EFFECTS OF CROSSOVER MODIFYING AGENTS ON RECOMBINATION IN INTRA- AND INTERLOCUS REGIONS IN *DROSOPHILA*^{1,2}

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CROSSOVER studies in *Drosophila* indicate that interference is highly positive within short genetic intervals (SUZUKI, BAILLIE and PARRY 1966). In microorganisms, however, high negative interference is observed in short regions, particularly in studies involving intralocus recombination (PRITCHARD 1955; CHASE and DOERMANN 1958). In order to resolve this contradiction, it might be assumed that the two classes of events, classical interlocus crossing over and intralocus exchange which is associated with negative interference in short regions, occur by different mechanisms (FREESE 1957). On the other hand, PRITCHARD (1960) invokes an hypothesis of short effectively paired regions within which multiple exchanges readily occur. Thus, by a single mechanism, he accounts for the correlation of intralocus recombination with high negative interference as well as classical interlocus crossing over.

If both intra- and interlocus crossing over result from a single mechanism, then treatments affecting crossover frequencies should alter exchange in both types of regions in the same way and to a similar extent. In studies of recombination between position pseudoalleles, LEWIS (1945) and GREEN (1955) found that intragenic crossing over was increased by the interchromosomal effects of inversion heterozygosity and by transfer of the alleles away from the centromere, respectively. Both of these conditions also increase intergenic crossing over. WATSON and BURDICK (1964) found that several different autosomal inversions had similar effects on intra- and interlocus crossing over in the *m-dy* region of the X chromosome in *Drosophila* and concluded that these results supported a single mechanism of crossing over. The present work extends the comparisons of modifications of crossing over in *Drosophila melanogaster* females in the two types of regions by use of several treatments having different physiological effects and known to affect crossing over. The results are consistent with the conclusions of WATSON and BURDICK (1964).

MATERIALS AND METHODS

Crossing over at the tip of the X chromosome was studied between the following markers (followed by their symbols and map positions as listed by BRIDGES and BREHME 1944): apricot—

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w^a 1.5, facet-notchoid— fa^{no} 3.0, split— spl 3.0 and ruby— rb 7.5. The position pseudoalleles, fa^{no} and spl are located within the Notch locus, fa^{no} being 0.03 units to the left of spl (WELSHONS 1958). The interlocus regions w^a - fa^{no} and spl - rb are designated as 1 and 3, respectively; the intralocus region from fa^{no} to spl , as 2. The possibility that intralocus crossover values might differ in *cis* and *trans* arrangements (ALTENBERG and BROWNING 1965) was tested by comparing crossover values in $w^a + + rb/+ fa^{no} spl +$ and $w^a + spl +/+ fa^{no} + rb$ females. In all other experiments, the genotype of the females tested was $w^a + + rb/+ fa^{no} spl +$. In each experiment, 12- to 18-hour old females were testcrossed individually to $w^a fa^{no} spl rb/Y$ males in shell vials. After six days (Brood I), the parents were discarded or transferred to a fresh vial. If transferred, the flies were again transferred after 3 days (Brood II) and discarded after six more days (Brood III). All offspring of each female were recorded separately for each brood. Of the six double crossover (DCO) classes possible, three ($w^a fa^{no} spl rb, + + + +$ and $+ fa^{no} + +$) could be readily recognized at all times; the other three classes were not screened for. Since only 24 DCOs were recovered in 556,325 offspring scored, the failure to detect the other 3 DCO classes will not alter the crossover values obtained. The recovery of DCOs has been discussed briefly (BAILLIE, ASTELL and SCHOLEFIELD 1966).

The following four conditions which are known to affect crossing over were tested: (1) *Autosomal inversions*. Crossing over was determined in Brood I of sibling females heterozygous for different combinations of the autosomal inversions, *SM1* and *Ubx*. (2) *Radiation*. Females were treated with 4000r of gamma-rays from a 6000 Curie Cobalt⁶⁰ therapeutic source. Non-irradiated controls were mated concurrently. Only Broods II and III were scored since PLOUGH (1924) and MAVOR and SVENSON (1924) showed that effects of radiation on crossing over do not appear until six days after exposure. (3) *Heat shock*. Females were placed in an incubator for 20 minutes at $40^\circ \pm 0.5^\circ\text{C}$ and mated after a recovery period of 2 hours at room temperature ($24^\circ \pm 1^\circ\text{C}$). Control females were maintained and mated at room temperature. Crossing over was scored only in Broods II and III. (4) *Mitomycin C*. Females were injected as described previously (SUZUKI 1965a) with mitomycin C (MC) dissolved in a 0.7N saline solution at a concentration of 100 $\mu\text{g/ml}$. Control females were injected with a 0.7N saline solution. Crossing over was scored in all three broods.

RESULTS

Crossover values in each test condition were compared with their respective controls since a chi-square analysis indicated that the control data were not homogeneous and therefore cannot be pooled. The data for all of the tests are shown in Table 1. The standard deviations are listed for the values in regions 1 and 3. 95% confidence limits for the values in region 2 were calculated by the method of STEVENS (1942). Crossing over in the fa^{no} - spl region was not different when the alleles were linked in the *cis* or *trans* positions (lines 1 and 2). However, crossover values were significantly higher in the two interlocus regions when w^a and rb were linked in *trans*. It cannot be said whether this does reflect a *cis-trans* difference or genotypic variation in the stocks used to generate the females.

Crossing over in all three regions was increased to a similar extent within each test of the autosomal inversions (lines 3 to 6). *SM1* had a greater effect than *Ubx* and the effect of *SM1* and *Ubx* together was greater than either inversion alone. Radiation (lines 7 to 10) and mitomycin C (lines 15 to 20) on the other hand, decreased crossing over significantly, the most striking effect occurring in Brood III. The recombination value in region 2 of Brood III in the radiation test is reduced but is not significantly different from the control. It would be of interest to determine whether the striking effect of mitomycin C and radiation on crossing

TABLE 1

Crossover values in the inter- and intralocus regions after treatment

Crossover percentages in each region									
Line	Test condition	Brood number	1		2		3		Total progeny
			percent	sd	percent	95% Confidence limits	Percent	sd	
1	<i>cis</i>	I	0.868	0.040	0.034	0.020–0.055	2.982	0.078	49,293
2	<i>trans</i>	I	1.034*	0.054	0.039	0.026–0.055	3.387*	0.070	68,753
3	+ +	I	0.846	0.047	0.029	0.014–0.051	2.953	0.094	38,306
4	<i>SM1</i> +	I	1.852**	0.077	0.071**	0.046–0.107	5.329**	0.131	31,000
5	+ <i>Ubx</i>	I	1.404**	0.067	0.057*	0.035–0.092	4.378**	0.118	31,477
6	<i>SM1 Ubx</i>	I	2.730**	0.105	0.117**	0.081–0.166	7.476**	0.174	24,758
7	Or	II	0.851	0.114	0.030	0.004–0.112	2.940	0.213	6,463
8	4000r	II	0.750	0.042	0.037	0.022–0.060	2.236**	0.072	42,927
9	Or	III	0.911	0.123	0.033	0.004–0.120	3.477	0.240	6,039
10	4000r	III	0.464**	0.035	0.019	0.008–0.039	2.079**	0.075	37,038
11	24°C	II	1.069	0.106	0.063	0.023–0.137	3.301	0.186	9,544
12	40°C	II	0.951	0.048	0.054	0.034–0.081	2.955*	0.085	40,886
13	24°C	III	1.148	0.128	0.000	0.000–0.052	3.515	0.223	7,056
14	40°C	III	1.099	0.059	0.031	0.015–0.057	3.224	0.100	32,042
15	saline	I	0.844	0.067	0.027	0.009–0.062	2.333	0.112	18,731
16	MC†	I	0.679	0.077	0.026	0.005–0.076	2.933**	0.160	11,488
17	saline	II	1.010	0.072	0.030	0.011–0.066	2.868	0.121	19,702
18	MC	II	0.693**	0.047	0.031	0.015–0.058	2.430**	0.088	31,767
19	saline	III	1.193	0.080	0.037	0.012–0.069	3.452	0.135	18,858
20	MC	III	0.768**	0.048	0.009*	0.002–0.026	2.821**	0.092	33,709

* These values differ significantly from control at the 0.05 level.

** These values differ significantly from control at the 0.01 level.

† MC: mitomycin C.

over primarily in mitotically dividing cells is related to their effects on DNA synthesis. Heat shock (lines 11 to 14) did not appear to have any effect on crossing over in any of the regions. This observation corroborates the reports that the X chromosome is refractory to temperature effect (PLOUGH 1917; MAVOR and SVENSON 1924).

DISCUSSION

These experiments were designed to extend the test of WATSON and BURDICK (1964) on the crossover response of inter- and intralocus regions by using more tightly linked markers, by selecting a portion of the X chromosome known to be more sensitive to factors modifying crossing over, by studying recombination in the two types of regions simultaneously in the same females and by using agents which affect crossing over in different ways. As expected, crossover frequencies were altered in different ways by the agents. The increase in interlocus crossing

over in response to autosomal inversions, the decrease after radiation and mitomycin C injection and the absence of effect after heat shock were, in each case, paralleled by an alteration similar in direction and magnitude in the intralocus region. These experiments reinforce the observations of WATSON and BURDICK (1964) and are compatible with the suggestion that crossing over within and between loci occurs by the same mechanism. It should be stressed, however, that these data are not critical proof of this concept and it is possible that two types of crossing over do, in fact, exist and happen to respond similarly to the agents tested.

It is noteworthy that inversions (SCHULTZ and REDFIELD 1951), radiation (MULLER 1925), mitomycin C (SUZUKI 1965b) and heat shock (PLOUGH 1917; GRELL and CHANDLEY 1965) are all recombinogenic in proximal regions of the autosomes yet vary in their effects on crossing over in the distal portion of the X chromosome. This points to the importance of considering a number of different chromosome regions when studies on induced changes in crossing over are carried out.

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SUMMARY

The effects of different agents on crossing over in interlocus (w^a - fa^{no} and spl - rb) and intralocus (fa^{no} - spl) regions were determined in *Drosophila melanogaster* females. Crossing over was not different in the intralocus region when the alleles were in *cis* or *trans* arrangements. Crossing over in both types of regions was increased by the autosomal inversions, *SM1* and *Ubx*, decreased by radiation (4000r) and mitomycin C (100 μ g/ml) and unaffected by temperature shock (20 minutes at 40°C). The data support the suggestion that crossing over within and between loci occurs by the same mechanism.

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