EVIDENCE ON THE BASIS OF THE CENTROMERE EFFECT IN THE LARGE AUTOSOMES OF DROSOPHILA MELANOGASTER[1]

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THE "centromere effect" in *Drosophila melanogaster* refers to the usual pattern of reduced crossing over adjacent to the centromere in normal females (DOBZHANSKY 1930a, 1930b, 1932). It has been shown by BEADLE (1932) that this low frequency of crossing over depends on proximity of the region to the centromere itself. That is, crossing over in a genetic interval can be decreased or increased by translocation to a position nearer to or farther from the centromere.

A recent study (THOMPSON 1963) has shown that the regular absence of crossing over in the small chromosome 4 of *D. melanogaster* is a kind of centromere effect, which somehow depends on the pairing of homologous centromeres. In that study, it was reported that chromosome-4 exchanges could be stimulated by the translocation of one 4 centromere onto a large fragment of chromosome 3. High nondisjunction of 4 indicated a breakdown in centromere pairing, so that distal associations in 4 probably occurred in the absence of centric pairing.

With this preliminary evidence that the centromere effect depends on homologous centromere pairing, it has seemed necessary to establish whether similar manipulations of the centric regions of the larger autosomes also produce quantitatively significant increases in the frequency of centric crossing over. Since the centromere effect was discovered and characterized on the basis of data on chromosomes 2 and 3, the demonstration of a dependence on centromere pairing in these autosomes might lead to an understanding of its actual nature.

METHODS AND RESULTS

Regions adjacent to the centromere in chromosome 3 were marked in normally arranged chromosomes with various combinations of the mutants scarlet (*st*), Wrinkled (*W*), inturned (*in*), radius-incompletus (*ri*), pink-peach (p^p) and curled (*cu*). Descriptions of all mutants are available in BRIDGES and BREHME (1944). Control linkage values were established by testcrossing female progeny from crosses of marker stocks with a Canton-S wild stock whose normal crossover properties have been established. The mutant combinations tested were *st in ri* p^p, *ri* p^p *cu*, and *st W*. A testcross of *st in ri* p^p/W females was also carried out. Crosses were of single day-old females from uncrowded stock cultures made on the standard Pasadena medium (LEWIS 1960) with WAGNER's Y-2 yeast. These females were first mated in vials for one day and transferred to bottles for the subsequent six days. A laboratory temperature of 22° ±1°C was maintained for all phases of the experiment.

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Normal chromosomes with similar combinations were also paired with the translocations T(2;3)B and T(2;3)bw^{V4}, and heterozygous females were tested for crossing over in the 3 centromere region. Conditions of culturing and mating were identical with those employed in the control series. The relative positions of markers and translocation breakpoints are shown in Figure 1. T(2;3)B has a break near the centromere of 3 in the right arm (DOBZHANSKY and STURTEVANT 1931) and carried the mutant Dichaete (*D*) as a nearby but easily separable marker in the left arm. T(2;3)bw^{V4} has a break in the basal heterochromatin of the left arm of chromosome 3 (GLASS 1933) and was marked only by the brown-variegated position effect of the translocation itself. This mutant effect provided a useful marker for the translocation breakpoint and adjacent regions.

Control crossover data from structurally normal chromosomes are summarized in Table 1, with the exclusion of females yielding fewer than 50 offspring. While these data show general agreement with established map values, there are indications of consistently lower frequencies of exchange in all crosses involving *W*. This might be interpreted as evidence for a small chromosomal aberration, previously undetected. In any event, the data are sufficiently heterogeneous to preclude their combination for single control values, and experimental comparisons have been made only with the appropriate control data, with or without *W*.

Data from crosses of females heterozygous for T(2;3)B are summarized in Table 2. The probabilities given in Tables 2 to 4 derive from the chi-square value for experimental and control data by region, in the form of a 2 × 2 test. The Yates correction for discontinuity of chi-square values was applied. Probabilities given are two-tail values, although these tests might properly be considered one-tail tests and the increases more significant than indicated. That is, the aim of the experiments was not merely to test for any differences in frequency of crossing over, but rather to test specifically whether translocation heterozygotes show *more* crossing over in these regions than normal strains.

The results with T(2;3)B are characterized by increases in the frequency of

TABLE 1

Control values of crossing over in the centromere region of chromosome 3

Females	Total crossovers (and percent) by region				Total progeny
		st-in	*in-ri*	*ri-p^p*	*p^p-cu*	
st in ri p^p/Canton-S		286(2.9)	28(0.3)	92(0.9)	.....	9878
ri p^p cu/Canton-S		.....	.....	30(0.6)	88(1.7)	5289
	st-W	*W-in*	*in-ri*	*ri-p^p*	*p^p-cu*	
st W/Canton-S	122(1.4)	.....	.....	.....	.....	8593
W/st in ri p^p	79(1.1)	26(0.4)	15(0.2)	33(0.5)	.....	7124

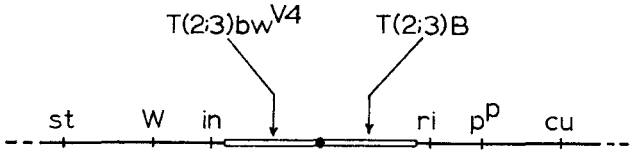

FIGURE 1.—The centromere region of chromosome 3, showing markers and the breakpoints of translocations.

TABLE 2

Comparison of crossover data from heterozygous T(2;3)B females with controls

Females	Total crossovers (and percent) by region				Total progeny
	st-in	*in-ri*	*ri-p*^{p}	*p*^{p}*-cu*	
T(2;3)B/*ri p*^{p} *cu*	.....	.....	27(0.9)	69(2.3)	2937
T(2;3)B/*st in ri p*^{p}	76(3.2)	2(0.1)	33(1.4)	.....	2352
Total percent	3.2	0.1	1.1	2.3	
Control (from Table 1)	2.9	0.3	0.8	1.7	
			P<.05	P<.05	

TABLE 3

*Comparison of crossover data from heterozygous T(2;3)*bw^{V4} *females and controls* (W *not present*)

Females	Total crossovers (and percent) by region				Total progeny
	st-in	*in-bw*^{V4}	*bw*^{V4}*-ri*	*ri-p*^{p}	
T(2;3;)*bw*^{V4}*/st in ri p*^{p}	172(3.6)	19(0.4)	6(0.1)	49(1.0)	4772
Control percent (from Table 1)	2.9 P<.05	0.3(*in-ri*)		0.8	

TABLE 4

*Comparison of crossover data from heterozygous T(2;3)*bw^{V4} *females and controls (where* W *is present)*

Females	Total crossovers (and percent) by region			Total progeny
	st-W	*W-bw*^{V4}	*bw*^{V4}*-ri*	
T(2;3)*bw*^{V4}*/st W*	193(1.7)	158(1.4)	.....	11270
Control percent (from Table 1)	1.3 P<.01	0.6(*W-ri*) P<.001		

crossing over in the *ri-p*^{p} and *p*^{p}*-cu* regions, presumably just distal to the breakpoint of the translocation. The *in-ri* region, in which the breakpoint is probably located, produced very few changes. Crossing over beyond the centromere, in the *st-in* region, is essentially normal.

Crossover data from females heterozygous for T(2;3)*bw*^{V4} are summarized in Table 3 (crosses not involving *W*) and Table 4 (crosses with *W*). It should be noted that in agreement with the findings of EILEEN SUTTON GERSH (DEMEREC, KAUFMANN and SUTTON 1939) the crossover data place the centromere between *in* and *ri*, rather than in the generally accepted position left of *in*. This is clearly indicated by an appreciable number of *st in bw*^{V4} and *bw*^{V4} *ri p*^{p} crossovers among offspring of T(2;3)*bw*^{V4}*/st in ri p*^{p} females. The break of *bw*^{V4} is known to be left of the centromere. Like the data for T(2;3)B, these results indicate increases in crossing over in regions of 3L just distal to the translocation

breakpoint, normal or higher than normal values in intervals beyond the centromere, and decreases only in the immediate vicinity of the centromere.

DISCUSSION

As a general pattern, crossing over in the presence of a heterozygous translocation is decreased markedly in the broken arm, especially in regions adjacent to the breakpoint, and increased slightly in the opposite arm (DOBZHANSKY 1930b; BEADLE 1933). The decreases adjacent to the point of translocation have been interpreted as due to incomplete synapsis, resulting from pairing conflicts (DOBZHANSKY 1931; DOBZHANSKY and STURTEVANT 1931), and might be expected regardless of the position of the break.

DOBZHANSKY (1930b) and BROWN (1940), however, have obtained data suggesting that a translocation with a break near the base of an arm reduces crossing over in the affected arm only slightly, if at all. BROWN was led to the conclusion that "When the break is near the centromere, the frequency of single chiasmata is normal or increased." It was of some consequence for the present study that these earlier data involved the use of *st* and *cu* as the markers nearest the centromere, since these loci are almost at the periphery of the centromere effect in chromosome 3. The use, in the present instance, of mutants as close to the centromere as possible has led to the demonstration that it is precisely in the regions nearest the breakpoint, on the distal side, that the most striking increases are obtained. The earlier data have shown that the increase disappears in more distal regions.

These increases in the frequency of crossing over just distal to a translocation breakpoint, when the break is located very near a centromere, have been substantiated by data of DR. EILEEN SUTTON GERSH (personal communication). Her crossover data were obtained from females heterozygous for the complex rearrangements $N^{264-100}$ *r1* and $N^{264-100}$ *r20*. These rearrangements have in common the detachment of nearly all of 3L from its usual centric association, in one case by translocation with 2L, in the other by a pericentric inversion of most of 3R. An insertion of X-chromosome material including the white locus marks the 3L breakpoint with a white-mottled position effect. With respect to the *st*–centromere region, they constitute arrangements analogous to T(2;3)bw^{V4}.

In all structurally heterozygous combinations, including $N^{264-100}$ *r1*/+, $N^{264-100}$ *r20*/+ and $N^{264-100}$ *r1*/$N^{264-100}$ *r20*, GERSH has found unexpectedly high frequencies of crossing over in the regions just distal to the 3L break. Most striking were the data from the latter cross, which produced 5.0 percent *W-in* crossovers and 2.0 percent *in-*w^m (breakpoint) crossovers (GERSH's data again place the centromere between *in* and *ri*). The combination of $N^{264-100}$ *r1* and $N^{264-100}$ *r20* represents the greatest structural complexity in the 3 centromere region, and it is quite possible that the 3 centromeres did not pair regularly. In this respect, the data are similar to those obtained by the author in his use of T(3;4)86D to produce chromosome-4 exchanges (THOMPSON 1963). That is, an increase in centric crossing over appears to correlate with low probability that the normally

adjacent centromeres have paired. Again, the interpretation might be developed that centromere pairing is responsible for the centromere effect. In the present studies involving T(2;3)B and T(2;3)bw^{V4}, however, there are strong indications that the 3 centromeres are pairing in spite of their involvement with translocations. The evidence for very regular pairing of centromeres rests on the normal or more than normal percentages of crossing over in intervals nearest the centromere in the untranslocated arm. GERSH also observed increases in the w^m(breakpoint)–p^p and w^m(breakpoint)–*Sb* regions when testing linkage with the $N^{264-100}$ *r20* translocation.

A possible argument against the acceptance of these increases in crossing over as genuine, both in the centromere region and intervals distal to the translocation breakpoint, is that crossover tetrads might be strongly selected for. It has been demonstrated that exchanges in translocation arms facilitate normal disjunction (DOBZHANSKY 1933; BROWN 1940). BROWN has shown, however, that the nontranslocated arm disjoins regularly regardless of participation in crossing over and that the selection of exchange products is not likely to bias crossover estimates in that arm. While it has been established that normal disjunction *is* facilitated by crossing over in the translocated arm, this mechanism of selection for crossovers is apparently countered by a lower efficiency of synapsis, due to pairing conflicts. Thus, heterozygous translocations having distal points of breakage invariably cause a considerable net reduction in crossing over in the translocated arm (DOBZHANSKY 1930b; BEADLE 1933), indicating that selection for exchanges is of minor importance.

With a break near the centromere, the pattern for most of the translocated arm is one of slight decrease (DOBZHANSKY 1930b) with normal or slightly increased values nearest the break (BROWN 1940). BROWN (1940) has shown by an extensive correlation of crossing over and nondisjunction that the latter frequencies are real, rather than apparent, high values. She has established that nondisjunction of the broken arm is minimal when the break is centric, and that the selection for crossovers is diminished correspondingly.

If the decreases in crossing over produced by more distal translocation breaks demonstrate an inescapable effect of synaptic conflicts, the present data suggest that reduced pairing of the basal translocation has been dramatically counteracted by removal of the centromere effect to produce a net increase in crossing over in the centric region. Actually, the capacity for exchange of paired centric regions may be of a much greater magnitude than the data indicate, if mixed pairing situations exist.

Another alternative which might explain this pattern of increased crossing over without reference to a specific role of the centromere, and which deserves momentary consideration, is the possibility that the involvement of two chromosomes in these translocations has produced a compensatory increase in chromosome 3 to match decreases in chromosome 2—a kind of interchromosomal effect on crossing over among the elements of the translocation. This pattern can be observed among translocations with one distal and one centric breakpoint (DOBZHANSKY and STURTEVANT 1931; GLASS 1933), but is not found among other

categories. Furthermore, the fact that it is always the member with a centric break that undergoes an increase in crossing over shows clearly that the centromere is involved in some way. While DOBZHANSKY and STURTEVANT (1931) demonstrated that the further inhibition of crossing over in one translocation member (by adding an inversion) leads to an increase in crossing over in the other member, it was clear that their results derived from the removal of pairing conflicts by eliminating synapsis in one pair of homologues, and are not related to centric increases observed with the translocation alone.

With this evidence that the presence of a basal translocation leads to appreciable increases in crossing over in the region just distal to the translocation breakpoint, what structural changes have been critical for the removal of the centromere effect? An earlier interpretation (THOMPSON 1963), made on the basis of chromosome-4 crossovers, was that the centromere effect depends on the pairing of homologous centromeres. It was suggested that in normal females the pairing of centromeres initiates a repulsion of these same centromeres, a repulsion occurring as a rule just before crossing over. Such an effect would leave the immediate centric region unsynapsed (or "desynapsed") and would produce the observable inhibition of adjacent exchanges, an inhibition which would diminish with increasing distance from the centromere.

In the present configurations, however, it appears that the 3 centromeres still pair regularly, although not in the same synaptic plane as the crossover intervals of the translocated arm. It is this last point which may be of greatest importance. When the basal translocation fails to eliminate centromere pairing, as with T(2;3)B and T(2;3)bw^{V4}, the repulsion phenomenon would presumably take place. Because of the change in planes of pairing, however, the movements of the centromeres would have no direct effect on pairing in the translocated arm, and crossing over should be released in that arm as if no centromere effect had ever been initiated (Figure 2). The increases in crossing over would, of course, be restricted to those centric regions in which the centromere effect is normally most marked.

This scheme for the centromere effect and its removal would apply to any rearrangement having a basal break, as long as the rearrangement does not otherwise interfere with pairing in the broken arm. One might predict on this basis

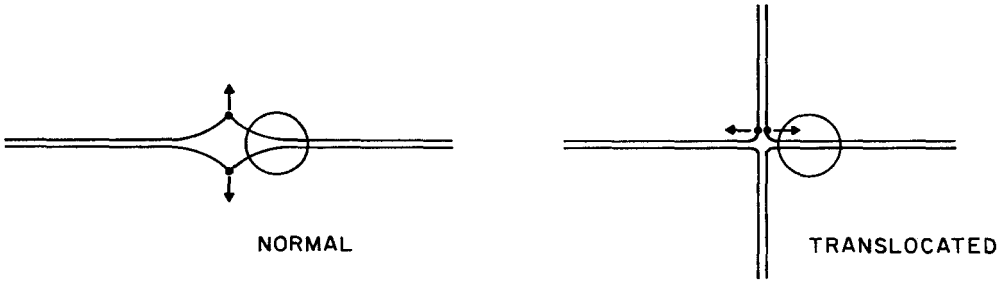

FIGURE 2.—Centromere repulsion in normal females and translocation heterozygotes. Circles designate centric regions tested for crossing over.

that a pericentric inversion having a break near a centromere should characteristically produce increases in crossing over just distal to the basal breakpoint (Figure 3). In addition to the $N^{264-100}$ *r1* data of GERSH previously referred to, this pattern has been reported by ALEXANDER (1952) for females heterozygous for the Plum-2 inversion. With a break just left of the centromere of 2, this pericentric inversion increases crossing over in the *b-pr* interval by about 50 percent. The centromere repulsion hypothesis offers a consistent interpretation for such observations, as well as for the results from translocation-bearing and normal females.

Since the regions most strikingly influenced by the centromere are precisely the same regions where increases in crossing over are detected in triploid females (REDFIELD 1930), in females carrying heterologous rearrangements (the interchromosomal effect, STEINBERG 1936), and in females subjected to temperature extremes (PLOUGH 1917), one is tempted to attribute all these phenomena to a single underlying cause. Under the hypothesis of centromere repulsion triggered by centromere pairing in normal females, the increase in centric exchanges in triploids could be accounted for with the assumption that pairing switchovers, known to be characteristic of triploid females (REDFIELD 1930), occur near the centromere in an appreciable fraction of nuclei (Figure 4). These changes in the

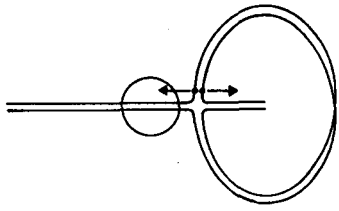

FIGURE 3.—Centromere repulsion in the presence of a pericentric inversion with basal breakpoint.

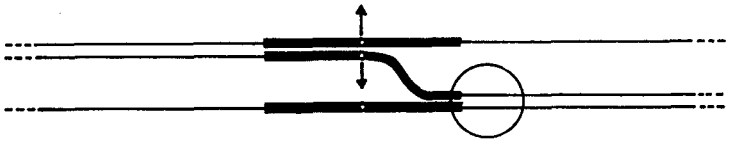

FIGURE 4.—Centric switchover of pairing in a triploid, as a possible basis for increased centric crossing over.

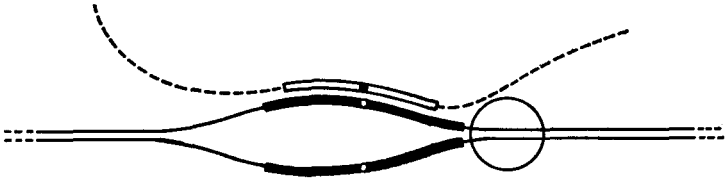

FIGURE 5.—Association of nonhomologous centromeres where the normal pairing of a heterologue (dotted line) has been hindered by a chromosome aberration.

association of homologues would have the same general effect as the presence of a basal translocation in that the two homologues involved in centromere pairing (and repulsion) would not always be the same pair of homologues engaged in pairing in the nearby centric regions. If the centric heterochromatin is a region of somewhat unspecific homologies, as appears to be the case, pairing switchovers might occur there with greater ease than elsewhere.

In essentially the same fashion, the increase in centric crossing over with heterologous rearrangements might be due to an association of nonhomologous centromeres, with adjacent switches to homologous pairing (Figure 5). It has been amply demonstrated that, with various kinds of structural heterozygosity, nonhomologous elements may segregate regularly from one another (e.g. GRELL 1959; GRELL and GRELL 1960; FORBES 1960; FROST 1961). The present model would have the advantage over the ingenious model of OKSALA (1958) of relating the interchromosomal effect to an increased likelihood of nonhomologous pairing. Whether paired nonhomologous centromeres repel or not, the predicted effect would be an increase of the same kind observed in triploid females.

Finally, the increases in centric crossing over observed at extremes of temperature (PLOUGH 1917) are consistent with the expectation that any precise mechanism for the repulsion (as well as for the attraction) of homologous chromosomes or centromeres would be sensitive to the conditions of their aqueous environment (YOS, BADE and JEHLE 1957; ZYRYANOV 1963). Thus, the centromere effect in normal circumstances may depend on an optimum temperature range, with a disruption of repulsion at either high or low temperature. This agrees with the variations observed by PLOUGH. Again, the postulated pairing-repulsion of centromeres offers a unified interpretation for a great body of hitherto puzzling crossover data.

SUMMARY

In the large autosomes of *Drosophila melanogaster*, the centromere effect (an inhibition of centric crossing over in normal females) not only depends on the pairing of homologous centromeres, but also requires that their pairing be in the same plane as the pairing of adjacent centric regions. With translocations or pericentric inversions having a basal break, including some in which the regular pairing of centromeres is strongly indicated, real and quantitatively significant increases in crossing over are observed in the region just distal to the basal breakpoint. These rearrangements typically form pairing configurations in which the centromeres and the region in question are paired in different planes and, presumably, in independent fashion.

On the basis of this evidence, it is suggested that the pairing of homologous centromeres initiates a repulsion of those same centromeres before the time of crossing over. This would account for the centromere's inhibitory effect in normal females and for the release of centric exchanges with translocations and pericentric inversions, since the movements of the centromere in these instances would not affect centric regions paired in another plane. The assumption of a pairing-repulsion of centromeres also provides a simple and straightforward model

for the increases in centric crossing over observed in triploid females and for the interchromosomal effect of heterologous rearrangements.

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