X-IRRADIATION OF THE DEVELOPING MALE GERM CELLS OF *TRIBOLIUM CONFUSUM*¹

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IN radiobiological experiments the two primary variables are the source of radiation and the organism irradiated, and the observed effects will usually be altered when either one or both variables are changed. A comparison of the altered effects leads to a more precise understanding of the events which ensue when radiant energy is absorbed by a living system, and for this reason it is important to have available information obtained from a variety of radiation sources as well as from a number of different organisms. At the present time a considerable portion of the information concerning the genetic effects of radiation has been derived from studies involving the mouse and various species of *Drosophila*, but these organisms are so different from each other that the data obtained may not always lend itself readily to comparison. The flour beetles, *Tribolium confusum* and *castaneum*, on the other hand, might prove to be useful in this respect since in many of their biological attributes, such as size and reproductive characteristics, they are similar to *Drosophila*, whereas in other ways, for example, chromosome number and life span, they are quite different.

There have been, however, only occasional studies made of the effects of radiations on *Tribolium*. About 40 years ago, DAVEY (1919) reported that small doses of X-rays seemed to increase slightly the life span of *T. confusum*. More recently CORK (1957) measured the changes in the longevity of *T. confusum* in response to different doses of gamma radiation, ROGERS and HILCHEY (1957) studied some of the responses of *Tribolium* to beta radiation, and PARK, DEBRUYN, and BOND (1958) compared the effects of X-rays on the productivity and longevity of males and females of *T. confusum* and *castaneum*. DUCOFF and WALBURG (1960) have attempted to evaluate the effectiveness of X-rays as a means for the production of phenocopies, and the depression of fertility in *T. confusum* males following exposure to fast neutrons was observed by McDONALD and LONG (1961).

Detailed studies of the differential radiosensitivity of male germ cells, such as have been made with *Drosophila* and mice (for example, see ALEXANDER, BERGENDAHL and BRITTAIN 1959; and BATEMAN 1958) have not yet been undertaken with *Tribolium*. The following report is an analysis of this effect of X-rays on *T. confusum* and an attempt to compare this response of the flour beetle with the same response in *Drosophila*.

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METHODS AND RESULTS

The strain of *T. confusum* used here originated from several prefertilized females captured in 1955. To analyze the effects of radiation on male fertility, the "brood pattern" technique was employed, whereby the treated male is mated sequentially with a number of virgin females. The percent of inviable eggs produced by the females is a reflection of the fertility of the irradiated male. This method, however, requires preliminary information concerning the onset of sexual maturity in males, for the fertility of a young male is likely to be a function of age as well as exposure to radiation. Data bearing on this problem are presented in Table 1. Adult males whose ages from the time of eclosion ranged from less than one to nine days were each placed in vials with a virgin female about nine days of age. Each vial contained five grams of the standard flour-yeast medium used for *Tribolium*. After 24 hours, the male was removed, but the female remained in the vial for another three days and was then transferred to a second vial for an additional four days. Six weeks later the number of offspring in each vial was counted. Unless otherwise stated, all experiments were conducted at a temperature of 30°C and a relative humidity of 50–70 percent.

Obviously, under these conditions, males do not attain complete sexual maturity until about three days after eclosion. Only one female was fertilized in group B, and in group C where the males were between two and three days of age when put into the vial and three to four days old when removed, only half the females were productive. Variation in the precociousness of males is suggested by the fact that two females in group D and one in group E were unproductive too. Apparently, most males are mature by the fifth day of their adult life, for all females in groups F and G were productive. A further observation is that the mean number of offspring per fertile female in group C (24.2) is significantly lower ($t = 5.6$, $P < 0.01$) than the mean number (81.9) in group

TABLE 1
The number of offspring produced by females mated with males of different ages

Group	Age of male in days	Vial number	Pair number									
			1	2	3	4	5	6	7	8	9	10
A	<1–2	1	0	0	0	0	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0
B	1–3	1	0	0	0	1	0	0	0	0	0	0
		2	0	0	0	0	0	0	0	0	0	0
C	2–4	1	36	0	0	10	3	32	11	0	0	0
		2	2	0	0	0	1	26	0	0	0	0
D	3–5	1	44	0	54	42	38	32	43	0	37	49
		2	40	0	44	50	46	19	35	0	39	43
E	4–6	1	41	28	39	39	40	35	0	53	41	41
		2	50	50	40	45	48	26	0	37	18	51
F	5–7	1	48	34	48	47	26	54	45	28	27	26
		2	47	39	38	46	13	61	47	36	35	16
G	6–8	1	32	38	44	31	8	34	49	38	51	41
		2	31	42	22	34	3	38	54	47	41	44

D. This means that a young male, even though able to inseminate a female, still cannot elicit her full reproductive response, but the cause of this has not been determined. As a result of these studies, in the experiments described below, males two to three days old were chosen for irradiation and then, to minimize the possibility of sperm accumulating in the male, were immediately placed with females.

Irradiation was accomplished through the use of a Pickett X-ray machine operated at 180 kv and 20 ma and containing 5 mm of Al added filtration. At a target to sample distance of 30 cm, the dose rate was approximately 145r/minute. Adult males confined in a small circular plastic container were irradiated, some for ten minutes (1450r) and others for 20 minutes (2900r).

Following irradiation, each male was placed in a five gram vial with a single female for two days, then transferred to a second female for another two days and this was repeated until each male had been paired with 12 different females over a period of 24 days. A sample of eggs, usually about 60, was obtained in two collections from each female over the eight days following the introduction of the male. The eggs were incubated in five gram vials and the number of adults obtained from the egg samples was recorded five to six weeks later. A control consisting of ten unirradiated males was handled in the same way except that only ten females were provided for each male. All females used were eight to ten days old. The data from these experiments are provided in Table 2.

In an effort to detect long term effects, five irradiated males (2900r) and five controls were kept for eight weeks following the time of irradiation and then placed individually with a female for four days. The number of adults obtained from eggs collected from these females was then determined. This data can also be found in Table 2.

TABLE 2
The percentages of eggs which failed to reach the adult stage

		Periods												Eight weeks later
		A	B	C	D	E	F	G	H	I	J	K	L	
Control														
Eggs		542	600	540	587	600	600	594	481	600	594	...	...	273
Percent														
inviabile		37	22	25	17	11	19	10	11	16	14	...	...	18
1450r														
Eggs		578	600	540	593	568	463	600	536	600	587	600	540	...
Percent														
inviabile		48	49	43	42	57	70	50	29	24	21	23	29	...
2900r														
Collection														
Eggs	1	258	300	314	263	295	300	300	230	290	261	297	285	267
	2	234	300	270	277	303	270	300	261	275	277	303	270	...
Percent														
inviabile	1	75	74	66	76	88	90	90	68	64	51	52	53	22
	2	84	68	64	72	87	89	88	75	63	47	54	54	...

DISCUSSION

The data in Table 2 and Figure 1 show that the percentage of inviable eggs

FIGURE 1.—A comparison of the radiation damage in the male germ cells of *T. confusum* (solid lines) and *D. virilis* (broken line). The *Drosophila* data was taken from ALEXANDER, BERGENDAHL and BRITAIN (1959).

obtained from females inseminated by irradiated males reaches a maximum value at 11 to 12 days following exposure (period F) and declines gradually to a minimal value at about the 20th day (period J). A similar response is known to occur in other organisms, for example, in *Drosophila*. As SCHMID (1961) has recently pointed out, the phenomenon is by no means adequately understood; but differential sensitivity of cells in the various stages of spermatogenesis as shown by the induction of dominant lethal mutations, and the depletion of sperm supplies as a result of degeneration of damaged germ cells are considered to be of prime importance. Probably the same explanations apply with *Tribolium*, but fecundity measurements of irradiated males will be needed before each component's contribution to inviability can be determined. Furthermore, an additional factor may be involved in high values found for periods A and B. The control also shows higher percentages at first, and this fact coupled with the information concerning the onset of maturity in *T. confusum* makes the data of the first and perhaps second period of doubtful value as indicators of radiation damage. Nevertheless, the effects of the radiation are not entirely obscured even in period A, for the percentages in the control, the 1450r, and the 2900r samples,

are clearly different. However, in view of the above facts, it may be advisable in future experiments to age the males for several days in the presence of females before irradiating them.

Information concerning the temporal sequence of events in spermatogenesis of *T. confusum* is not yet available, so that the various stages of spermatogenesis cannot be assigned with certainty to the different periods shown in Figure 1. However, observations of Feulgen stained serial sections of the testes of one day old males have revealed the presence of all stages of spermatogenesis, including sperm, and since the irradiated males were at least two days of age when treated, it is certain that all the various cell types were exposed. If it is assumed, on the basis of evidence from other organisms, that early spermatids and meiotic cells are most sensitive to radiation, then period F and perhaps E and G include those cells, while periods A through D represent sperm and later spermatids, and H through L represent spermatogonia. Bearing in mind the assumptions involved, an attempt has been made to compare the response of *T. confusum* with that of *D. virilis* when irradiated with 2000r of 200 kv X-rays (*Drosophila* data taken from ALEXANDER, *et al.* 1959). In Figure 1, the highest percentage point of ALEXANDER's data (early spermatids) was made to coincide with the highest point of the *Tribolium* data (period F), and the other points were then plotted at appropriate distances on either side. The impression is gained that the responses of the two organisms are, in general, quite similar. There is some indication that the early spermatid and meiotic stages of *D. virilis* are more sensitive than the comparable stages of *T. confusum*, but definite conclusions must be deferred until more extensive *Tribolium* data become available.

The dosage-response curves plotted in Figure 2 suggest a departure from linearity within the periods H through L. This may be due in part to the high control level caused by the questionable values obtained for periods A, B, and C. However, even if these are eliminated and the control value is reduced as a consequence to about 14 percent, the dosage-response of cells in periods H through L still appear to be nonlinear. The average value for these periods at 1450r is about 11 percentage points over the reduced control, whereas the average value at 2900r exceeds the control by about 44 points. Thus, doubling the dosage approximately quadruples the damage in premeiotic cells. This information suggests a second difference between the response of *Tribolium* and *Drosophila* to X-rays, for ALEXANDER, BERGENDAHL and BRITTAIN (1959) found the production of dominant lethals in the premeiotic cells of *D. virilis* to be a linear function of dose.

It is possible that differential sensitivity is the explanation for the male fertility changes noted in *T. confusum* by PARK, DEBRUYN and BOND (1958). They found that the fertility of males receiving 2000 and 3000r of X-rays declined to a minimum at 12 to 15 days following treatment and then underwent a gradual increase. Since in their experiments the male remained with the same female continuously, and the female is able to store sperm, it would be necessary to assume either that the male inseminated the female only once when first placed with her, or that she was inseminated repeatedly but used each new batch of sperm preferentially. The latter explanation seems more likely, especially since

FIGURE 2.—The response of male germ cells to different doses of X-rays. Periods presumed to represent: mature sperm and late spermatids (●), early spermatids and meiotic cells (○), and premeiotic cells (△).

SCHLAGER (1960) has demonstrated the existence of sperm precedence in *T. castaneum*.

The data in Table 2 have been arranged so that the individual four-day egg collections and adult counts from every period appear separately. This provides an opportunity to explore the possibility that irradiated sperm may recover with time, for sperm fertilizing second collection eggs had been stored by the female during the preceding four days while sperm for first collection eggs were being utilized. Therefore, if sperm are able to recover, a higher percentage of viable eggs might be expected in second collection eggs than in those of the first collection. The evidence available here, however, gives no indication of recovery. Viability is not consistently better in either egg collection and, in fact, when all periods are combined, the proportion of viable and invivable eggs in the two collections are not significantly different ($\chi^2 = 0.28$, $P = 0.60$). The possibility of sperm recovery occurring, however, should not be abandoned until a more thorough investigation is made over a longer period of time. This should prove to be entirely practicable with *Tribolium*, for the female of *T. confusum* can obtain from a single insemination sufficient sperm to fertilize her eggs for more than three months.

Finally, there is no indication in Table 2 of any extended effect of the radiation, such as the sterile period found in mice (BATEMAN 1958), or the prolonged depression of fertility which occurs in *Tribolium* following higher dosages (PARK, DEBRUYN and BOND 1958). Although the percentage of inviable eggs obtained from females inseminated by males eight weeks after irradiation is slightly higher than the control percentage, this difference is not significant ($\chi^2 = 0.975$, $P = 0.32$).

SUMMARY

Young adult males of *T. confusum* have been shown to require about four days, following eclosion, to reach sexual maturity. Subjecting two or three day old males to 1450 or 2900r of X-rays depresses their fertility, as evidenced by the increase in the percentage of inviable eggs produced by their mates. This inviability, probably due in part to the induction of dominant lethals, reaches a maximum value 11 to 12 days following irradiation and then declines. In general, the pattern of response suggests that the various stages of spermatogenesis of *T. confusum* are differentially sensitive to X-rays. No evidence was found for recovery of irradiated sperm stored by females, or of extended effects on the fertility of the treated males.

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