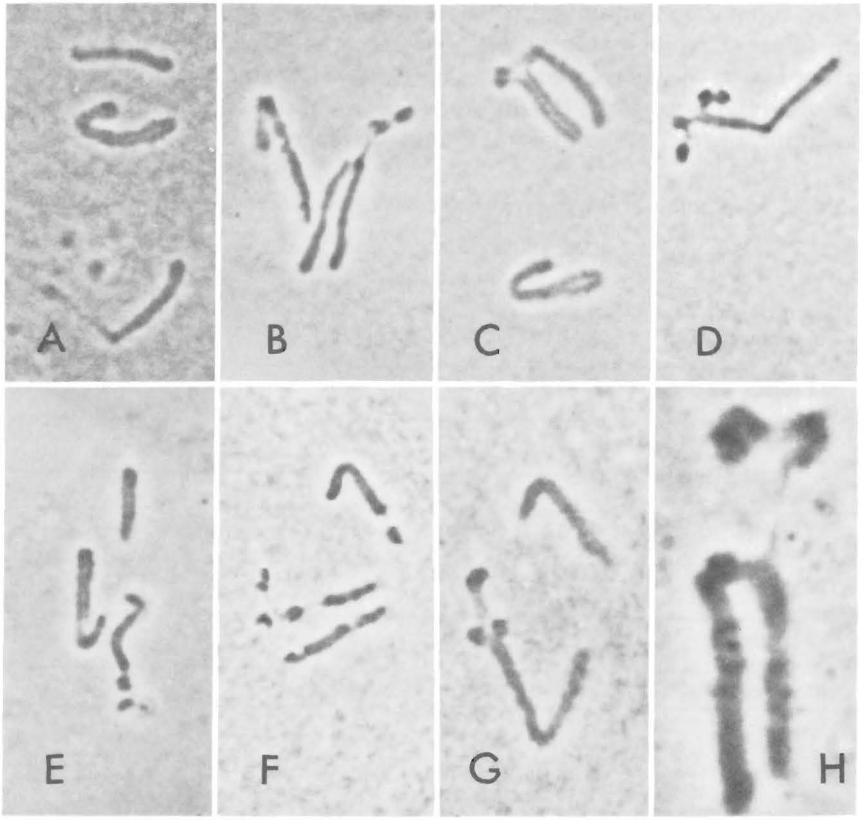

FIG. 11.—*X*-to-autosome translocates at M I. A. *X* broken near to its centromere, and one of the fragments translocated on an autosome (bivalent). Uncertain whether all four chromatids involved. B. Single break of *X* near to its centromere, and one of the chromatids translocated to one autosomal chromatid that might belong to a very short second arm. *Y* slightly beaded. C. Double break and deletion in an autosome, single break in *X*; all four chromatids involved in translocation. D. Single break of *X* near to its end, and interchange of broken chromatids with those of two different autosomes. E. *X* showing a double break near to its centromere, and chromatid interchange with an autosome near to the end. F. In *X*, several gaps, whole-arm deletion, and interchange of the remaining arm with an autosome. Double gap in *Y*. G. This is most probably a reciprocal chromatid interchange between *X* and a fragment of *Y*, although autosomal material may be also involved. H. Chromatid interchange between *X* and autosome; for *X* this looks like subchromatid translocation. A and G 250r+430h; B, D, and E 500r+36h; C 250r+288h; H 500r+12h.

XYA. It is to be noted that many of the translocates involving sex chromosomes contain more than the minimum of interchanges necessary for keeping them together.

Although 500r constantly produced more translocations than 250r, the difference was statistically significant only in the case of *XYA* (table 5).

Free fragments at M I were also scored. These may have arisen by translocations or by deletions. The difference between 250r and 500r was not significant (1.63 and 1.65 fragments per beetle respectively).

Manifestation of the translocations follows the same pattern as gap manifestation (fig. 10, table 6).

SEGREGATIONAL ERRORS

As is to be expected, aberrant chromosomes face serious trouble in anaphases, especially in meiosis. Some of the segregational errors ob-

TABLE 4.—*Dose dependency of five different translocation types scored at M I*

			Number of translocates per beetle													
			0	1	2	3	4	5	6	7	8	9	10	11	12	13
XA[1]	250r	16 beetles:	5	5	3	2				1						
		24 *XA*:		5	6	6				7						
	500r	63 beetles:	20	12	8	8	4	2	2	3	2	1				1
		147 *XA*:		12	16	24	16	10	12	21	16	9				11
YA[2]	250r	16 beetles:	6	2	5	2		1								
		23 *YA*:		2	10	6		5								
	500r	63 beetles:	10	10	15	8	2	3	2	2	1					
		121 *YA*:		10	30	16	8	15	12	14	8					
XY	250r	16 beetles:	5	4	5	1				1						
		24 *XY*:		4	10	3				7						
	500r	63 beetles:	27	9	12	4		3	2	2	2			1		1
		126 *XY*:		9	24	12		15	12	4	16			11		13
XYA[3]	250r	16 beetles:	13	2	1											
		4 *XYA*:		2	2											
	500r	63 beetles:	29	18	7	4	2	2				1				
		71 *XYA*:		18	14	12	8	10				9				
AA	250r	16 beetles:	15	1												
		1 AA:		1												
	500r	63 beetles:	49	8	4	2										
		22 AA:		8	8	6										

[1] Includes one *XAA*/250r, 6*XAA*/500r, and2*XAA*/500r.

[2] Includes 2*YAA*/500r.

[3] Includes 6*XYA*/500r and 3*XYAAA*/500r.

served in radiated *O. cyanipennis* are shown in figs. 16 to 19. They include dicentrics that lag in anaphases and have the tendency to form bridges that either persist, or yield nondisjunctively. Persisting bridges have been seen outside of the fusome (fig. 18 c, f).

Segregational errors accumulated in spermatids are very common (fig. 19). The bundles of spermatids that have just started to elongate are of help in estimating gross damage to nuclei. The exceptionally low number -16 - of spermatids per cyst facilitates such an analysis. In preparing squashes the bundles are often broken, and a separate study using a more

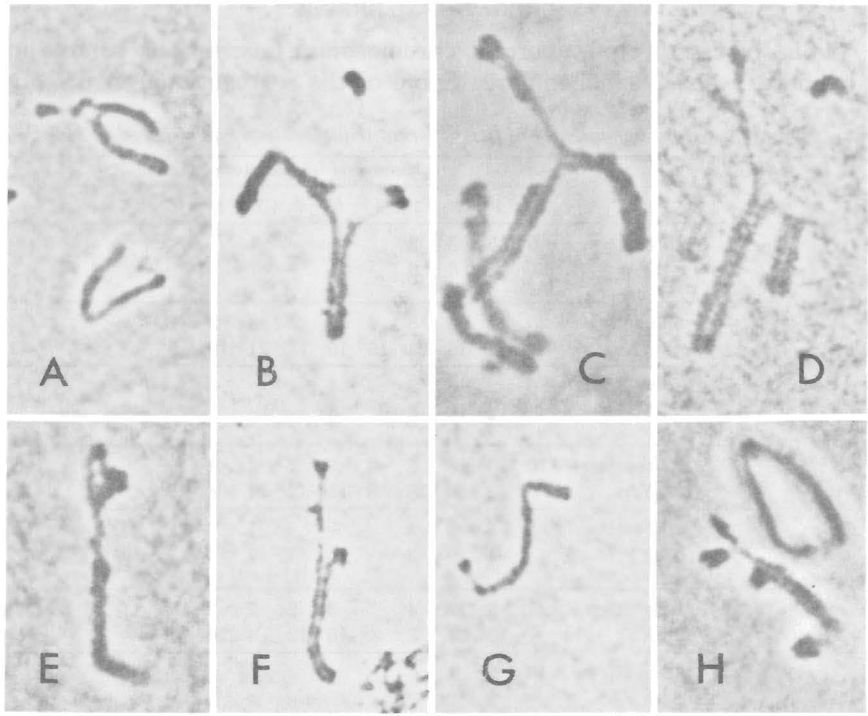

FIG. 12.—*Y*-to-autosome translocations at M I. A and B. Chromatid interchange following a single break in an autosome and in *Y*. C and D. Like above, but only one chromatid involved in both chromosomes. E. Single break near to one end of *Y*; both ends joined to chromatids of a doubly broken autosome (univalent); this case could also be interpreted as intercalation of a small *X* fragment to the *Y* chromatid, or, even better, as an unequal interchange between the *Y* chromatids. F. Double break at one end of *Y*, single break in an autosome; autosomal fragment translocated on one chromatid of *Y*, bivalent on the other. G. As in F, but involving both chromatids of both chromosomes. H. An *Y* translocate that has consumed 2 autosomal bivalents. The structure of the upper part is near to the case in F, that of the lower part resembling G. A, C, and E to G 500r+60h; B 500r+84h; D 250r+60h, H 500r+24h.

delicate squashing would be necessary to obtain a more precise measure of this.

Radiation-damaged bundles may differ from healthy ones both by the number and by the size of their nuclei (fig. 19 b, c). Only the numerical variation was analyzed. Deviations from 16 nuclei per bundle were not observed in a control material of 20 beetles. A slight rise was observed in radiated beetles, although there was no significant difference between the 250r and 500r groups. Up to 50 hours postradiation time, little change

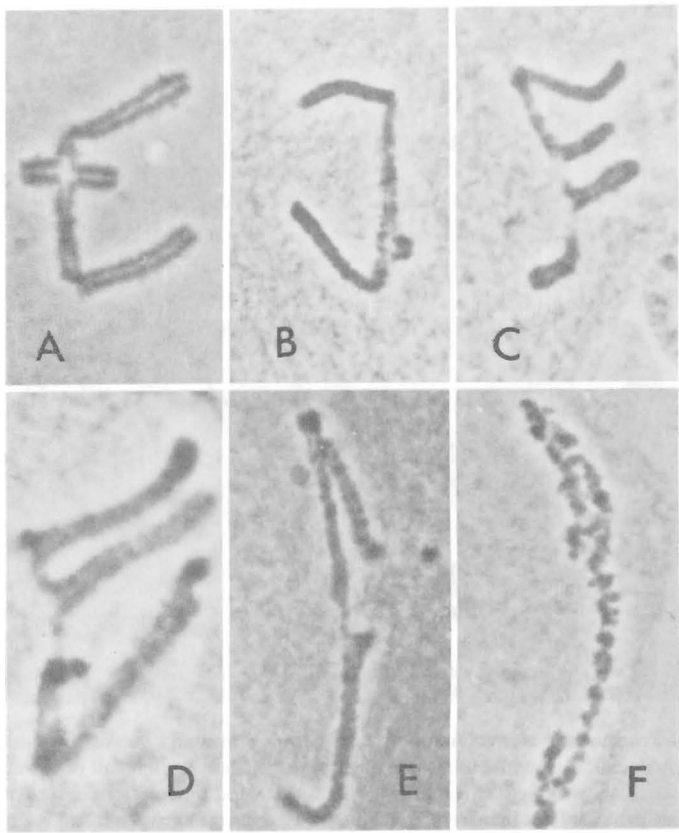

FIG. 13.—Chromatidal translocations between *X* and *Y*. A to C. All four broken ends involved. D. One *X* chromatid broken near to its centromere, and one *Y* chromatid broken near to the end of the short arm; two of the broken ends interchanged. E. One chromatid broken subterminally in both sex chromosomes. Interchange, and corresponding small fragment. F. This case could be similar to E, but beading caused by high dose renders the mutual involvement unanalyzable. A 250r+288h; B and D 250r+60h; C and E 500r+24h; F 3kr+12h.

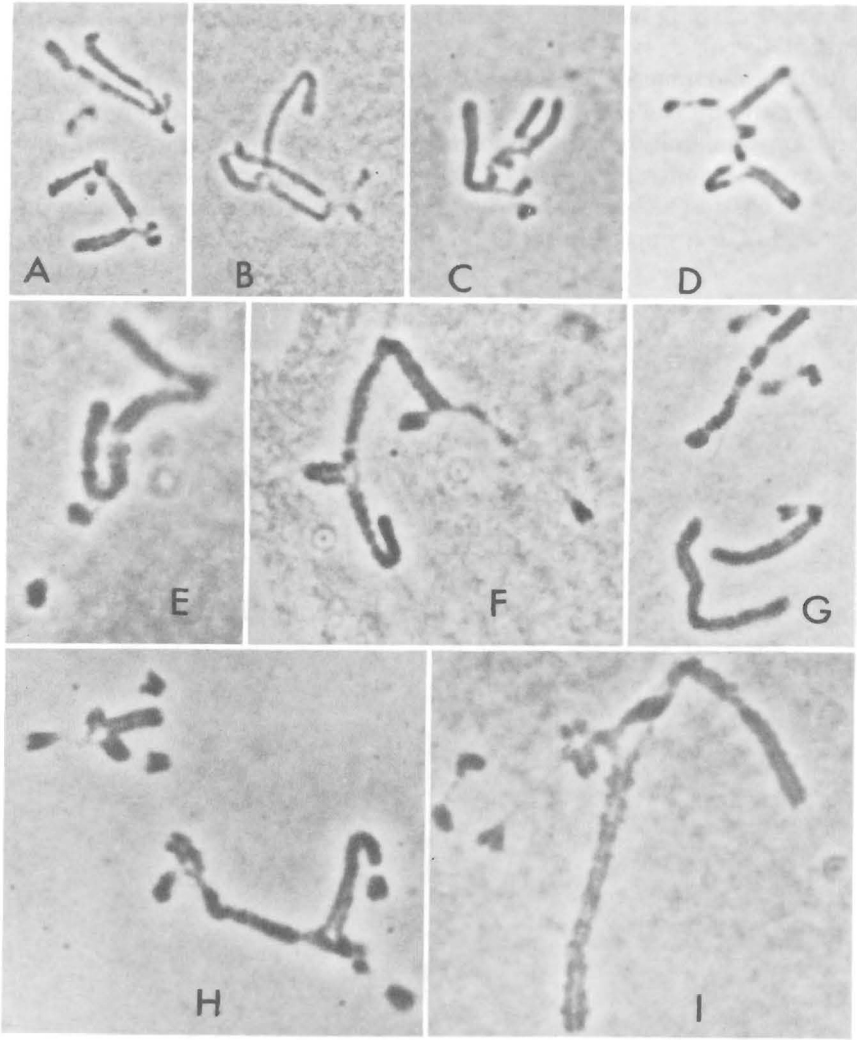

FIG. 14.—Translocations involving both sex chromosomes and one or more autosomes. A. The *Y*-translocate (below) includes two autosomal bivalents as a result of two chromatid translocations. *X* shows single chromatid break and several pale regions. Bivalent near to its centromere probably not involved. B. Chromatid exchange between distal region of long arm of *Y* and a median arm region of *X*; in addition, one autosomal bivalent incorporated due to chromatid interchange with procentric region of *X*. C. Here the basic configuration has been a cross with very unequal arms, formed between the sex chromosomes by a chromatid interchange. The short arm of the cross has incorporated an autosomal bivalent by chromatid interchange, and the same has occurred, nearer to the cross, in one of the long arms. D. Probably two autosomal bivalents incorporated to the *XY*-translocate. The uninvolved arm of *X* is out of focus. E. Translocation nature of this configuration is

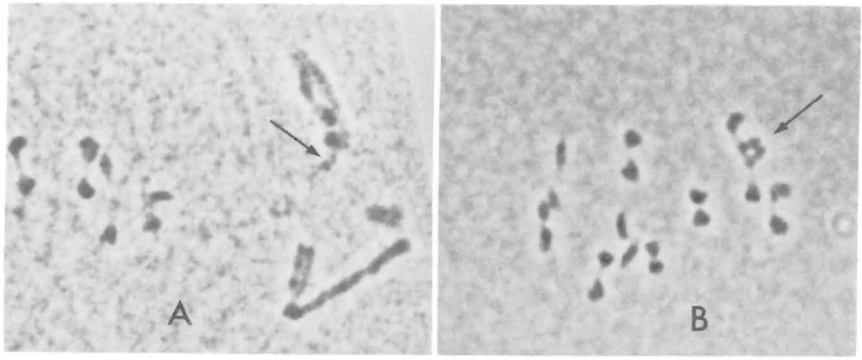

FIG. 15.—Autosomal aberrations. A. Two neighboring bivalents have lost the compact region (including centromere and the minute second arm) of one of their chromatids. The remaining fractured ends may have joined mutually (uncertain). The resulting fragment might be the one seen in the sex spindle (arrow), where there is also a larger fragment of sex chromosomal origin. B. A cross-shaped translocate (arrow), result of one chromatid interchange between two bivalents.

TABLE 5.—*Dose dependency of four translocation types involving sex chromosomes. Some traits based on table 4*

	250r				500r			
	XA	*YA*	*XY*	*XYA*	*XA*	*YA*	*XY*	*XYA*
Translocates per beetle	1.50	1.44	1.50	0.06[1]	2.33	1.92	2.00	0.35[1]
Translocates per M I	0.028	0.027	0.028	0.005	0.051	0.042	0.044	0.025
Percentage beetles affected	68.75	62.50	68.75	0.06	68.25	68.25	57.14	0.22
Percentage MI affected[2]	2.83	2.76	2.83	0.47	5.10	4.20	4.37	2.46

[1] $t = -3.48^{x}$

[2] Number of sex chromosome translocations = number of affected M I.

uncertain. The chain could be formed merely by intertelomeric adhesion between *X* and *Y*, and centric adhesion between *Y* and autosome. F. Subdistal chromatid translocation between the longer arm of *Y* and one of the arms of *X*. Another subdistal chromatid break of *X* has incorporated an autosomal bivalent by chromatid interchange affecting, probably, the minute second arm of the autosome. G. Provided that *X* and *Y* of this cell are intact, there is a long extra sex chromosome fragment that has exchanged chromatids with procentric region of an autosome. H. This complicated configuration includes up to 4 autosomal bivalents translocated on mutilated *X* (above) and *Y*. I. *X* and *Y* have interchanged chromatids subdistally, the result being a cross with unequal arms (as in fig. 13 B). The longer of these has incorporated an autosomal bivalent through a chromatid interchange. A, C, D, and H 500r+36h; B and E 500r+60h; F 500r+24h, G 250r+430h, I 125r+72h.

was observed. The highest frequency observed was at 80 to 100 h post-radiation time (fig. 8).

DISCUSSION

ON THE CHARACTERISTICS OF THE ABERRATIONS

The qualitative picture of chromosomal damage produced by gamma rays in *O. cyanipennis* is similar to the aberration picture induced by 75 to 200r of X-rays in *Tradescantia* microspores reported by Sax in his classical papers (26, 27, 28). Doses under 1000r have produced similar results in numerous insects (cf. 3, 8, 9, 10, 18), whereas doses over 3000r produce complicated lesions difficult to analyze, and still higher doses, "pulverization" of the chromosomes.

The number of gaps increases with increase in dose. If most of the single and double gaps are the result of single hits, the increase should be

TABLE 6.—*Sex chromosome translocations per beetle in relation to postradiation time*

h	Beetles		*XA*		*YA*		*XY*		*XYA*		Totals	
	250r	500r	250r	500r	250r	500r	250r	500r	250r	500r	250r	500r
1–6		13		0.15		0.77		0.62		0.62		2.16
12	4	4	1.00	1.00	1.33	0.75	1.67	3.00	0.33	1.75	4.33	6.50
24–50		12		4.00		2.67		4.25		2.25		13.17
60–72	4	8	2.00	3.63	2.25	3.00	3.25	2.98	0.25	1.63	7.75	11.24
80–100	4	8	1.00	2.25	2.00	1.88	1.00	1.25	0.50	1.00	4.50	6.38
120–170		10		3.10		2.50		2.60		0.50		8.70
190–440	4	5	0.50	3.80	0.50	3.20	0.50	0.40		1.50	1.50	8.90

directly proportional to dose (6, 28). The increase obtained is somewhat larger than expected (4.38:10.03 and 2.43:5.76 in tables 2 and 3, respectively). Sex chromosome translocates, except perhaps *XYA* (tables 4 and 5) do not follow a two-hit dosage curve. Because the material is heterogeneous (age, season, spermatogenetic stage), further analysis was not attempted.

The so-called subchromatid aberrations must now be interpreted in a new way, since a chromatid is composed of a DNA mononema as a maximum of strandedness (1, 14, 25, 30, 32). Because such aberrations are induced in late prophase, the advanced chromosome condensation can limit the interchange to only the lateral loops of the coiled mononema.

Several studies (5, 9, 11, 22) emphasize the significance of spatial interchromosomal relationships at the time of radiation. In interphase, the Rabl-orientation is one such spatial factor. The same orientation also supposedly occurs in the earliest spermatocytes I, but is replaced by the bouquet (35) in the prophase. During the interphase (and the Rabl-

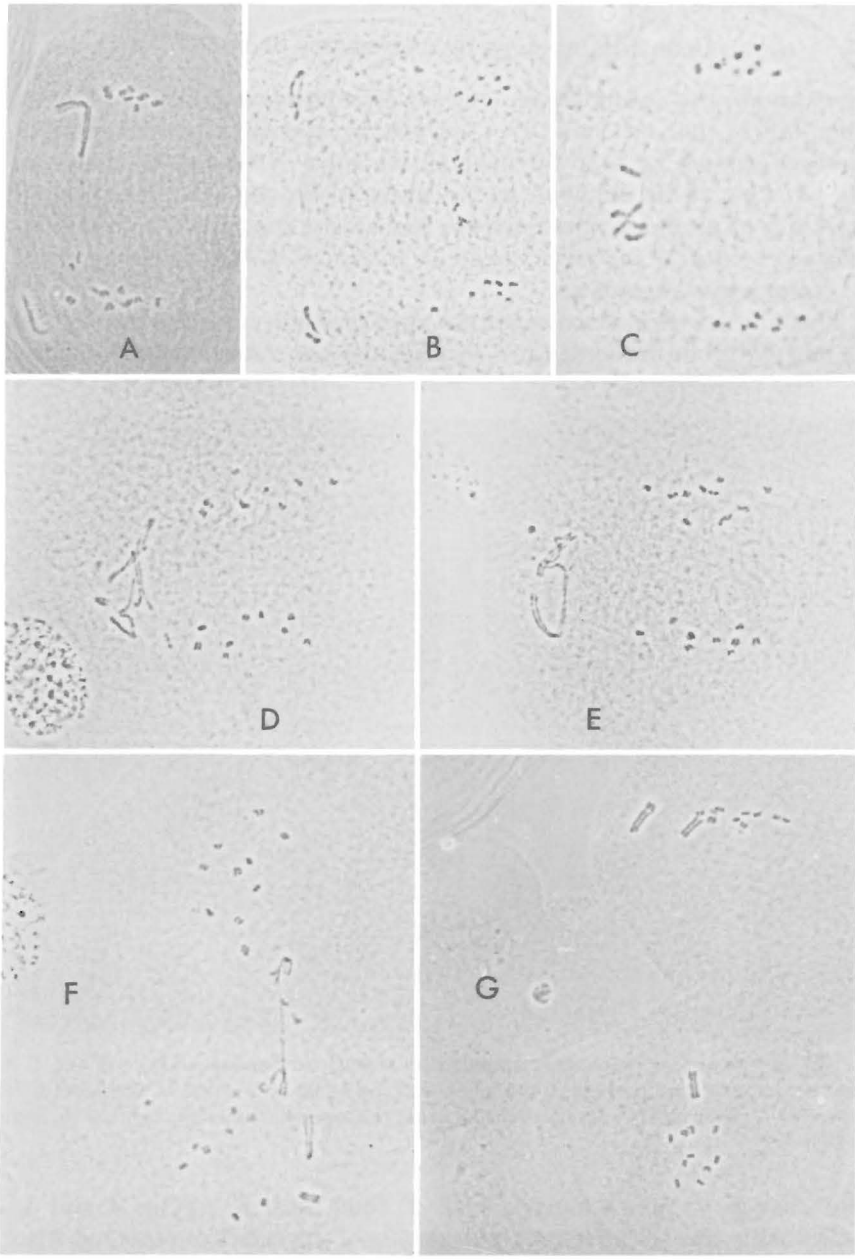

FIG. 16.—Segregation irregularities in the first meiotic division. A to F, A I; G, T I. A. Dicentric *X*A-translocate forming a bridge. B. Two lagging autosomal bivalents or dicentrics. C. Dicentric *XY*-translocate forming a bridge. D. *XY*A-translocate segregating to centric and acentric fragments of various sizes. E. Late dissolving of a cross-shaped *XY*-translocate. No dicentric chromatids. F. Dicentric *XY*-bridge, plus uneven distribution of various sizes of sex chromosome fragments. G. One of the fragmented *X*-arms segregated together with *Y*. Autosomal involvement in the *X*-fragment probable. A and G 500r+84h; B 500r+6h, C 125r+72h, D to F 500r+60h.

orientation), the decondensed, long sex chromosomes probably fill more than half of the nucleus in *O. cyanipennis,* spatially approximating to most autosomes. Later in the bouquet the loops of the sex chromosomes are too long to fit unfolded in the nucleus. On the other hand, wavy structure of prophase chromosomes that might contribute to interchromosomal contacts and interchange (9) is limited by the shortness of the *O. cyanipennis* autosomes.

Thus the sex chromosomes are the most probable interchange partners for any of the chromosomes of *O. cyanipennis.* An autosome has a slightly

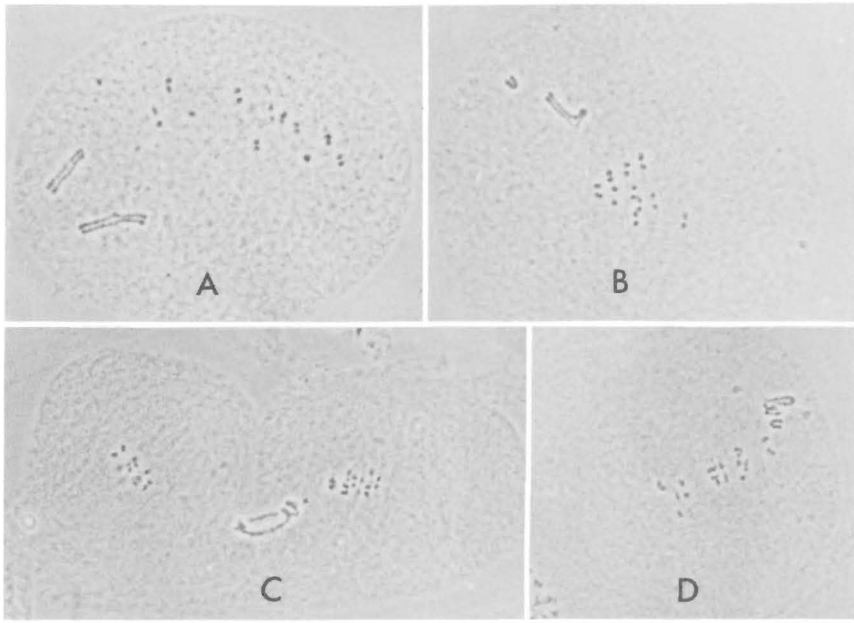

FIG. 17.—Results of segregation irregularities at M II. A. Nondisjunction of *X* and *Y*. 9 autosomes and 3 fragments. B. *Y* and a fragment, probably of *X* origin. C. Persistent *XY*-bridge. D. Various nondescript autosomal and sex chromosome fragments. A 500r+1h, B to D 500r+60h.

less chance to be exchanged with *Y* than with *X* (tables 4 and 5), presumably due to the length relationship of the sex chromosomes. Also, *Y* resulted slightly less gapped than *X* by 500r, but 250r produced controversial results (tables 2 and 3). Autosomes participated in translocations nearly as often as the sex chromosomes (*cf.* length relationships on pages 118–120), but only 6.8% of the simple exchanges where autosomes took part were between autosomes only. Contrary to this, *XY* translocates occurred in 32% of the simple exchanges where the sex

chromosomes took part. If the distribution of the breaks is random, the chromosome length seems to be of prime importance in determining the translocation partnerships in *O. cyanipennis*. In some grasshoppers that have much smaller *X* chromosomes, the *XA* translocations were much less frequent than in *O. cyanipennis* (20).

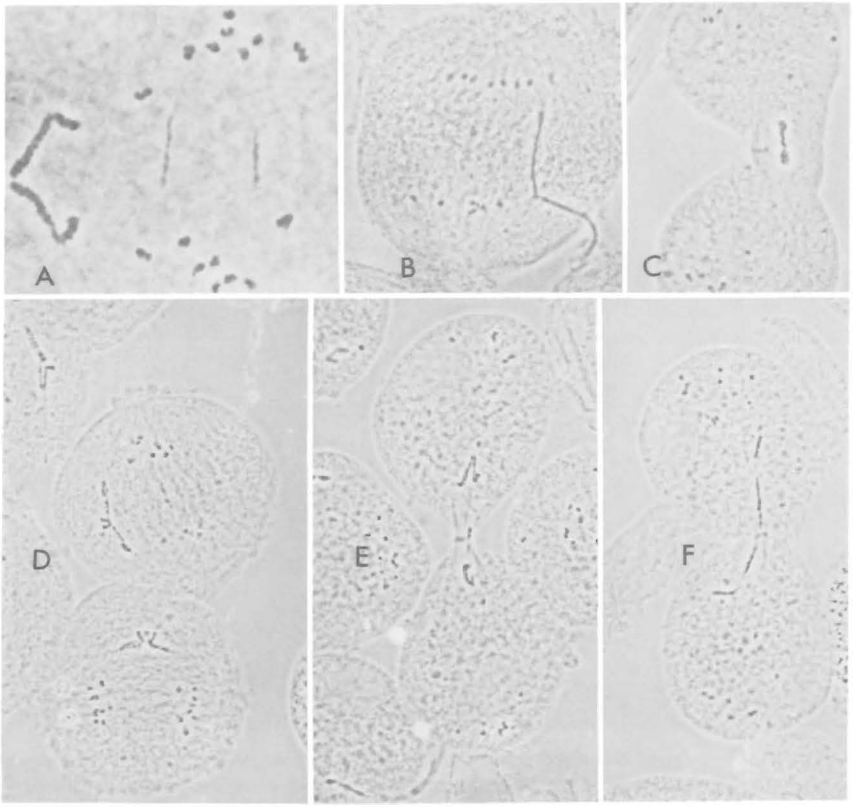

FIG. 18.—Further segregation irregularities in the second meiotic division. A. Autosomal anaphase bridges. B. Single, undercondensed *X*-chromatid at A II. C. Late sex chromosome bridge, left outside of the fusome. D. Sex chromosome bridges. E and F. Further sex chromosome bridges. These bridges may become located within the fusome. A 250r+430h, B 500r+60h, C 250r+12h, D to F 500r+6h.

Practically all of the males had chromosome defects at the radiation levels used in this study. The fact that the gonial chromosomes seemed unaffected is in accordance with numerous earlier experiments (*cf.* 24, 30). One reason for this is the relative rarity of these cells in adult Oedionychina testes. This, combined with high frequency of cell death and mitotic delay in radiated gonia (17), might suffice to explain the

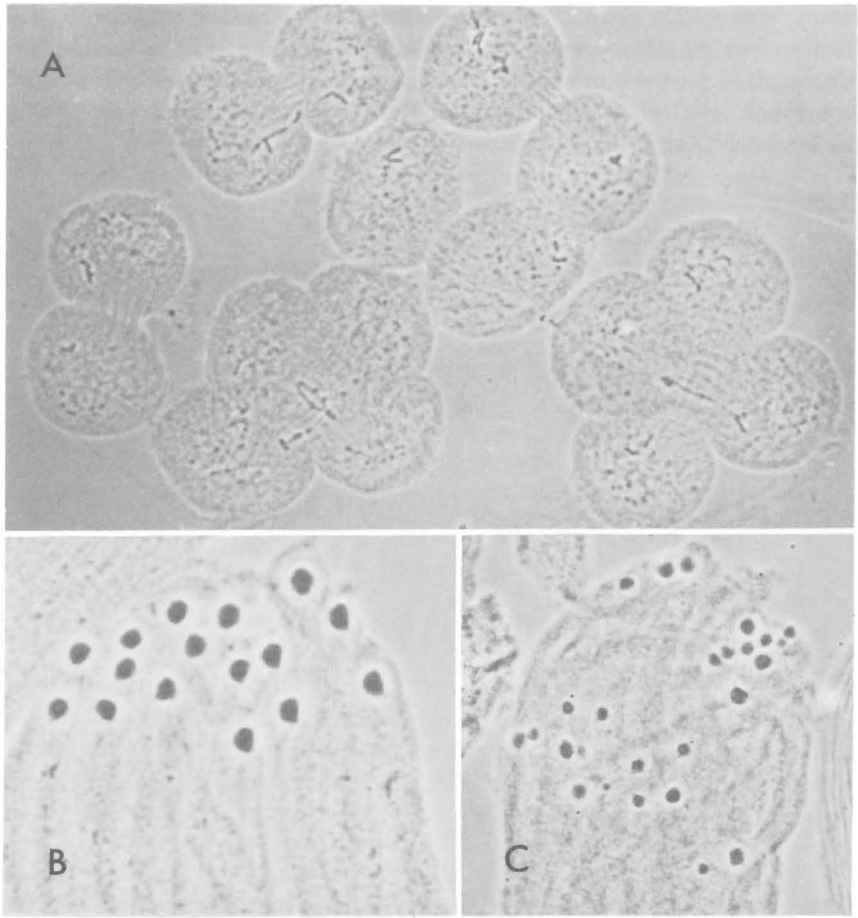

FIG. 19.—Results of disturbed meiotic segregation. A. Late A II, entire cell contents of a spermatocyst. Each one of the 16 spermatids has its own, aberrant karyotype. B and C. Elongating spermatids, head region of entire bundles. B has remained normal, with 16 nuclei of equal size. C has 25 nuclei of various sizes. A 500r+12h, D 500r+84h, C 250r+60h.

negative findings. As dying gonocysts were not observed, the delay seems to be more important than the death.

TIMING OF THE INDUCTION AND MANIFESTATION OF ABERRATIONS

Delay or reversion in the cell cycle is a general radiation effect. In *O. cyanipennis,* the frequency of M I is abruptly dropped 6 h after radiation, and increases very slowly thereafter. There seems to be a blocking effect of radiation in the late prophase of spermatocyte I as in grasshopper neuroblasts (3). Even 720 h (30 days) after radiation, chromatid gaps

appear (38) and these are hard to explain as other than lesions produced in the same cell where they were found.

The aberration frequency at M I is roughly complementary to the M I frequency (figs. 8 and 10). All lesions begin to manifest at M I 4 to 6 h after treatment, although there are some very early observations (fig. 17 A). According to timing of PM I and M I in vitro (32), these first-come lesions should have been induced well before the chromosomes condensate from the diffuse stage. Actually, the manifestation limit must be closer to M I, because the observed 4 to 6 hours correspond to a phase where the cell cycle was already delayed. The contraction stage, lasting about one hour before the congression for M I begins, apparently does not facilitate other interchanges except "subchromatid interchanges," despite the close packing of chromosomes. As M I is highly radiosensitive in most meiotic systems (6), usual types of lesions are presumably induced then, but because they are not immediately observable, they escape the scoring. The delay of manifestation seems to be operative at other stages as well (17). Thus Mazumder and Manna (20), scoring at diplotene in a grasshopper, found a similar time interval before first lesions became recognizable. The delay in the cell cycle obviously counteracts the delay in lesion manifestation: when the delayed cells reach the scoring stage, the lesions are already recognizable.

For the time being, since timing of *Omophoita* spermatogenesis has not been studied in vivo and in detail, the following tentative interpretation of the manifestation of aberrations seems plausible:

1. First to appear are "subchromatid interchanges" (= interchanges between peripheral loops of condensed mononema), because they are induced in late PM I cells that do not suffer from delay effects.

2. Second to appear are gaps and translocates. The present results suggest that these aberrations are induced only 24 to 50 h before M I, which interval appears to be too short a time when considering that the long diffuse diplotene (the dominant phase of the testes) is intercalated in between. Only timing the events of spermatogenesis can place this into the proper perspective as to whether the induction of the gaps actually occurs in G_1 and S phases.

Unexpectedly, the frequency peak of translocates coincides with that of the gaps. As translocations occur throughout the middle and late prophase, which are also most affected by delay (17), it seems that the lateness of this increase in frequency is due to delay. Middle and late prophase is occupied by the diffuse diplotene, during which the spermatocyte I is engaged in reorganization of "nuage" material inherited from spermatogonia, and in synthesis of similar new material (36, 39). Sex chromosomes are presumably engaged in this synthesis, and it is feasible that their activity is related to an increase in radiosensitivity. On

the other hand, the larger nuclear size might diminish the chances for translocation during this stage (11). Granted that duration of diffuse diplotene is much longer than the time when the gaps are induced, and that translocations can be induced during the entire course of diplotene, the curve of the translocate timing should be broader than the gap curve, but it is not (fig. 10).

SPERMATID NUCLEI AS INDICATORS OF GROSS CHROMATIN DAMAGE

Morphological abnormalities have been induced in sperm cell heads by radiation (2) and by chemicals (40). Wyrobek et al. (41) have shown that Robertsonian or reciprocal translocations and arm deficiencies are not sufficient to cause such abnormalities in mice. Spermiogenesis is controlled by the preceding diploid genotype to such extent that even chromosomeless sperm cells can develop normally (16), therefore it seems possible that nuclei with high variation of chromosomal content might develop into normal-looking sperm heads. However, this is not the case in the harlequin lobe of the pentatomid testes, where spermatid nuclei are variable in size (29), nor in strains of *Drosophila melanogaster* that have unequal meiotic segregation (12). It is also generally known that single chromosomes or chromosome fragments separated from the main nucleus form micronuclei. It is on this basis that the number of nuclei varies in *O. cyanipennis* spermatid bundles: irregular segregation sometimes forms supernumerary and undersized nuclei. Because there is a correlation of the number of micronuclei with the number of aberrations induced in mammalian bone marrow cells, rapid and reliable tests based on the number of micronuclei have been developed for detecting gross chromosomal damage (13, 19). Similarly, in Oedionychina, micronuclei frequency could be used to check the gross damage in the spermatid bundles.

FEASIBILITY OF PRODUCTION OF *OMOPHOITA* STRAINS WITH ALTERED SEX CHROMOSOMES

Because, with a random distribution of breaks, the longer monocentrics suffer average larger losses by deletion than short ones, the Oedionychina sex chromosomes are destined to heavy loss by radiation (33). Indeed, all sizes of sex chromosome fragments up to the length of whole arms were seen. Many of the centric fragments should survive if these chromosomes are heterochromatic, as they appear to be. On the other hand, the mechanism of distance pairing and segregation is not affected by radiation, except when complicated by chromosome fusions.

When Oedionychina autosomes have recognizable second arms, these tend to be allocyclic (fig. 1)., i.e. the autosomes are diphasic. The heterochromatic arms of the diphasics are perhaps generally dispensable to some degree in Coleoptera (31). Thus monocentric translocates formed

by an interchange between a sex chromosome and the allocyclic arm of a diphasic have a greater chance for producing neo-sex chromosome systems. A case related to this is known from Morro-do-Ferro in Brazil (the highest intensity natural radiation site in the world), where a pairing formula 9 II + XY has been established in a still unidentified Oedionychina species through transposition of autosomal pairing segments (=euchromatic arms) to the tips of the large X and Y (37).

As the size and general health of Coleopteran progenies are little affected by acute doses lower than 1000r (see 38 for references), production of Oedionychina with sex chromosomes altered by radiation would appear to be feasible. The genetic effects of the sex chromosome aberrations, induced profusely even with 125r, are expected to be slight, due to heterochromasy. Also recessive lethal mutations induced at these dose levels are insignificant (7). Our experiments concerning postradiation survival of Oedionychina (38) show that the beetles are rather demanding as to their nutritional requirements and as to the rearing conditions. The main question to be solved before the study can continue is how to successfully rear these beetles.

Meiosis acts as a sieve that eliminates mechanically incompetent aberrations that may survive mitosis. Functioning gametes derived from germ line cells radiated prior to M I have thus already passed one efficient selection process. Aberrations induced in spermatids and spermatozoa are not submitted to the mitotic and meiotic sieves until the next generation. Thus it might be advisable in cases like Oedionychina, where the rearing is difficult, to produce aberration-bearing progenies starting from gametes radiated as gametocytes. As yet it is unknown when such sperm becomes available. From the present results is appears that the highest frequency of translocations (induced at diffuse diplotene) reaches the elongating spermatid stage 3 to 4 days after treatment (figs. 8 and 10).

RESUMEN

Dosis agudas de 250 y 500r produjeron aberraciones cromosómicas prácticamente en todos los machos adultos tratados. Las metafases de los espermatogonios se encontraron normales. En la espermatogénesis, las primeras aberraciones se manifestaron en la primera metafase de 4 a 6 h después de la irradiación. Al comenzar, las secciones pálidas ("gaps") fueron más comunes; secciones sencillas, así como dobles, llegaron a la I M simultáneamente, y alcanzaron su frecuencia máxima de 24 a 50 h después de la irradiación. Aunque supuestamente inducidas en una fase más tardía que las pálidas, las translocaciones alcanzaron su frecuencia máxima en la M I de 60 a 72 h después de la irradiación. Esto se debe a una tardanza del ciclo celular en las células tratadas durante la etapa de diploteno difuso. Los efectos inducidos en la profase más tardía fueron los contactos intercromosomales pegajosos, así como también intercambios entre las espirales periféricas del cromonema ya bastante compactado (= "intercambios subcromatidales").

La frecuencia de las secciones pálidas, así como de las translocaciones, aumentó según la dosis. Tres y 12 kilorröntgens produjeron agregaciones y "pulverización" de los cromosomas.

Los cromosomas sexuales largos son los que con más probabilidad translocan con cualquiera de los cromosomas del cariotipo. Translocaciones interautosomales ocurrieron solamente en 6.8% de todas las translocaciones.

Las aberraciones inducen irregularidades de segregación en ambas anafases de la meiosis. Como resultado, se forman núcleos supernumerarios y de tamaño excepcional, además de la variación de menos escala en el contenido cromatínico de los núcleos, que parecen ser normales.

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