THE EFFECTS OF HETEROLOGOUS REARRANGEMENTS ON INTER-LOCUS AND INTRA-LOCUS RECOMBINATION IN *DROSOPHILA MELANOGASTER*¹

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THE relationship of a recombinational event within a gene and crossing over between linked genes has been explored by many investigators in recent years. Attempts have been made to discover whether a single exchange-mechanism exists to produce both inter-locus and intra-locus recombination. In several instances (for review see FREESE 1957; NELSON 1962), results have been obtained which indicate that separate exchange mechanisms are at work producing the two types of recombination. The work of PRITCHARD (1960), and DORN and BURDICK (1962), however, supports a single exchange-mechanism theory. These investigations were made using diverse genetic organisms and the final solution to this paradox may show that some organisms employ two or more exchange mechanisms while others exhibit only one such recombinational mode.

To date the resolution of the miniature-dusky complex in *Drosophila melanogaster* has, with a few reservations, yielded results which seem best interpreted by assuming that a single mechanism exists both within the complex and between it and other linked genic sites.

This study was conducted to obtain further information concerning the mode of inter- and intra-genic recombination in the region of the X chromosome where the miniature-dusky complex is located. To do this, we have employed the widely known if not well explained interchromosomal crossing over effect exerted by heterologous structural rearrangements carried heterozygously in the genetic background.

MATERIALS AND METHODS

The experimentation was conducted in two phases, one of which permitted measurement of inter-locus crossing over, while the second allowed observation of recombination within the miniature-dusky complex. For the inter-locus study a triple *cis*-phase heterozygote consisting of the following recessive markers was used: vermilion (*v*, BRIDGES and BREHME 1944), dusky^{60k} (*dy*^{60k}, *Drosophila Inform. Serv. (D.I.S.)* 35: 45) and garnet (*g*, BRIDGES and BREHME 1944). These three loci are positioned on the X chromosome at 33.0, 36.2, and 44.4. The intra-locus exchange rates were assayed with a *trans*-phase heterozygote—*v* + *dy*^{60k} +/+ *m*^{61e} + *g*. The two pseudoalleles *m*^{61e} (*D.I.S.* 36: 38) and *dy*^{60k} represent the current leftmost and rightmost elements in the map of the *m-dy* complex. The outside markers *v* and *g* were used in these intra-locus crosses to determine if the wild-type winged progeny arose from reciprocal recombination, nondisjunction or reverse mutation.

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The following six structural rearrangements were introduced into the major autosomes either singly or, in the case of *SM5-TM3*, in combination: *In(3L)D* (Dichaete); *In(2LR)Rvd* (Revolutoid); *Ins (3L + 3R)P* (Payne); *In(2LR)Gla* (Glazed) (BRIDGES and BREHME 1944); Second multiple-5 (*SM5*, D.I.S. 29: 75); and Third multiple-3 (*TM3*, D.I.S. 34: 51).

The introduction of inversions into females in which the separate kinds of recombination were measured was accomplished by the same scheme of matings. Thus, a degree of control over the background other than the marked inversion chromosomes was possible. Figure 1 shows the cross schemes for the inter-locus crosses (A), and for the intra-locus crosses (B). In the inter-locus crosses each F_1 female received one complete maternal set of homologues and a like paternal contribution. In the intra-locus crosses a comparable degree of background uniformity has been obtained.

From the inter-locus study it was possible to clearly distinguish the eight separate progeny categories derived from this three gene arrangement, and so visual classification and counting of each category was employed. However, the intra-locus progeny classes (16 in number) were designed so that only the wild-type winged progeny, clearly distinguishable from the other short winged offspring, were counted to represent one half of the crossovers between the two pseudoalleles. These wild-type individuals were selected by scanning the progeny. Subsequently,

FIGURE 1.—Mating schemes used to synthesize heterozygous females in which inter-locus or intra-locus recombination was measured. Only the chromosome 2 schemes are shown; the chromosome 3 schemes were identical except for the inversion markers followed. $+^a$ indicates the source (stock a) of the unrearranged chromosome. Similarly, $+^b$ and $+^c$ indicate stocks b and c.

they were test-mated to determine their genetic constitution. The remaining flies were collected in ethyl alcohol, dried, and weighted on a Mettler microbalance to estimate their number. The intra-locus recombination rates were determined by doubling the number of wild types recovered and dividing this figure by the estimated total number of progeny. Appropriate standard errors were calculated for the derived crossover rates.

In order to obtain unconfounded data, we mated single 24-hr old females with two of the appropriate males in half-pint glass bottles on a cornmeal-molasses-agar medium. Crosses were maintained in a controlled temperature room at $25 \pm 1^\circ\text{C}$.

RESULTS

The results of both the inter-locus and the intra-locus portions of this experiment are presented in Table 1, where the rearrangements have been listed in order of increasing amount and complexity. Standard errors are shown for each recombination percentage value.

It will be noted that inversions *D* and *Rvd* failed to alter significantly either the inter-locus or the intra-locus exchange rates. The five remaining rearrangement systems produced significant increases in the *v* to *g* region. Each of these, with exception of *Gla*, caused a significant increase in both interstitial segments (*v* to *dy*^{60k} and *dy*^{60k} to *g*). One can see the additive effect upon both types of recombination by observing Items F, G, and H of Table 1. For all three inter-locus regions and the intra-locus regions, the *SM5-TM3* effect is greater than either the *SM5* or the *TM3* effect singly.

The experimental design of the intra-locus phase incorporated a progeny testing device to ascertain the X chromosome make-up of the wild-type winged progeny. Of the 156 recombinants recovered all but one revealed a *v*++ *g/v m*^{61e} *dy*^{60k} *g*, or *v*++ *g/Y* genotype, indicative of a single crossover between the pseudoallelic elements. The remaining female revealed a *v*+++/*v m*^{61e} *dy*^{60k} *g* constitution, which resulted from an intra-locus exchange accompanied by an exchange between *dy*^{60k} and *g*. It is of interest to note that this double-crossover type resulted from a simultaneous crossover in the larger of the two inter-genic regions, which is the more likely multiple exchange type. In addition, this double crossover occurred in the *SM5-TM3* cross where the intra-locus recombination rate was elevated the most.

The pattern of results for the intra-locus phase shows in general an increasing trend from *D* to *SM5-TM3*. There is, however, one noteworthy exception to this trend, the *SM5* intra-locus value. This value indicates a nonsignificant decrease in *m*^{61e} to *dy*^{60k} crossing over.

DISCUSSION

The presence of *In(3L)D* in chromosome 3 neither enhances the crossover frequency "between" genes nor significantly affects the exchange rate within the *m-dy* complex. *Rvd*, although involving a displacement of a greater amount of the background, is also ineffective in enhancing between-gene recombination, but it tends to increase the intra-locus rate. The Payne inversions enhance sig-

TABLE 1
*Comparison of the inter-locus and intra-locus recombination rates in the v-g region
when various rearrangements are present in the autosomes*

Item	Rearrangement	Inter-locus recombination rates				Intra-locus recombination rate		
		Number of progeny	$v-dy^{60k}$	$d_y^{60k} \cdot g$	$v \cdot g$	Estimated Number progeny	$m^{61e} \cdot d_y^{60k}$	
A	Control	14,199	3.085 $\pm$.146	6.593 $\pm$.211	9.677 $\pm$.252	67,508	.0593 $\pm$.0094	
B	<i>In(3L)D</i>	5,475	3.174 $\pm$.239	6.127 $\pm$.328	9.301 $\pm$.400	48,588	.0576 $\pm$.0109	
C	<i>In(2LR)Rcd</i>	5,029	3.091 $\pm$.249	6.161 $\pm$.343	9.252 $\pm$.415	56,514	.0956 $\pm$.0130	
D	<i>Ins(3L+3R)P</i>	4,660	4.056* $\pm$.291	8.369** $\pm$.401	12.425** $\pm$.494	54,730	.0841 $\pm$.0124	
E	<i>In(2LR)Gla</i>	5,706	4.452** $\pm$.275	7.115 $\pm$.345	11.567** $\pm$.432	52,032	.0730 $\pm$.0118	
F	<i>SM5</i>	7,061	4.320 $\pm$.244	8.101** $\pm$.329	12.420** $\pm$.401	45,586	.0395 $\pm$.0093	
G	<i>TM3</i>	10,956	4.582** $\pm$.201	7.667* $\pm$.258	12.249** $\pm$.320	52,559	.0761 $\pm$.0120	
H	<i>SM5-TM3</i>	1,706	5.803** $\pm$.572	10.786** $\pm$.765	16.589** $\pm$.928	38,419	.1145* $\pm$.0174	

* These values differ significantly from control at the .05 level.
** These values differ significantly from control at the .01 level.

nificantly v - dy^{60} - g crossing over and show a tendency to increase within-gene recombination. The same general statement is possible regarding *Gla*.

The multiple-break rearrangements *SM5* and *TM3* show large intergenic enhancements. *TM3* shows a strong but nonsignificant intra-locus rate increase. The combination of *SM5-TM3* has a stronger enhancing effect than either of the rearrangements separately. This dual rearrangement system, which disturbs virtually the entire background, causes the m^{61e} to dy^{60k} region to be enhanced by approximately 80 percent above the control rate.

The response to heterologous rearrangements exhibited by the m - dy complex is qualitatively analogous to the inter-locus response. This is particularly true for the minimal and maximal degrees of background disturbance. The pattern of response produced by intermediate degrees of disturbance is left somewhat less well defined by these data. Our population sizes (approximately 50,000 flies) may not be large enough to discriminate the intermediate responses. The v to g region tends to show a minimal heterologous inversion effect (SCHULTZ and REDFIELD 1951).

If we assume that the presence of structural heterozygosity in the chromosomes produces a force, or physiological condition, which acts upon recombination in other, isosequential, chromosomes, then the likelihood that this "force" will affect two diverse mechanisms of exchange in the same quantitative manner is remote. It is more plausible that comparable results would be obtained when this force is affecting just one exchange mechanism operating to produce inter-locus and intra-locus recombination. To date the strongest evidences for separate mechanisms are the inferences that may be made from interference properties (LEWIS 1963). That is to say, the theory of separate inter-genic and intra-genic mechanisms has been evoked to explain clear cases of negative interference in lower organisms.

However, evidence suggestive of negative interference has not been adduced in the fine structure analysis of the m - dy complex in *D. melanogaster*. In our investigation only one case of multiple exchange was noted.

The data obtained by DORN and BURDICK (1962) for outside marker segregations and crossover frequencies support a single inter-locus and intra-locus exchange mechanism. In addition, the recent confirmation of the production of the reciprocal crossover product within the m^{61e} - m region lends further support to a single classical mode of recombination (SHLESER and BURDICK 1964).

The preponderance of evidence, therefore indicates that a single classical mechanism of crossing over functions in the areas within and adjacent to the m - dy complex on the X chromosome of *D. melanogaster*.

SUMMARY

An experiment was performed to ascertain if the various elements of a pseudo-allelic series respond to heterologous inversions in a manner comparable to linked loci present in the same general area of the X chromosome. The data indicate that inter-locus and intra-locus recombination respond in the same qualitative manner

to these interchromosomal modifiers of crossing over. We conclude that a single recombination mechanism is sufficient to account for both inter-locus and intra-locus recombination in the region of the *m-dy* locus.

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