SPERMATOGONIAL CROSSING OVER IN *DROSOPHILA* *MELANOGASTER* AND *DROSOPHILA ANANASSAE*

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FRIESEN (1936) induced crossing over in *Drosophila* males by X rays and found that most of the exchanges were in a preferred region, the complementary cross-over classes were very unequal, and the percent recombination varied greatly from individual to individual. According to him, the data were sufficient to demonstrate the occurrence of spermatogonial crossing over. WHITTINGHILL (1937) found that induced crossing over in *Drosophila* males differs from normal female crossing over in that, in the former it occurred in a preferred region, double and multiple crossover classes were rare, and the complementary cross-over classes were unequal. COOPER (1946) observed similarities in induced crossing over and the oogonal disjunction of attached-X chromosomes. This led him to conclude that the induced crossovers were spermatogonial in origin. WHITTINGHILL (1947) reported that in *D. melanogaster* the presence of Curly inversions heterozygous for *rucuca* had no effect upon induced crossing over in males. This lack of interchromosomal effect was consistent with the lack of any effect upon the rare disjunction of attached-X chromosomes which is at least sometimes oogonal. WHITTINGHILL (1955) induced crossing over in males heterozygous for a chromosome of standard sequence and a Curly inversion chromosome carrying lethals. The correlated recoveries of only one crossover chromosome and a lethal-free noncrossover chromosome established the occurrence of crossing over long before meiosis, which might have resulted in the death of the sister cell carrying a lethal borne on the noncrossover chromosome and another crossover chromosome. LINDSLEY (1955) gave evidence for the occurrence of spontaneous spermatogonial exchanges between the X and the Y chromosomes. MORIWAKI (1936) reported induced crossing over in early pupae of *D. ananassae*.

Occurrence of radiation induced spermatogonial crossing over has been reported by several workers (CHANDLEY 1962; SAVHAGEN 1961; OLIVIERI and OLIVIERI 1963, 1964; FAHMY and FAHMY 1964). In almost all these experiments adult males were treated, where the population of spermatocytes dominates over that of spermatogonia. Moreover the main criterion utilized to distinguish the spermatogonial crossovers from the meiotic ones is the time of their appearance in the testcross progeny, which may have meiotic crossovers also. This screening is not always easy because in some particular broods a mixture of both these types of crossovers may be obtained (FAHMY and FAHMY 1964).

In the present work spermatogonial crossing over is studied by irradiating early developmental stages where spermatocytes are not present. Experiments were performed at various doses with *D. ananassae* and *D. melanogaster*.

MATERIALS AND METHODS

In *D. ananassae* a second-chromosome triple recessive stock *cu b se* (curled wings, black body, sepia eye) was used. The markers are described by RAY-CHAUDHURI, SARKAR, MUKHERJEE and BOSE (1959). In *D. melanogaster* a third-chromosome double recessive stock *se cu* was used. The wild stock was Oregon-R. for *D. melanogaster*, and a wild strain serialized as a8 was used for *D. ananassae*. It was observed earlier that males heterozygous for this combination do not undergo any spontaneous crossing over.

Wild females inseminated by recessive males were allowed to lay on standard *Drosophila* medium in 2 inch petri dishes for an hour. The petri dishes were irradiated 24 or 36 hours after eggs were laid. The target distance was 10 cm under a 1mm Aluminum filter. The tube operated at 115 kv and 4 ma. The doses received were 1000r, 1500r or 2000r. Pupae were taken from the petri dishes and from these, heterozygous males were collected within 2 hours after their eclosion. These were grown for 48 hours and then individually mated with six to eight virgins for 2 days. This was followed by a 2-day rest in separate vials after which they were supplied with a second set of virgins for the second brood. Whenever possible four to six such broods were obtained with a 2-day rest between the successive broods. The testcross progeny was scored for the frequency of crossing over. Altogether seven such experiments were performed, of which three were with *D. melanogaster* and four were with *D. ananassae*. Twenty-six males were irradiated in each experiment, except one with *D. melanogaster* where 30 males were tested. The same stocks were used for the control experiments. Wild virgins were mated to recessive males in mass matings. In each control experiment, 20 heterozygous males were collected within 4 hours after emergence. These were grown for two days and then individually mated to the first set of virgins as in the treated series. Six broods were scored similarly, with a 2-day rest between the successive broods. Only one control experiment was performed with the first experiment for each species.

RESULTS

In the first experiment 24-hr old embryos were irradiated with 1000r, both in *D. melanogaster* and in *D. ananassae*. The experiment with *D. melanogaster* was successful in that 153 crossovers were obtained, but in *D. ananassae* crossing over occurred in a single male, giving only four crossovers. This male was found sterile or did not produce any progeny in all the broods except the second one, in which the four crossovers were recovered from a total of 248 flies. A higher dose, 1500r was therefore given to the same stage. This also failed to produce any crossovers in the 26 treated males. Also, most of the males were completely sterile. Another experiment was performed where 1500r was given to 36-hr old embryos. Although a good number of progeny was obtained in this case, no crossover was recovered. Therefore a still higher dose, 2000r was administered. Unfortunately, even with this dose crossing over could not be induced in any of the 26 treated males. Experiments with *D. ananassae* were thus a failure. The control experiments did not show any crossovers.

In *D. melanogaster*, when the 24-hr old embryos were irradiated with 1000r, exchanges occurred in two males giving 153 crossovers. Out of these, 151 crossovers were from one male alone. Crossovers continued to appear in all the five

tested broods in this male, but all these were *cu* crossovers, the complementary class *se* being totally absent (Table 2, last column). Both the complementary classes were however recovered from another male where crossing over occurred. The experiment was repeated. The exposure was delayed 2 hours in the replication because of electric failure, resulting in the treatment of 26-hr in place of 24-hr old embryos. Exchanges occurred in four males (Table 2, center), but crossovers did not continue to appear throughout the tested period in any of these males as in the earlier experiment. In one of the four males, 26 *se* crossovers appeared without any complementary type. Two others gave equal complementary classes, 57:63 and 1:1.

In both experiments, many males did not produce any progeny. Some died after giving very few offspring. This was perhaps because too early stages were irradiated, and therefore a somewhat later developmental stage, 36 hours after laying, was exposed to 1000r. This experiment was also expected to give some idea of the sensitivity of the spermatogonia at these two developmental stages. Only three males died during this experiment and only one male was found sterile throughout the experiment. The total number of flies observed was also greater than in the earlier experiments (Table 1, line 3). Moreover, crossing over was induced in six males. Out of these six, five males gave from one to nine crossovers each, only in the earlier broods (Table 2, left three columns). The complementary classes were equal. In one male, both of the complementary types appeared in the first brood only. From the second brood onwards the *cu* crossovers ceased to appear, whereas *se* crossovers continued as long as the male was tested, resulting in a pronounced inequality (117:8) of the complementary classes.

DISCUSSION

In *D. melanogaster* the testes contain only spermatogonia up to 24 hours of larval life (COOPER 1950; see also HANNAH-ALAVA 1965). The embryos irradiated in the present experiments thus contained no later stages than spermatogonia

TABLE 1

Induced crossing over in males of Drosophila at different stages with variable doses

Expt. No.	Species	Stage irradiated; hours of laying	Dose	Males tested	Males with crossovers	Total crossovers	Total progeny
1	<i>D. melanogaster</i>	24	1000r	30	2	153	20,942
2	<i>D. melanogaster</i>	26	1000r	26	4	175	22,814
3	<i>D. melanogaster</i>	36	1000r	26	6	142	33,623
4	<i>D. ananassae</i>	24	1000r	26	1	4	8,720
5	<i>D. ananassae</i>	24	1500r	26	0	0	4,195
6	<i>D. ananassae</i>	36	1500r	26	0	0	15,733
7	<i>D. ananassae</i>	36	2000r	26	0	0	2,359
Total				186	13	474	108,386

TABLE 2

Spermatogonial crossing over in successive broods from 12 se cu/++ males of Drosophila melanogaster

BROOD	1000 P. 36 HOURS AFTER LAYING												1000 P. 26 HOURS AFTER LAYING												1000 P. 24 HOURS AFTER LAYING															
	NON CROSSOVERS				CROSSOVERS				NON CROSSOVERS				CROSSOVERS				NON CROSSOVERS				CROSSOVERS				NON CROSSOVERS				CROSSOVERS				NON CROSSOVERS				CROSSOVERS			
	++	se	cu	se	cu	++	se	cu	se	cu	++	se	cu	se	cu	++	se	cu	se	cu	++	se	cu	se	cu	++	se	cu	se	cu	++	se	cu	se	cu					
I	189	152	5	4		196	121	3	0		147	151	1	0		166	124	8	6		189	119	22	26		216	187	0	18											
II	181	128	0	0		173	152	0	0		0	0	0	0		208	126	0	4		178	126	19	14		168	194	0	48											
III	222	141	0	0		216	154	0	0		0	0	0	0		266	200	0	9		129	88	5	13		164	165	0	53											
IV	132	81	0	0		156	159	0	0		0	0	0	0		81	60	0	0		162	123	11	10		169	149	0	22											
V	171	146	0	0		214	168	0	0		0	0	0	0		-	-	-	-		171	135	0	0		147	72	0	9											
VI	102	71	0	0		84	62	0	0		0	0	0	0		-	-	-	-		147	128	0	0		-	-	-	-											
TOTAL	997	719	5	4		1039	796	3	0		147	151	1	0		721	510	8	19		976	717	57	63		864	777	0	151											
I	192	91	3	8		232	197	0	0		166	137	1	0		145	126	7	0		141	128	1	1		162	152	1	1											
II	194	125	4	0		134	120	2	1		217	174	0	0		212	161	16	0		75	75	0	0		201	177	0	0											
III	214	195	8	0		176	158	0	0		161	106	0	0		277	164	3	0		0	0	0	0		107	67	0	0											
IV	225	167	51	0		114	91	0	0		0	0	0	0		86	46	0	0		0	0	0	0		162	127	0	0											
V	125	94	27	0		0	0	0	0		0	0	0	0		115	61	0	0		0	0	0	0		109	58	0	0											
VI	238	189	44	0		96	71	0	0		0	0	0	0		122	113	0	0		0	0	0	0		-	-	-	-											
TOTAL	1188	859	117	8		752	617	2	1		544	417	1	0		967	671	26	0		216	201	1	1		741	581	1	1											

and the observed exchanges were therefore spermatogonial exchanges, provided they were immediately produced by the agent.

MULLER (1916) reported the first case of spermatogonial crossing over in *Drosophila*. It was pointed out that this crossing over must have occurred once very early in the embryonic development of the male to affect all his mature germ cells. In the early embryonic development of *D. melanogaster*, a group of cells originating from several nuclei are extruded from the caudal end of the embryo. These are pole cells. A few of these are later enclosed in the gonads and form the primordial germ cells. The germ cells, thus, are not linearly descended from any single apical cell ((SONNENBLICK 1950; TIHEN 1946). BRIDGES and MORGAN (1919) reported a case of spermatogonial crossing over at a somewhat later stage affecting only a part of the spermatogonial cells. In our experiments, although early developmental stages are irradiated, the number of recovered crossovers is only a fraction of the total progeny scored.

In almost all males except one, crossovers appeared from the first brood. MEYER (1954) irradiated earlier stages and reported that crossovers appear more frequently in later broods. In the present work, in some males crossovers appeared in all the tested broods. This indicates that the cells carrying crossover chromosomes continued multiplication for a considerable time. According to COOPER (1950), two types of gonial cells appear in the testes of *Drosophila*. One type is characterized by not occurring in groups and by undergoing asynchronous mitoses. These are primary spermatogonia. The other type consists of secondary spermatogonia (indefinitive and definitive spermatogonia of HANNAH-ALAVA 1965). TIHEN (1946) suggested that all the primary spermatogonia of a just-

hatched larva behave in an analogous fashion to the cambial cells of higher plants. According to him, a primary spermatogonium at each division gives rise to one primary spermatogonium and one secondary spermatogonium. The secondary cell forms its nest of 16 spermatocytes and eventually produces 64 spermatozoa. A nearly constant reserve of primary spermatogonia is thus maintained along with the continued production of new secondary spermatogonia. SAVHAGEN (unpublished, quoted by YTTERBORN 1962), proposed that a primary spermatogonium may divide into two primary cells or into two secondary cells. Those which divide into two primary spermatogonia may later divide in a similar manner into two primary spermatogonia or into two secondary cells, or they may follow the cambial-cell type of division into one primary and one secondary cell.

If crossing over is induced in a primary spermatogonium dividing according to TIHEN's hypothesis, one crossover chromosome will pass to a primary spermatogonium and its complementary crossover to the secondary spermatogonium, which will ultimately form at the most 64 spermatozoa. The primary spermatogonium with its crossover chromosome on the other hand, will continue the cambial like divisions and at each such division will produce 64 spermatozoa. This may result either in the pronounced inequality of crossover classes or in the absence of one complementary class. Such inequality has been earlier reported in many cases of spermatogonial exchanges by other workers and in the present work in the progeny of some positive males (Table 2, 117:8, 26:0, 0:151). However, in one male equal numbers of complementary types appeared, which can be explained by suggesting that the primary spermatogonium with an exchange divided once into two primary spermatogonia which followed the cambial type of division continually so as to produce equal numbers of complementary classes (Table 2, 57:63). Another possibility is that following anaphase separation of one of the kinds described by STERN (1936) both the crossovers passed to one daughter primary spermatogonium which continued to produce equal numbers of reciprocal types. A continued appearance of clusters has also been reported by PURO (1964), who suggested that this continuation can be used as a genetic criterion for differentiation between definitive and predefinitive spermatogonia. Appearance of a few crossovers in the progeny of some males (Table 2, 5:4, 2:1) might be due to death of the primary spermatogonia after following such divisions for a few generations. Single crossovers, 1:0 etc., can be due to crossing over in secondary spermatogonia.

Individual variation in crossover values is frequently associated with spermatogonial crossing over, so much so that it has become a criterion for detection of the phenomenon (WHITTINGHILL 1955). Such variation is evident in the present work, where crossing over did not occur in a majority of the males, but where it did, there is a great difference from male to male in the number of crossovers produced. This difference ranges from 1 to 125, 2 to 120 and from 2 to 151 in the three experiments. On the other hand, the individual variation is insignificant when crossing over occurs in spermatocytes. KALE (unpublished) treated newly emerged males by 1000r of X rays and mated them to six sets of virgins in successive broods as in the present study. Here the complementary classes from

adult males were equal and the variation in crossover production of treated males was not significant. The data suggested that the observed crossovers were meiotic in origin.

SLIZYNSKA (1963) measured X-ray sensitivity of male germ cells of *D. melanogaster* to chromosome breakage by scoring structural changes in the salivary glands of larvae from irradiated fathers. It was found that the spermatozoa and the spermatids in most or all males are equally sensitive to X rays, while in regard to spermatogonia the males represent a heterogenous population consisting of positive and negative males. Statistical analysis by WOOLF (1963) confirmed this observation. SLIZYNSKA (1963) suggested that these spermatogonia undergoing mitoses at the time of treatment will show high sensitivity because of the noncondensed state of chromosomes at that time. Thus the variation in sensitivity of spermatogonia both between and within individual males is tentatively attributed to the presence of spermatogonial mitoses. Alternatively, the variation between males may be a consequence of the clonal origin of gonial exchanges.

Inequality of complementary crossover classes is one of the criteria used to detect the occurrence of spermatogonial crossing over. Our observations on some of the males extend evidence for such inequality, but the equality observed in a majority of positive males indicates that chances are greater for equal complementary classes in gonial crossing over. However, the difference in the number of males with equal and with unequal complementary classes (equal 8: unequal 4) is not statistically significant.

According to OFTEDAL (1964) low doses are more efficient than higher ones in the induction of mutations in heterogenous populations. It was suggested that the decrease in efficiency with higher dose takes place through the killing of a class of highly sensitive cells which constitute about 10% of the total spermatogonial cell population. Low doses kill fewer of the sensitive cells and therefore lead to higher mutation yields. In *D. ananassae*, we failed to induce crossing over with high doses which might have caused the death of a large number of sensitive spermatogonia. Experiments with low doses may give interesting results both in *D. ananassae* and in *D. melanogaster*.

A comparison of the results of experiments 1 and 4 with *D. melanogaster* and *D. ananassae* respectively (Table 1), shows that at the same dose and stage, in *D. ananassae*, very few progeny could be scored. Also the number of fertile males was much smaller than in *D. melanogaster*. This may be due to the difference in developmental rates of the two species. It has been observed in this laboratory that at $22 \pm 1^\circ\text{C}$, *D. ananassae* develops more slowly than *D. melanogaster*. KIKKAWA (1938) reported a similar observation. Stages irradiated in *D. ananassae* might be too early in development, when compared to those of *D. melanogaster*.

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SUMMARY

Crossing over has been induced by irradiating early developmental stages of *Drosophila*. Great individual variation has been observed in crossover values. The complementary crossovers are very unequal in some of the positive males. Continued appearance of crossovers in the progeny of the positive males throughout the tested period is attributed to the multiplication of primary spermatogonia behaving in an analogous fashion to that of the cambial cells of higher plants.

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