# COMPARATIVE EFFECTIVENESS OF DDT SELECTION METHODS IN *DROSOPHILA MELANOGASTER* MEIGEN

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Insect resistance to a variety of poisons has been known since the early part of the century. Brown (1957, 1958) has summarized the widespread resistance of various insects to the newer synthetic insecticides. This study bears on two questions concerning evolution of resistance to DDT by *Drosophila melanogaster* Meigen. It is of importance (1) to know whether a mixed population of flies known to contain genes (from several sources) conferring resistance to DDT, would show different rates of gain and level of resistance under different methods of selection applied under similar culture conditions, and (2) to determine the maximum degree of resistance obtainable by selection of such a population.

## Methods and Materials

Three strains of DDT-resistant *Drosophila melanogaster* were taken from stock culture in October, 1959 and mixed to form one heterogeneous stock. The three lines, HL2-top (Bennett, 1960), Brown eye-R (Crow, 1954), and ORS-1001 (King, 1957), had been cultured and tested earlier by Bennett (1960, and unpublished data). The mixed stock was then divided among three population cages.

The flies used in this study were raised in 8 dram straight-walled glass shell vials (25 x 95 mm), each of which contained approximately ¾ of an inch of food medium, and polyethylene population cages (Bennett, 1956). The standard food medium consisted of 18 g of agar, 60 cc of sugar, 100 cc of brewer's yeast in 1000 cc of water. For the first five generations, 5 cc of propionic acid was added as a mold inhibitor, for the last 5 generations, 15 cc of 10% Moldex in alcohol was substituted. Culture vials were seeded with dry yeast (Schlitz, brewer's yeast) before use. This study covered a ten month period (October, 1959 to July, 1960).

In the ninth generation tests, a disproportionately large kill occurred when the temperature in the incubator rose from the normal of 25° to 29° C (a known cause of increased mortality, Barker, 1957).

The "holding food" was similar to the above described "regular" food except that the yeast was omitted. The test vials contained filter paper impregnated with DDT crystals deposited from acetone solution. A test set consisted of three such vials with concentrations of 1, 25, 625 $\mu$ g DDT/cm$^2$. Data from each test set covered three concentrations and $LD_{50}$ value were thus established. General testing procedures followed those used by Bennett (1960), and Coomes and Bennett (1960). The $LD_{50}$ value of a particular test set was based on the performance of at least nine and nor-

mally 18 flies. At least three flies per vial were tested and not more than six flies per vial were used.

In the sib-selection line, values were obtained for each sibship based on one test set. These values provided the basis for selection of the sibships to provide parents for the next generation. The values presented here are based on summation of survival values at each concentration in all of the test sets used for a particular line or cage in each generation.

All testing was done on female flies as Crow (1954) has shown that the results obtained are more reproducible than when males are used. The females tested were of varying ages. Those from the progeny of pair matings raised in standard food vials were approximately two days old.

## Methods of Selection

Tests were made on each of the three cages for two consecutive generations to determine a base point of DDT resistance for each cage.

Cages 1 and 2 were designated as control cages and cage 3 as the permanent DDT cage A DDT vial (3,050 $\mu$g DDT/cm$^2$) was attached to this cage throughout the period of study, beginning with test generation one. A new DDT paper was introduced twice, in the fifth and ninth generations. Eight to twelve food vials were attached to each cage. Samples were obtained from each cage at every generation by etherizing the whole cage.

After determining the base point of resistance, a direct selection line was established by taking the top 30 females which survived the highest concentrations of DDT from the sets from cage 3. Males were taken directly from cage 3. Of the 30 females, six were placed in each of five vials along with males. After a two-day period of egg laying, the flies were transferred into another five vials, providing a larger population of flies for testing. Each succeeding generation was established in the same way without going back to cage 3. Thus, female survivors of a given test were used as parents of the next generation and were mated to untested males of the same generation. This provided direct selection for DDT-resistance exhibited by survivors (and male offspring of the previous generation's survivors) that had been raised in standard food vials.

The sib-selection line was established with 60 pair matings in standard food vials from cages 1 and 3. Thirty females from cage 1 were mated to 30 males from cage 3. The reciprocal crosses were also made, insuring a comparable sample from both cages. Thus, each of 60 pairs of flies was allowed to produce one progeny (sibship, family) in one food vial. Of the 60 the 40 largest progenies were tested for DDT tolerance. Nine to 18 females were taken from each progeny and tested. After the results were recorded, the progenies were ranked according to their DDT tolerance, based on the performance of the females tested. The top 20 progenies were broken down into the "top five" and the "next 15". From the top five cultures showing most resistance, 30 pair matings were made, using six pairs from each progeny. The next

15 progenies each contributed two males and two females (30 pair matings) to the next generation. For the first five generations males and females were taken from the same progenies, thus inbreeding by brother-sister mating. In the last five generations, the males and females from each progeny were mated serially. In each case the flies were mated with one pair to each food vial. The result was 60 vials, each with a pair of parents, to produce 60 progenies, 40 of which would be tested the next generation. This indirect selection was repeated in each generation. Individuals of the germ line were never exposed to DDT.

In each generation, 40 test sets were made from the sib-lines raised in vials, 20 test sets from the direct lines raised in vials, 20 test sets from control cage 1, and 20 test sets from cage 3, the permanent DDT cage. Any remaining test sets were used for the testing of control cage 2. Cages 1 and 3 never required 20 test sets, so in each generation a number were used for control cage 2.

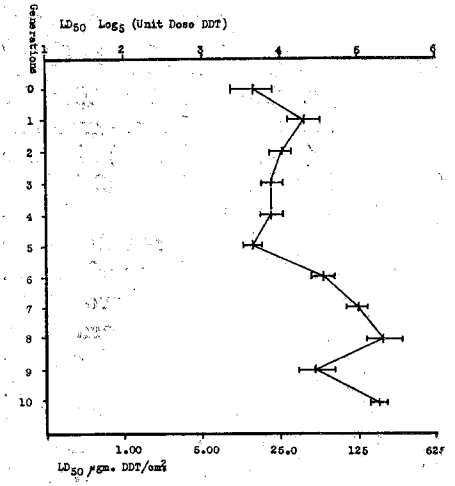


FIG. 1.—Results of testing sib-selection line for DDT tolerance for 18 hour period. Mean and 95% confidence limits indicated.

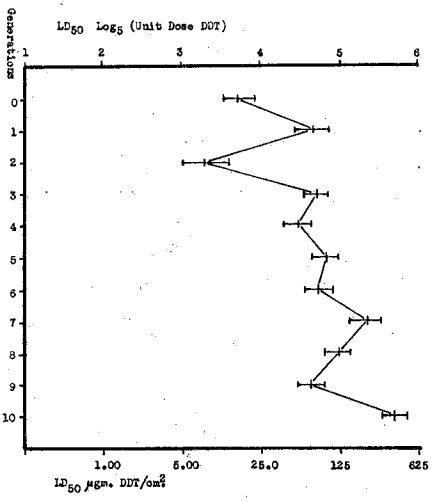


FIG. 2.—Results of testing direct selection line for DDT tolerance for 18 hour period. Mean and 95% confidence limits indicated.

## RESULTS

The principal results of this study are summarized in Figures 1, 2, and 3.

The course of selection through 10 generations of the sib-selection line is shown in Figure 1. During the first five generations of selection, brother-sister pairs were used as parents. This inbreeding prevented any net gain in resistance. Starting with the parents of generation 6, random mating was instituted. All of the observed gain of resistance in this line occurred following this change of mating system.

The direct selection results are shown in Figure 2. No change of mating system occurred in this line

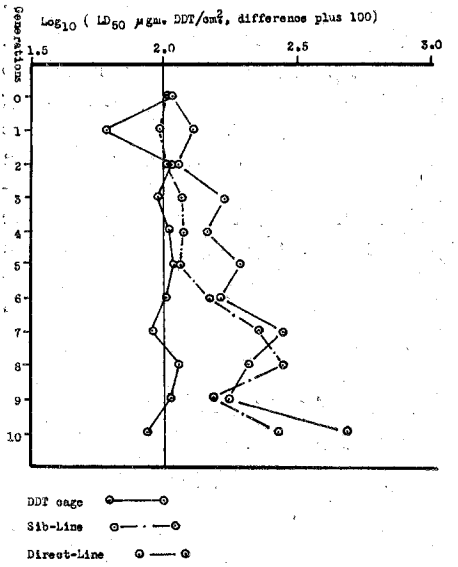


FIG. 3.—Results of the difference (plus 100) between the values (In $\mu$ g DDT/cm²) of the control cages and the experimental lines tested for DDT tolerance for 18 hour period.

and, despite gross fluctuations, the gain was more evenly distributed through the selection period.

Figure 3 presents the material in different form, and with the results from cage 3 for comparison. The base line in Figure 3 was established by taking the combined test results of the two control (unselected) population cages (1 and 2), subtracting that value for each generation from the values of the other indicated lines, adding 100 to eliminate negatives, and expressing the results as logarithms to the base 10.

## DISCUSSION

The second objective of this study, to produce a selected line of *Drosophila melanogaster* more resistant than any previously tested, was clearly achieved. The $LD_{50}$ of the direct selection line in the 10th generation was 5.70 ($\log_5$ unit dose DDT; 385.6 $\mu$ g DDT/cm²) and of the sib-selection line, 4.81 ($\log_5$ unit dose DDT; 189.92 $\mu$ g DDT/cm²). In a comparative study of resistant lines from Japan and two laboratories in the U.S. (Bennett, 1960) the two most resistant stocks showed values of 4.50 and 5.49 ($\log_5$ unit dose DDT; 55.9 and 276.8 $\mu$ g DDT/cm²). Thus the most resistant line in this study was nearly 40% more resistant than any previously reported line. The three DDT resistant lines that were the progenitors of the starting population in this study had the following $LD_{50}$'s when tested in 1957 (Bennett, 1960): ORS 1001, 5.49 (276.8 $\mu$ g DDT/cm²); Brown-eye-R, 3.68 (14.99 $\mu$ g DDT/cm²); HL2-Top, 3.35 ($\log_5$ unit dose DDT; 67.8 $\mu$ g DDT/cm²). The values achieved by direct selection surpassed the highest value shown by these lines in the past. This is interpreted as indicating that some degree of integration of the resistance factors of the parental lines had been achieved, combining separate resistance mechanisms for a superior total resistance.

Comparative tests using the mosquito test kits provided by the World Health Organization showed that the least resistant parental line used here required several times the DDT exposure recommended for resistant mosquitoes to achieve a significant kill. Thus it is apparent that *Drosophila melanogaster* has achieved much higher tolerance to this insecticide than have mosquitoes (Coomes and Bennett, 1960).

The primary objective of this paper has been only conditionally satisfied. The final degree of resistance exhibited by the direct selection line was considerably higher than that of the sib-selection line. The increase in DDT resistance over that of the starting population was 23-fold for the direct-selection line and 13-fold for the sib-selection line. However, the inbreeding in the first five generations of selection in the sib line prevented any net increase in resistance, so the 13-fold gain was attained in the final 5 generations. This compares with a 5.5-fold increase for the direct line in the first five generations and a 4-fold increase in the final five generations. Thus one could argue that sib-selection had demonstrated greater rate of gain during the final five generations than direct-selection produced in either 5 generation period.

The evidence thus does not provide a delineation of the relative effectiveness of the two selective methods. It is clear that both methods can be highly effective under the conditions used.

Figure 3 shows that the population in the DDT Cage did not gain in resistance during this study. This population was highly resistant at the start and it appears that the rate of kill (observed to be very low) produced by the DDT lined vial in the cage was so low as to provide no effective degree of selection. This is of interest because Bennett (1960) had attempted earlier to compare effectiveness of sib-selection in pair matings in vials with direct-selection in population cages (of a different design than those used here). The comparison did not seem a good one at the time, but was used as the only one available in the data at hand. In this study we have been able to make a partial comparison using flies raised in vials on the same batches of food. We know that a difference in population density existed in the culture vials of the direct and sib-selection lines. In future work the differences of population density and of mating pattern will have to be dealt with.

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## Summary

A heterogeneous population of *Drosophila melanogaster* was produced by mixing three DDT-resistant strains. This hybrid population was divided into sub-populations by culturing them in two control population cages and a third population cage containing a permanent DDT lined vial. A sib-selection line and a direct-selection line were tested for DDT resistance each generation. Selection was carried on for ten generations. During the first five generations the sib-line was inbred (brother-sister pair matings) whereas the last five generations out-breeding (between families within the line) was used.

At the end of the selection period, the DDT cage population showed a DDT tolerance 0.46 times that of the starting population. The sib-selected

line showed no increase in the first five generations due to inbreeding, but a 13-fold increase in tolerance was attained by five generations of out-breeding and selection. The direct-selection line reached a 23-fold increase in resistance at the end of ten generations of selection. However, in the five generations of effective selection, the sib-selection line increased resistance by more than twice as much as the direct-selection line in a comparable five generations.

The direct-selection line attained a higher degree of tolerance to DDT than any single resistant strain tested earlier. Thus the different resistant genotypes were recombined in a way which yielded a higher degree of resistance than had been attainable by any one selected line.

In this study sib-selection appears to be a more effective method of selecting DDT-tolerant genotypes than the direct-selection method.

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