SELECTION FOR REDUCED CROSSING OVER IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Selection was practiced for reducing crossing over between the third chromosome genes *Sb* and H^2 of *Drosophila melanogaster*; the method employed was to select the repulsion double heterozygotes $Sb+/+H^2$ every generation. Two replicate selection lines were maintained. After 24 generations of selection, Line 1 showed no significant difference from the control, although the regression of recombination value on generation was significant. In generation 20, Line 2 had a significantly lower recombination value than the control, as well as having a highly significant regression coefficient. No chromosome rearrangements were involved in the response. It was concluded that there was substantial genic variability in the frequency of crossing over between *Sb* and H^2 in the base population.

THE existence of genetic variation in the frequency of crossing over is of interest to population geneticists on account of the selection pressure for tighter linkage between interacting polymorphic loci (FISHER 1930). The importance of chromosome rearrangements, especially paracentric inversions, in this respect has long been recognized, but the role of genic modifiers of recombination has not been so well documented. Recent experiments in which artificial selection for modified recombination values was practiced have demonstrated the existence of genic variation in this character (CHINNICI 1971a, b; KIDWELL 1972a, b). The purpose of this report is to present similar evidence, using a new type of selection scheme which is suitable for organisms with no crossing over in one sex.

Let A and B be two dominant markers with homozygous lethal effects, located on the same chromosome. Let the frequency of recombination between A and B be r (for the sex in which recombination occurs). The scheme practiced by us consists of intercrossing $A+/+B$ repulsion heterozygotes together in each generation. It is easily seen that the only recoverable types are $A+/+B$ (nonrecombinants), and $A+/++$ and $+B/++$ (recombinants). The nonrecombinants and recombinants are in the ratio $(1-r)$: r. Only the nonrecombinants are used to set up the next generation, so there is a strong selection pressure against genotypes with high recombination values.

This scheme is related to the "simulated natural selection" experiment of KIDWELL (1972b), where coupling double heterozygotes were used. It involves less labor in breeding and scoring.

MATERIALS AND METHODS

The experiment involved the markers *Sb* and H^2, located at positions 58.2 and 69.5 respectively, on the right arm of chromosome 3 of *Drosophila melanogaster*.

Flies were grown at 25 ± 1° in ⅓-pint milk bottles, with a yeast-agar-sucrose medium containing propionic acid as a mold inhibitor.

Selection for reduction of crossing over between *Sb* and H^2 was carried out using a heterozygous background of X and second chromosomes, which was constructed as follows. About 30 fertilized wild-type females were trapped in the greenhouses of the Nuffield Unit of Medical Genetics, Liverpool in April 1972. These were used to found a laboratory stock *ND*, maintained by mass mating. The heritability of sternopleural bristle number in this stock was 0.44, $t_{48} = 2.63$ ($p < 0.02$). This is in the range of values usually found for this character, so it was concluded that the *ND* stock probably contained a normal level of genetic variability, and would be suitable as a base population. It was found, however, that inversions were present on chromosome 2 in this stock. Since inter-chromosomal effects of inversions on crossing over would have affected the course of selection, the following procedure was adopted to obtain an inversion-free stock. Forty single-female lines were started from the *ND* stock; each was divided into 3 sublines, which were inbred by brother-sister mating for 3 generations. Between 20 and 30 larvae from each line were then examined for the presence of inversion heterozygosity. Nine lines were apparently inversion-free. To check whether any of these were in fact homozygous for an inversion, 20 virgin females were collected from each, and crossed with males from the standard *Oregon-R* wild-type stock. The salivary glands of at least 30 larvae from each cross were examined. We finally obtained 7 lines free of chromosome rearrangements, each descended from a different member of the original set of 40. One was discarded because of low fertility, and replaced with a line from *Oregon-R*.

The marker genes were transferred separately onto the background of X and 2nd chromosomes from each of the six *ND* lines, and from *Oregon-R*, using the breeding program shown in Figure 1. The balancers *Binscy*, *SM5* and *Ubx* ^{130} are standard (Lindsley and Grell 1968); Px^4 is described by Whittle (1969). Seven lines of *Ubx/Sb* and Ubx/H^2 were thus obtained; all the members of each group of seven were then intermated in every possible way, and equal numbers of the F_1 progeny used to found the final stocks of *Ubx/Sb* and Ubx/H^2. These were maintained in mass culture.

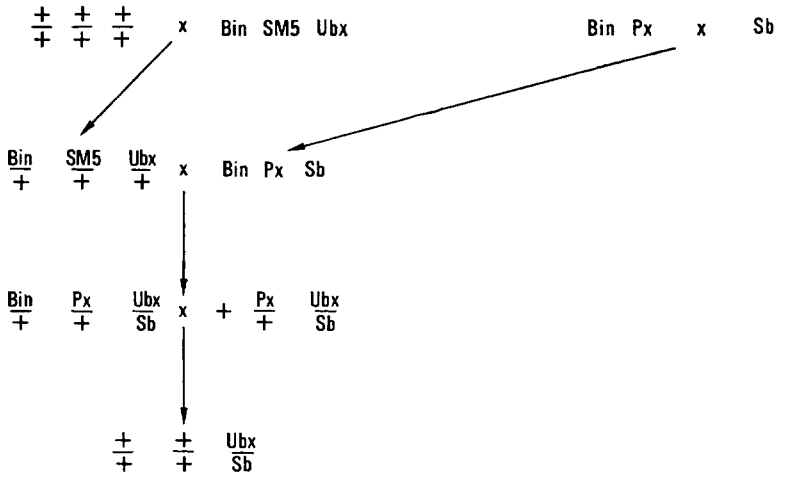

Figure 1.—This shows the breeding program for transferring *Sb* onto the *ND* background (designated by +).

To initiate the selection lines, the *Sb* and H^2 stocks were intercrossed, and the $Sb+/+H^2$ progeny collected. Two replicate lines were maintained. Forty doubly heterozygous individuals of each sex were used to set up each generation of each line. The males were discarded 24 hours after setting up matings, and half the females were transferred to a fresh bottle. The females were allowed to deposit eggs for 4 days and were then discarded. In each line, at least 300 flies were collected every generation in order to estimate the recombination fraction between *Sb* and H^2 reasonably accurately. The flies were collected as virgins over 36 hours. Selection was practiced for 24 generations in Line 1 and for 21 generations in Line 2.

RESULTS

Figures 2 and 3 show the recombination values in Lines 1 and 2 plotted against generation of selection. The regression coefficients and their 95% confidence limits were 0.22 ± 0.17 and 0.28 ± 0.10 for Lines 1 and 2, respectively. The respective regression coefficients were significant at the 2% and 0.1% levels, by the *t*-test. Since measurements of the amount of recombination in the unselected stocks were not carried out every generation, it is conceivable that the changes in the amount of recombination were due to environmental changes. To test this possibility, controlled measurements of the recombination value were carried out. The unselected *Ubx/Sb* and Ubx/H^2 were intercrossed and $Sb+/+H^2$ virgin females crossed with *Oregon-R* males. At the same time (generation 20), $Sb+/+H^2$ females from each of the selected lines were crossed with *Oregon-R*. Twenty bottles of six-pair matings were set up for each of the crosses and the progeny emerging over 3 days were classified. The results are shown in Table 1.

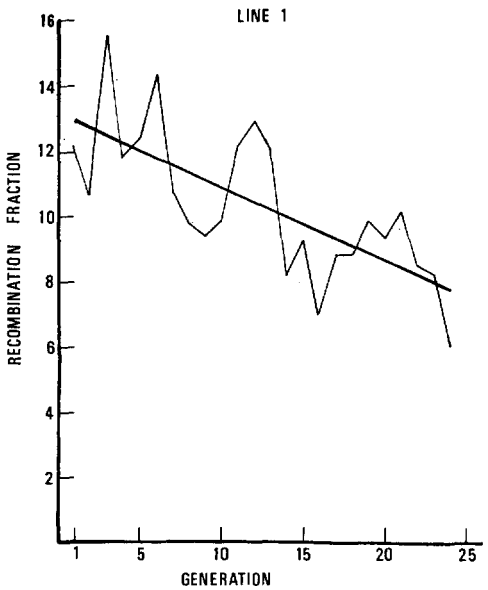

FIGURE 2.—This shows the recombination fraction (per cent) between *Sb* and H^2 in Line 1, plotted against generation of selection, together with the fitted regression line.

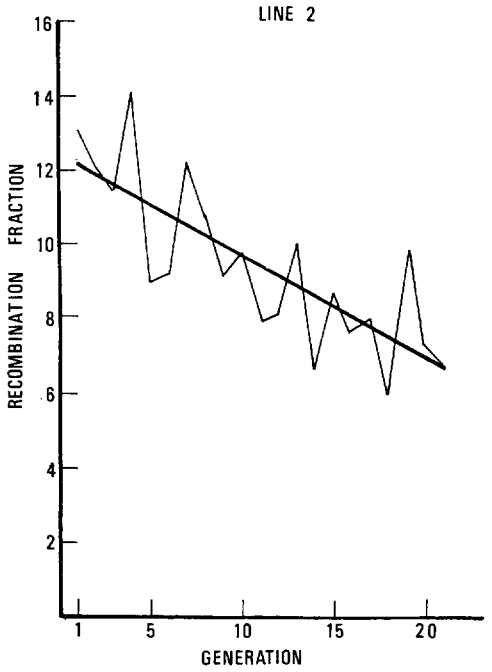

FIGURE 3.—This is the same as Figure 2, except that the response in Line 2 is graphed.

TABLE 1

The results of the controlled measurements of recombination in generation 20

Line no.	Selected lines					Control				
	Sb+	*+H²*	*SbH²*	*++*	*r*(%)	*Sb+*	*+H²*	*SbH²*	*++*	*r*(%)
1	456	468	40	54	9.2	908	1124	110	134	10.7
2	836	793	64	83	8.3	..	..	..	..	..

The amounts of recombination in the control and the selection lines were compared by 2×2 χ^2 tests. Line 1 did not differ significantly from the control, whereas Line 2 did ($p < 0.01$). The fact that the regression for Line 1 was significant suggested that the failure to detect a difference in the χ^2 test might have been due to inadequate numbers. The test was therefore repeated using generation 23 of Line 1. Totals of 1921 and 2087 individuals from Line 1 and the control were counted; the respective recombination values were 8.69% and 9.58%, which do not differ significantly.

There is therefore good evidence that Line 2 responded to selection, leading to a reduction in recombination of about 2.5% compared with the control, at generation 20. The evidence about Line 1 is equivocal. The response to selection must have been due to genic modifiers of recombination; in generations 7 and 19, 50 larvae from each line were examined for chromosome rearrangements: none were found.

DISCUSSION

Our results show that it is possible to reduce the amount of crossing over between two genes as a result of selection for genic modifiers of recombination. This is in agreement with the findings of CHINNICI and of KIDWELL referred to earlier; the rate of response obtained by us is broadly similar to those in their experiments. Since the X and second chromosomes contained most of the genetic variability in these lines, it seems likely that the bulk of our response is due to genes on these chromosomes, but this is a point of secondary importance.

LEWONTIN (1971) showed that, with constant genotypic fitnesses, the optimum value of the amount of recombination between interacting genes in a polymorphic multi-locus system is zero. The existence of inversions, and such devices as the absence of crossing over in the males of many Drosophila species, suggests that many species have gone part of the way toward this state. With the ample genetic variability available for modifying the rate of crossing over, as demonstrated by this and other experiments, one may ask why non-zero cross over values should occur at all. Two answers can be given to this question. Crossing over could be an essential component of meiosis, which cannot be dispensed with entirely. This seems unlikely, in view of the performance of male Drosophila. The second explanation is that there is an intermediate optimum for recombination in a population exposed to temporally varying selection coefficients in multilocus systems (e.g. MATHER 1943). This seems more attractive, but a selection pressure of this sort is likely to be long-term and correspondingly weak.

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