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Radiation-Induced Increases in Fitness in the Flour Beetle Tribolium confusum

Abstract. *Polygenic mutations were induced in an inbred strain of* Tribolium confusum *by exposure to 500 roentgens of gamma radiation. Female progeny of irradiated males bearing induced mutations in the heterozygous state produced significantly more viable offspring than control female progeny of nonirradiated males.*

Wallace (*1*) demonstrated that the genetic fitness of x-irradiated organisms under certain conditions is increased. He found that mutations, induced in the second chromosome of *Drosophila* by an x-ray dose of 500 r, brought about a significant net increase in viability for the bearer in the heterozygous condition. Wallace used a Cy L/Pm balanced lethal stock to facilitate the isolation and maintain the integrity of individual second chromosomes from wild populations of *Drosophila*. He compared the viability of flies homozygous for a specific wild-type second chromosome with that of flies carrying wild-type second chromosomes identical except for mutations induced in one of the homologues. He interpreted this observed net increase in viability as indicating that radiation-induced polygenic mutations, most of which would be deleterious in the homozygous condition, produced sufficient cumulative heterotic effects in the heterozygous condition to more than counterbalance induced, dominant, deleterious mutations (*1–3*). Contrary views and experimental evidence interpreted as a contradiction of Wallace's results have been reported by Falk (*4*) and Muller and Falk (*5*). We have tested the hypothesis of Wallace in a different genetic system, that of *Tribolium confusum* (*6*). Heterozygosis was induced by irradiating males of an inbred strain (Berkeley CF I 1) and crossing them with full-sibling nonirradiated females. Female progeny of this cross, having received an irradiated set of chromosomes from the male parent, would be heterozygous for any mutations induced in the gonial cells at loci homozygous in the inbred strain and transmitted to them.

Irradiated males and their mates were taken as pupae from the 38th generation of a strain perpetuated by crosses between full siblings with a theoretical coefficient of inbreeding (F) far in excess of 0.99. Experimental and control beetles were taken from a series eclosing during a single week. Between 1 and 2 weeks after eclosion, 16 male parents were irradiated in a single dose with 500 r from a Co^{60} source of γ-rays. An equal number of control male parents received identical treatment except for irradiation itself. Immediately after irradiation, irradiated and control males were placed with genetically marked females for a period of 2 weeks to permit mating and consequent exhaustion of irradiated spermatozoa, spermatids, and spermatocytes. Then, the irradiated and control male parents were paired with full-sibling females. Pairs were maintained in individual vials with whole-wheat flour as the medium, and were transferred to fresh medium weekly for 7 weeks. The sex of the progeny of irradiated and nonirradiated males was determined in the pupal stage; the sexes were isolated and allowed to mature for 1 week following eclosion. Before the test cultures were established, beetles were maintained at approximately 29°C and 70 percent relative humidity on a diet of whole-wheat flour supplemented with killed and dried yeast (5 percent), except when being irradiated, transferred, or otherwise handled.

Test cultures were set up in petri dishes (60 mm in diameter by 15 mm deep) in 7 g of medium; each culture consisted of three males (of nonirradiated parents) and three females. The females in experimental cultures were progeny of irradiated males; those in control cultures were progeny of nonirradiated males. In order to maintain identical relationships between mates in experimental and control cultures, males and females within a culture were never siblings but were descendants of the same grandparents—that is, double first cousins. Fifty experimental and fifty control cultures provided the basis for comparison of productivity. Cultures were established as rapidly as young adults became available, with no attempt to have all parental pairs equally represented in cultures. Thus, parental pairs provided progeny for cultures in direct proportion to their own productivity.

Adults of test and control cultures were transferred weekly to petri dishes containing fresh medium. The measure of fitness recorded was total live progeny (adults and pupae) surviving 8 weeks after the parents were removed from a given culture. By this time, and under the experimental conditions employed, all but a very few pupae had completed metamorphosis. Accordingly, the component of fitness measured was compounded of adult fecundity, egg fertility, and progeny viability under conditions which allowed minimum competition and reduced the possibility of cannibalism.

Two points of importance are noted here: (i) test and control cultures were maintained purposefully at suboptimal conditions (25°C and low, variable room humidity), and (ii) the progeny counts that were compared were based on the productivity of cultures during the first 3 weeks.

On the basis of total productivity for the 3-week test period, cultures containing female progeny of irradiated males produced a mean of 135.28 progeny per culture. By comparison, control cultures containing female progeny of nonirradiated males produced a mean of 118.34 progeny per culture.

This amounts to an increase in productivity by progeny of irradiated males of 14.3 percent over control productivity. An analysis of variance test indicated that the observed difference was significant ($F = 5.99$; $p < .02$).

Analyses of weekly counts indicated that the important differences between experimental and control cultures occurred in the number of progeny produced during the 2nd and 3rd weeks (Table 1). Productivity for the 1st week was similar for experimental and control cultures, with the latter producing almost 7 percent more progeny than the experimental cultures. The difference, however, was not significant ($F = .86$; $p > .3$). For the 2nd and 3rd weeks, the female progeny of irradiated males produced, respectively, 22 percent and 23 percent more progeny than the controls. For these two comparisons, the differences are highly significant ($p < .005$ in both cases; $F = 11.41$ and $F = 9.46$, respectively, for week 2 and week 3).

The similarity of the productivity of experimental and control cultures during week 1 is interpreted as indicating that the two groups may increase to maximum productivity (in the 2nd week) at about the same rate. That is to say, experimental and control groups may exhibit similar initial rates of acceleration in productivity of progeny, but controls may attain their maximum daily productivity rate (and cease acceleration) at a lower level (and earlier) than experimental cultures. Differences between the productivity means of the experimentals and the controls during weeks 2 and 3 are largely responsible for the observed differences in total productivity. From statistical analyses of these differences, it is concluded that they reflect real differences in ability to produce viable progeny. The increased productivity of female progeny of irradiated males is interpreted as being due to the effects of induced polygenic mutations in the heterozygous condition, and thus to overdominance. The possibility that such induced increases in productivity may be due to dominance (complete or incomplete), while not precluded by the experimental design employed, is taken as improbable. Such a hypothesis would require that radiation-induced dominant mutations resulting in increased fitness either are more frequent or exhibit higher expressivity than induced dominant deleterious mutations. On theoretical grounds, it seems rather unlikely that such is the case.

Table 1. Mean weekly productivity of live offspring per culture by female progeny of irradiated males (experimental) and female progeny of unirradiated males (controls), 50 replicates in each set.

Mean productivity ± S.E.		
Week 1	Week 2	Week 3
Experimental culture		
31.14 ± 1.69	55.68 ± 2.07	48.46 ± 2.22
Control culture		
33.24 ± 1.52	45.70 ± 2.10	39.38 ± 1.94

A test period of 3 weeks was chosen on the basis of high positive correlations between pair productivity during the first 3 weeks and pair productivity during the subsequent 3 weeks for both irradiated and control cultures in a series of experiments. It was established in earlier studies by Crenshaw and Lerner (*7*) that the productivity rate of inbred strains of *T. confusum* was maximum and more or less constant during the first 6 weeks, and fairly closely correlated with total productivity. The strain employed in the study described here, CF I 1, appears to terminate its productivity period at about 14 weeks after eclosion.

We also examined the possibility that the high productivity of progeny of irradiated males might reflect the effect of a less crowded environment in the larval stages for experimental than for control females. Counts of the progeny produced by irradiated and control males revealed that the irradiated males actually produced more progeny, although not significantly so, than non-irradiated sibling males. Thus, control rather than experimental progeny would be expected to receive any benefit to be derived from the slight difference in crowding between the two groups. Care was taken to rear all animals under relatively uncrowded conditions.

In the experiment described here, the beetles were kept under conditions (25°C and low, variable relative humidity) that are different from and result in lower productivity of progeny than the conditions (29°C and 70 percent relative humidity) under which stocks are maintained. In an earlier experiment in which the beetles were kept under suboptimal conditions, unusually high (but not significant) differences were found between the productivity of progeny of irradiated males and the (lower) productivity of controls. In a series of experiments conducted over the past 3 years under standard conditions, the progeny of irradiated males have always shown higher "face-value" productivity (not significantly so in any single experiment) than progeny of control males. However, the difference between the experimental and control animals has never been as great relatively as in the experiments carried out under suboptimal conditions, both in the earlier study and that described here. It seems reasonable to infer that induced polygenic overdominance may be of even greater fitness value to the bearer under conditions of stress than under normal conditions. These results support the conclusion that induced heterozygosity may increase fitness, and suggest that the overdominance induced may be of especial value in providing the bearer with genetic flexibility that permits greater adaptive response to changed environmental conditions.

The relative magnitude of the difference between the performance of experimental cultures and that of controls reported here far exceeds that found by Wallace (*1, 3*) in his studies, and may be accounted for, in part, by the fact that Wallace's experiments were carried out under standard laboratory conditions whereas ours were not. It is also quite possible that the observed differences reflect inherently different responses to radiation by *Drosophila* and *Tribolium*. An additional factor that may be of importance is that Wallace induced heterozygosity in only one otherwise homozygous chromosome pair while other chromosome pairs in the experimental and control flies were not homozygous. In our investigation, all chromosome pairs were homozygous except for radiation-induced mutations present in an entire genome of the test females.

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