A POSITIVE CORRELATION BETWEEN CROSSING OVER WITHIN HETEROZYGOUS PERICENTRIC INVERSIONS AND REDUCED EGG HATCH OF DROSOPHILA FEMALES[1]

PAUL A. ROBERTS[2]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received January 23, 1967

EXTENSIVE chromosomal polymorphism, probably due to pericentric inversions, has been reported in animals as dissimilar as grasshoppers (WHITE 1958) and mice (OHNO *et al.* 1966). Inversion polymorphism is also prevalent in natural populations of many Drosophila species, but rarely are the inversions pericentric. The infrequency with which pericentric as compared with paracentric inversions are found in natural populations of Drosophila has been attributed to reduced fertility of females heterozygous for inversions that include the centromere. In Drosophila females heterozygous for paracentric inversions (centromere not included in inversion), dicentric bridges and acentric fragments that result from single crossing over within the inversions remain in the polar nuclei of the female and do not lead to reduced fertility (STURTEVANT and BEADLE 1936). There is, however, no known mechanism through which duplicated and deficient chromatids resulting from single and certain types of double crossing over within pericentric inversions can be eliminated without leading to reduced fertility.

Nevertheless, heterozygous pericentric inversions have been reported in natural populations of *Drosophila algonquin* (MILLER 1939), *Drosophila robusta* (CARSON and STALKER 1947), and *Drosophila ananassae* (FRIERE-MAIA 1954). ALEXANDER (1952) tested females heterozygous for two long pericentric inversions of the second chromosome for egg-hatch reduction in order to determine whether appreciable numbers of aneuploid gametes were produced by crossing over within the inversions. She found that egg-hatch reduction was less than 10% for each inversion tested and concluded that heterozygotes for pericentric inversions may not be at so much of a selective disadvantage as had been supposed.

In the present experiments, recombination and egg hatch were compared for females heterozygous either for one of three pericentric inversions or for a paracentric inversion of the metacentric third chromosome of *D. melanogaster*. As genetic theory predicts, only females heterozygous for the two pericentric inversions in which there were appreciable amounts of crossing over had their fertility reduced (by 25% and 50%).

MATERIALS AND METHODS

Figure 1 shows the extent of the inversions tested and the relation of inversion breakpoints to

[1] Research sponsored by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.

[2] Present address: Department of Zoology, Oregon State University, Corvallis, Oregon 97331.

TABLE 1

Recombination and fertility in heterozygotes for pericentric and paracentric inversions of chromosome 3

Inversion	No. of progeny scored	Region 1 *ve-h* No.	%	Region 2 *h-th* No.	%	Region 3 *th-cu* No.	%	Region 4 *cu-bx* No.	%	Region 5 *bx-e* No.	%	Region 6 *e-ro* No.	%	Region 7 *ro-ca* No.	%	Double crossovers within inversion No.	%	Inversion constitution of parents ♀	♂	Hatch of eggs laid by experimental and control females No. of eggs	% hatch*
Control	3818	1040	27.2	708	18.5	167	4.4	340	8.9	594	15.5	881	23.1	439	11.5	..	..	..	..	.....	.....
In(3LR)190	3243	357	11.0	27	0.83	0	0	0	0	27	0.83	554	17.1	467	14.4	0	0.00	*190*/+	+/+	1523	88.0±4.1
																		+/+	*190*/+	1373	88.2±4.6
In(3LR)165	2875	28	0.97	22	0.76	58	2.0	273	9.5	418	14.5	773	26.9	403	14.0	18	0.62	*165*/+	+/+	1990	68.0±4.5
																		+/+	*165*/+	1481	93.0±3.3
In(3LR)269	5330	1454	27.3	900	16.9	115	2.2	92	1.7	73	1.4	56	1.1	9	0.17	161	3.02	*269*/+	+/+	1960	48.9±5.1
																		+/+	*269*/+	1562	91.0±3.5
In(3L)299	1691	0	0.0	2	0.12	34	2.0	237	14.0	250	14.8	415	24.5	209	12.4	2	0.12	*299*/+	+/+	1894	90.6±2.9
																		+/+	*299*/+	1697	86.5±3.9

* With 95% confidence limits.

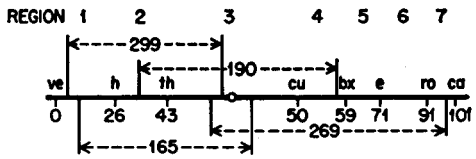


FIGURE 1.—Diagram of the inversions studied and the relation of their breakpoints to markers on chromosome 3.

the markers used. The inversions were recovered in a search for crossover suppressors in female offspring of Canton-S males that had received 4000r of X rays (ROBERTS 1965). For crossover studies, males heterozygous for an inversion and the balancer chromosome, Ubx^{130}, were crossed to females homozygous for the following recessive markers on chromosome 3: veinlet (*ve*, 0.2, longitudinal wing veins interrupted), hairy (*h*, 26.5, extra hairs), thread (*th*, 43.2, aristae thread-like), curled (*cu*, 50.0, wings upcurved), bithorax (*bx*, 58.8, balancers enlarged), ebony (*e*, 70.7, body color black), claret (*ca*, 100.7, eye color). Non-*Ubx* F_1 females were crossed to males of the marker stock, and the progeny were scored for recombination.

Egg-hatchability studies were conducted by crossing males heterozygous for a pericentric or paracentric inversion and the balancer chromosome, Ubx^{130}, to Oregon-R (Ore-R) females. Females carrying the inversion to be tested (*Ubx*+) were crossed to Ore-R/Canton-S hybrid males. The reciprocal cross, Ore-R/Canton-S females × *In(3)*/Ore-R males, served as controls. Females of the desired constitution were mated and allowed to lay eggs on standard Drosophila cornmeal food to which bakers' black paste dye had been added. One day after removal of females from this medium, the numbers of collapsed and uncollapsed eggs were scored.

RESULTS

Table 1 gives the egg hatch and the amount of recombination, by region, of control females and of females heterozygous for the inversions diagrammed in Figure 1. A breakdown of the recombinant types is given in Table 2, and it is from this table that the probable double crossovers within each inversion were selected and entered in Table 1. Because single crossovers within pericentric inversions are duplication-deficient and are not recovered, the recombinants scored as single crossovers in regions 4 and 5 of *In(3LR)269* must have resulted from multiple exchanges, including one inside and one outside the inversion in region 3. Similar considerations apply to the five single crossovers recorded for region 2 of *In(3LR)165* heterozygotes (and to a few of the doubles listed in Table 2), which must have been recovered because of additional undetected exchanges in region 3. These recovered double crossovers, which represent 2- and some 3-strand double exchanges within the inversions, permit an estimate of the amount of recombination occurring within the pericentric inversions.

The inversions studied here are illustrated in Figure 2. *In(3LR)190* includes mostly regions of 3L and 3R adjacent to the centromeric heterochromatin, regions that have a relatively long cytological length in salivary gland chromosomes but a short genetic map length (*th-cu* = 7 crossover units out of 106 total for chromosome 3; *th*, located in section 72 and *cu* probably in section 86 of the salivary map = approximately ⅓ the cytological length of salivary chromosome 3). With the inversion breakpoints in the adjacent regions 2 and 4, little recombination within the inversion was anticipated, and, in fact, no recombinants were recovered

TABLE 2

Recombinant types recovered from inversion heterozygotes

		Control	*In(3LR)269*	*In(3LR)165*	*In(3L)299*	*In(3LR)190*
No crossover	. .	943	2961	1259	723	1939
Single crossover	1	471	1293	10	0	242
	2	283	750	5	0	17
	3	46	28	19	12	0
	4	130	2	157	154	0
	5	271	4	274	185	22
	6	401	0	585	307	497
	7	166	0	248	136	397
Double crossover	1–2	30	128	3	.	2
	1–3	14	5	2	.	.
	1–4	48	.	2	.	.
	1–5	93	2	1	.	2
	1–6	178	1	.	.	47
	1–7	87	3	.	.	64
	2–3	10	.	6	.	.
	2–4	46	.	.	.	.
	2–5	63	.	.	.	1
	2–6	124	2	.	1	4
	2–7	52	1	.	.	3
	3–4	2	21	.	.	.
	3–5	25	26	3	4	.
	3–6	21	18	9	10	.
	3–7	12	.	5	5	.
	4–5	7	24	2	1	.
	4–6	23	18	54	53	.
	4–7	19	3	34	26	.
	5–6	35	5	62	30	2
	5–7	27	.	55	29	.
	6–7	14	.	43	11	3
Triple crossover	123	3	.	.	.	.
	124	6	2	.	.	.
	125	10	1	.	.	.
	126	18	.	.	.	.
	127	8	.	.	.	.
	134	2	3	1	.	.
	135	1	2	1	.	.
	136	6	2	.	.	.
	137	7	.	2	.	.
	145	5	2	1	.	.
	146	13	5	2	.	.
	147	9	2	2	.	.
	156	12	.	.	.	.
	157	9	.	.	.	.
	167	5	.	.	.	.
	234	2	3	1	.	.
	235	2	4	.	.	.
	236	2	1	2	.	.
	237	.	.	2	.	.
	245	.	3	.	.	.

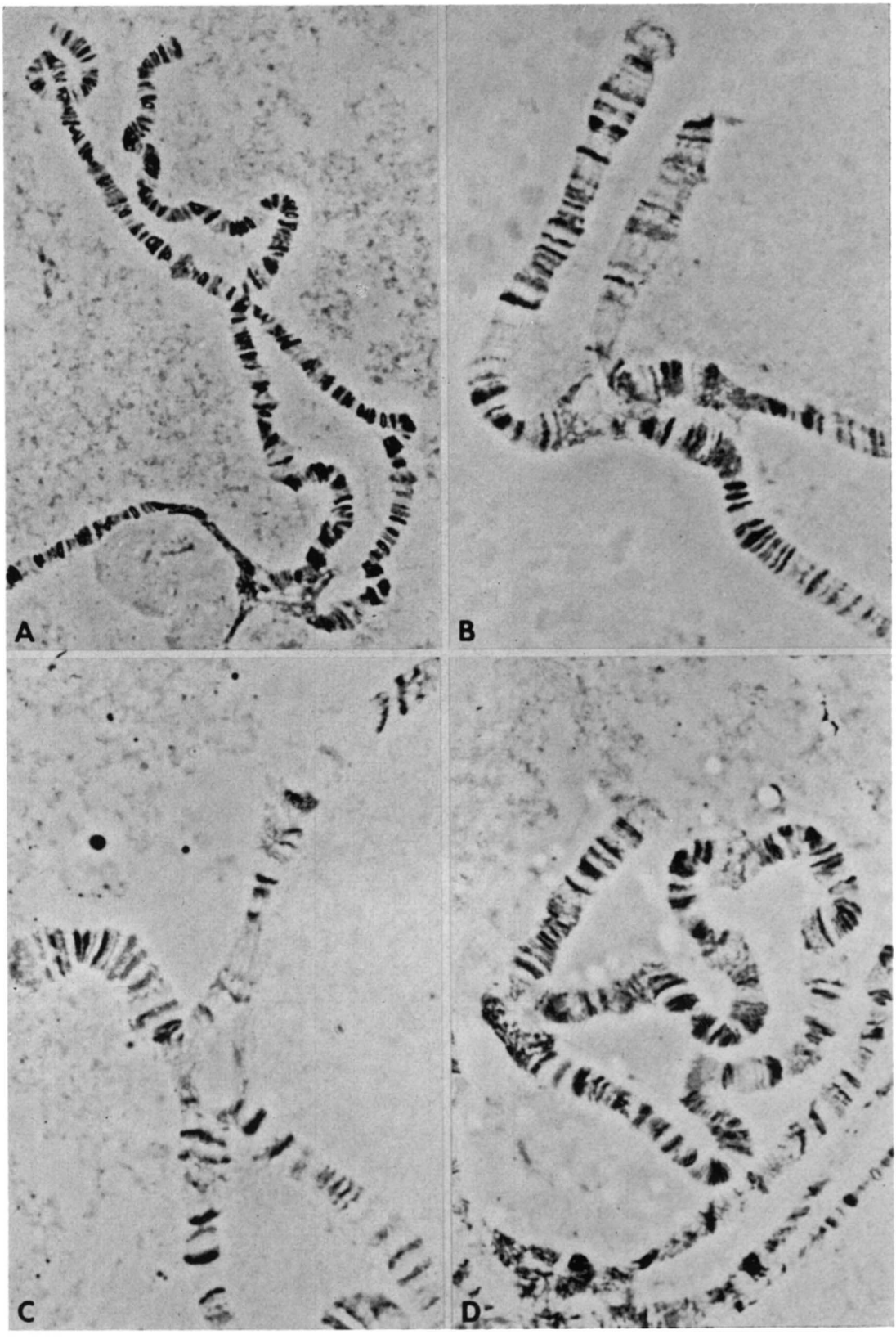
246	14	2	.	.	.
247	8	.	.	.	.
256	10	.	.	.	.
257	11	.	.	.	.
267	.	.	.	.	.
345	.	.	1	.	.
346	1	.	.	1	.
347	.	.	.	.	.
356	1	.	1	.	.
357	4	.	1	.	.
367	.	.	.	1	.
456	1	.	6	.	.
457	1	.	4	1	.
467	.	.	3	.	.
567	.	.	3	.	.
Quadruple crossover types, by region and number (in parentheses)					
	1236	1234	1256	2346	.
	1246	1236	2345	.	.
	1256(2)	1246	2346	.	.
	1257	.	4567	.	.
	1347	.	.	.	.
	1356	.	.	.	.
	1357	.	.	.	.
	1367	.	.	.	.
	1456	.	.	.	.
	2367	.	.	.	.
Total	3818	5330	2875	1691	3243

(Table 1). The percent hatch of eggs laid by females heterozygous for this inversion is the same as that of the control females.

On the other hand, females heterozygous for pericentric inversions that have appreciable amounts of recombination within their boundaries have their fertility markedly reduced. *In(3LR)165*, with 0.62% doubles recovered, lowers fertility by approximately ¼ (68%/93% = 73%) when heterozygous in females but not appreciably when the inversion is contributed to half the zygotes by the male parent (egg hatch is usually about 90% even in hybrids between two strains, and control values for the different inversions studied here are not significantly different from one another). There is more opportunity for recombination within *In(3LR)269* than within *In(3LR)165* because the distal breakpoint of *269* is closer to the tip of 3R than the distal breakpoint of *165* is to the tip of 3L. Almost ten times as many crossovers are, in fact, recovered within *269* as in *165*, and the egg hatch is reduced by another 20%, so that the fertility of females heterozygous for *In(3LR)269* is about half that of the control females (49%/91% = 54%).

DISCUSSION

The most likely explanation for the positive correlation between the amount of recombination occurring within heterozygous pericentric inversions (esti-


A
B
C
D

mated by recoverable double crossovers) and the degree to which egg hatch is lowered is that duplication-deficient chromatids are produced by crossing over within the inversions and enter functional egg nuclei. The duplication and deficiency of large blocks of genes in embryos receiving such single-exchange, or, 4- and (⅔ of the) 3-strand double-exchange chromosomes would be expected to lead to embryonic death and failure of the egg to hatch. Failure of *In(3LR)165* and *269* to reduce egg hatch when transmitted to the zygote by males (in which there is no crossing over) (Table 1) suggests that embryonic lethality results from recombination in inversion heterozygotes rather than from lethal genes associated with the inversions.

The magnitude of the lethal effects of crossing over in *In(3LR)165* and *269* (25 and 50% reductions in egg hatch) contrasts with the effects of the Glazed and Plum^2 inversions of the 2nd chromosome studied by ALEXANDER (1952). Heterozygosity for the Glazed inversion produced less than 10% reduction in egg hatch, but no crossovers within the inversion were recovered. The probable explanation for the lack of recombination within the Glazed inversion is that it had been induced in a second chromosome already carrying *In(2L)Cy* (GRELL 1962). Although the presence of *In(2L)Cy* on the Glazed inversion would make recombination within the pericentric inversion infrequent, thereby accounting for the near normal fertility of heterozygous females, an explanation for the minimal effect of the Plum^2 inversion on fertility is lacking. Females heterozygous for the Plum^2 inversion had only a 10% reduction in fertility despite the recovery of 1% doubles within the inversion (ALEXANDER 1952); *In(3LR)165* reduced fertility of heterozygous females by 20% although fewer doubles within the inversion were recovered (0.65%).

The present findings in *Drosophila melanogaster* are, however, comparable to results obtained by MORGAN (1950) in *Zea mays*. In this plant, heterozygosity for pericentric inversions led to both pollen abortion (20 to 28%) and ovule abortion (12 to 25%). Plants heterozygous for a paracentric inversion had an average pollen abortion of 25% but only 4% ovule abortion; this difference between pollen and ovule abortion was attributed to exclusion of dicentric single-crossover chromatids from functional megaspores (but not from microspores) just as they are excluded from functional egg nuclei of Drosophila oocytes (STURTEVANT and BEADLE 1936). There was no apparent egg lethality resulting from heterozygosity for the autosomal paracentric inversion studied here, *In(3L)299* (Table 1), but only two double crossovers were recovered within the inversion.

Heterozygotes for pericentric inversions should be at a selective disadvantage unless crossing over within the inversion is suppressed, either by the nature of the inversion or by an additional crossover suppressor. Many grasshopper species are polymorphic for rearrangements, probably pericentric inversions, that change the

FIGURE 2.—Polytene configurations of inversion heterozygotes.

A, *In(3LR)190*[*In(3LR)69F;89D*]. B, *In(3LR)165*[*In(3LR)64C;83C*].

C, *In(3LR)269*[*In(3LR)78C;98F*]. D, *In(3L)299*[*In(3L)63C;80*].

position of the centromere. The absence of chiasmata between the centromeres of heterozygotes has been interpreted as evidence of total suppression of crossing over in the inverted regions; consequently, no duplication-deficiency chromatids are produced and the fertility of heterozygotes should be normal (WHITE 1958). In contrast with the behavior of pericentric inversions in grasshoppers, the present data indicate that heterozygosity for pericentric inversions does not prevent crossing over in Drosophila (suggesting that loop formation occurs in oocytes as in the salivary nuclei illustrated in Figure 2) and that crossing over within such inversion heterozygotes leads to reduced fertility.

One would not expect to find polymorphism for pericentric inversions in Drosophila populations unless the inversions include only regions that ordinarily have a low frequency of spontaneous recombination (for example, *In(3LR)190*) or are associated with additional structural heterozygosity. One naturally occurring pericentric inversion (in *D. algonquin*) (MILLER 1939) appears, in fact, to be maintained by association with an arrangement that differs from it by two overlapping paracentric inversions. The pericentric inversions reported in *D. ananassae* (FRIERE-MAIA 1954) are found with low frequency, but CARSON and STALKER (1947) have described a pericentric inversion in *D. robusta* that is found throughout the northern range of the species, and in certain areas with high frequency (e.g. 30%). The position of the breakpoints leads one to suspect that recombination within this inversion is low, but detailed studies have not been reported.

WHARTON (1943) has presented evidence that in the course of evolution in the genus Drosophila acrocentric chromosomes may have been transformed into metacentrics and has pointed out that this might be accomplished most simply through pericentric inversion. Females heterozygous for the two pericentric inversions studied here that alter the position of the centromere [*In(3LR)165* and *In(3LR)269* change V-shaped chromosomes to J-shaped ones] have their fertility reduced by 25 and 50%. Such pericentric inversions should be, at least initially, at a pronounced selective disadvantage in natural populations of Drosophila unless additional crossover suppressors are present.

The assistance of MISS MARY C. STEWART is gratefully acknowledged.

SUMMARY

A positive correlation between the amount of crossing over within pericentric inversions and the degree of egg-hatch reduction of heterozygous females suggests that embryonic death is caused by duplication-deficiency chromosomes formed as a consequence of crossing over within the inversions. Drosophila females heterozygous for pericentric inversions in which there is appreciable recombination should be at a pronounced selective disadvantage.

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