THE SUPPRESSION OF CROSSING OVER IN INVERSION HETEROZYGOTES OF DROSOPHILA PSEUDOOBSCURA

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Observations and experiments on natural and artificial populations of *Drosophila pseudoobscura* have shown that within a given population flies of which the third chromosomes differ in gene arrangement (inversion heterozygotes) often possess higher adaptive values than those of which the homologous pair has the same arrangement in both chromosomes (inversion homozygotes).[1, 2] In many localities the inversion heterozygotes outnumber the homozygotes. The welfare of the populations of these localities, therefore, seems to depend upon the maintenance of a high adaptive level in the inversion heterozygotes relative to the homozygotes. In other localities, however, the populations consist mainly or entirely of inversion homozygotes. In these the adaptive level of the homozygotes must be at least tolerably high.

In theory, two genetic mechanisms could bring about adaptive differences between and among the inversion homozygotes and heterozygotes. First, the action of genes in development may be altered simply by differences in their order within the chromosome (position effect). Second, chromosomes with different gene arrangements may be essentially alike but may carry different complexes of genes which make their possessors physiologically and adaptively different. At the same time they might be co-adapted in such a way as to produce the higher adaptive level of the inversion heterozygotes. Of these two mechanisms the first, position effect, may or may not play a rôle in the evolution of the third chromosome inversions of this fly. However this may be, the second is both effective and important. For if the superior adaptiveness of inversion heterozygotes were due to position effect alone, then each arrangement would be expected to have the same relative superiority regardless of the geographic origin of the chromosomes concerned. This is not the case. The evidence shows that inversion heterozygotes are adaptively superior to the corresponding homozygotes only if the chromosome pair has been drawn from the same or neighboring localities. They are no longer superior when each member of the pair is drawn from distant populations (as for example, from southern California and the central Sierra Nevada). The gene contents of the chromosomes from these populations are not co-adapted to each other in such a way as to produce adaptively superior heterozygotes when combined.[3]

The biological function of inversions in the natural populations of

Drosophila and their principal rôle in the evolution of these insects might be conceived to be the suppression of crossing over between gene complexes which have reached an adaptive equilibrium. Crossing over would destroy these complexes and would result in gene combinations of different adaptive values, but would be prevented by the binding effect of the inversions. Any inversion which is intrinsically neutral, that is, which produces no position effects, would accordingly spread through a population provided that it would guard from disintegration a gene complex well adapted to the habitat concerned. This would be particularly important where the highest adaptive values are found in heterozygotes. The degree to which such gene complexes are maintained by the binding effect of inversions would accordingly depend upon the degree to which crossing over is suppressed. The present study was designed to secure this information.

TABLE 1

RECOMBINATION OBSERVED IN THE CROSS $\frac{or\ Sc\ pr\ cv}{\text{WILD}}$ ♀ × *or Sc pr cv* ♂

		STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
0	wild type	2035	4473	3748	2708
	or pr cv	769	1251	873	558
1	*or*	1781	175	14	3
	pr cv	738	62	1	2
2	*or pr*	357	2	..	..
	cv	425	7	..	..
1,2	*or cv*	260	..	..	..
	pr	311	4	..	..
Total		6676	5974	4636	3271

Three wild strains of *Drosophila pseudoobscura* carrying Standard, Arrowhead and Chiricahua gene arrangements, respectively, and a strain carrying the Tree Line arrangement were employed. The former were originally collected at Piñon Flat, San Jacinto Mountains, California, the latter was collected at Mather, California, in the Sierra Nevada. The inversion heterozygotes of both localities are known to be adaptively superior to the homozygotes. The third chromosomes bearing the gene arrangements designated have been described previously.[4] Flies of each strain were outcrossed to a strain homozygous for the third chromosome recessives *orange* (*or*), *purple* (*pr*), *crossveinless* (*cv*) and the dominant *Scute* (*Sc*). The *or Sc pr cv* chromosome is known to have the Standard arrangement. F_1 females resulting from this cross, were then testcrossed to *or Sc pr cv* males. The progeny counts of the testcrosses are summarized in table 1. The gene *Sc* was disregarded in the counts because its manifestation in heterozygous condition is variable in different strains.[5]

The observed percentages of recombinations are as follows:

INTERVAL	STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
or-pr	46.3	4.0	0.3	0.15
pr-cv	20.3	0.2	0	0

Because the distance in the chromosome between the genes *or* and *pr* is so great that considerable double crossing over must take place in the flies with Standard chromosomes, another experiment was arranged. The four wild type strains were crossed to flies which carried a third chromosome with the recessives *or* and *pr* and the dominants *Blade* (*Bl*) and *Sc*. F_1 females showing *Bl* and *Sc* were selected from the progeny and testcrossed to homozygous *or pr* males. The progeny counts of this testcross are shown in table 2.

TABLE 2

RECOMBINATION OBSERVED IN THE CROSS $\frac{or\ Bl\ Sc\ pr}{\text{WILD}}$ ♀ × *or pr* ♂

		STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
0	wild type	1933	3555	7483	2218
	or Bl Sc pr	1147	1954	3046	725
1	*or*	313	99	27	7
	Bl Sc pr	220	54	21	..
2	*or Bl*	562	17	1	..
	Sc pr	408	9	2	..
3	*or Bl Sc*	892	6	..	..
	pr	922	11	..	..
1, 2	*Bl*	3	..	..	..
	or Sc pr	6	..	..	..
2, 3	*Bl Sc*	81	..	..	..
	or pr	88	1	2	..
2, 3	*Sc*	84	..	..	..
	or Bl pr	105	..	..	..
Total		6764	5706	10582	2950

The following percentages of recombination have been computed from the data in table 2.

INTERVAL	STANDARD	ARROWHEAD	CHIRICAHUA	TREE LINE
or-Bl	10.5	2.7	0.5	0.2
Bl-Sc	17.3	0.5	0.03	0
Sc-pr	32.1	0.3	0.02	0

The standard map distance obtained in our experiments between the loci furthest apart, *or* and *cv*, is 80.2 units, or slightly higher than the figure 68.4 units, obtained by Tan[6] from somewhat less extensive data. It may also be noted that Tan's experiments were conducted at 25°C., ours at ±18°C. The location in the cytologically visible chromosome of the genes used in our experiments can be inferred from the chromosome map published by Tan.[7] As seen in the salivary gland cells, the third chromosome is a ribbon-like structure which for purposes of description has been subdivided into nineteen more or less equally long sections numbered 63 to 81, inclusively. The gene *or* lies in section 65 at a distance from the centromere of about one-tenth the length of the whole chromosomes. The centromere is in section 63. The gene *cv* lies somewhere in sections 79–81, close to the free end of the chromosome. The map distance of 80 units between *or* and *cv* therefore represents most of the genetic length of the third chromosome. Taking into account the fact that some double crossing-over has remained undetected, and also that between one-tenth and one-fifth of the cytological chromosome contains no known genetic markers, the linkage map of this chromosome is probably less than 100 units long.

The total frequency of recombination found in Standard/Arrowhead heterozygotes is about 4.0 per cent, in Standard/Chiricahua 0.5 per cent and in Standard/Tree Line only 0.2 per cent. Arrowhead differs from Standard by a single inversion which includes sections 70–76 or about one-third the length of the chromosome.[4] Tan[7] places the gene *Sc* within, and *pr* outside and distal to the Arrowhead inversion, but his data regarding *pr* are inconclusive. *Bl* and *or* certainly lie between the inversion and the centromere. In Standard/Arrowhead heterozygotes less than one-fiftieth of the recombination normally taking place in the *pr-cv* interval is permitted, and about one-fifteenth of recombination between *or* and *pr*, most of it in the *or-Bl* interval. In other words, an inversion exerts a relatively greater suppressive effect on recombination in the part of the chromosome which lies distal to it (between the inversion and the free end of the chromosome) than it does on recombination between the centromere and the inversion. This agrees with the data of Sturtevant[8] for *Drosophila melanogaster*.

The Chiricahua and Tree Line arrangements differ from the Standard each by a triple inversion which extends from about the middle of section 68 to section 79, inclusive, or about six-tenths of the length of the chromosome as seen in the salivary gland cells.[4] Our data show that recombination in Chiricahua/Standard and Tree Line/Standard is almost wholly suppressed in the whole of the third chromosome. The small amount still permitted, less than six-tenths per cent, is concentrated between the genes *or* and *Bl*, a negligible amount being found elsewhere. Particularly noteworthy is the fact that crossing-over is very strongly suppressed between the centromere and the inversions, the result, probably, of interference by the inversions with meiotic pairing.[9] No recombination whatsoever is detectable in the inverted part of the chromosome. (The three *or pr* flies recorded in table 2 among Standard/Chiricahua and

Standard/Tree Line heterozygotes are most likely the result of contamination; otherwise, they must represent triple crossovers, one between *or* and the inversion and a double to include the locus of *pr;* the individuals concerned were not tested further.)

The amount of crossing over within the inverted parts of the chromosome cannot be determined from our data, since single crossing-over in paracentric inversions does not result in detectable recombination.[10] However, single crossing-over in multiple inversions may result in deficiencies and duplications which produce unviable zygotes. The Standard/Tree Line heterozygotes, for example, have a long paired region which extends from section 68 to 74 (see Fig. 3 in Dobzhansky[4]). Crossing-over in this region would result in chromosomes which would act as lethals in zygotes. Whether such crossing over actually takes place is unknown; in neither case would viable chromosomes carrying gene recombinations normally borne in Standard and in Tree Line (or Chiricahua) appear. The possibility cannot be excluded that in inversion heterozygotes chiasmata may be localized in the immediate vicinity of the centromere, but crossing-over at the centromere would give no recombinations of the genes in Standard, Tree Line and Chiricahua chromosomes.

Summary.—Heterozygosis for inversions found in the third chromosome in natural populations of *Drosophila pseudoobscura* reduces the frequency of recombination of genes located in the chromosome to a small fraction of the normal value. Recombination is strongly prevented not only for genes within the inverted sections, but also for those which lie between the centromere and the inversion, and between the inversion and the free end of the chromosome. Inversion is therefore a powerful means of holding together gene combinations which confer upon their carriers superior adaptive properties.

[1] Dobzhansky, Th., *Evolution,* **1,** 1–16 (1947).

[2] Dobzhansky, Th., and Levene, H. Unpublished data.

[3] Unpublished data.

[4] Dobzhansky, Th., in Dobzhansky and Epling, Carnegie Inst. Washington, Publ. 554, 47–144 (1944).

[5] Helfer, R. G., *Genetics,* **24,** 278–301 (1939).

[6] Tan, C. C., *Ibid.,* **21,** 796–807 (1936).

[7] Tan, C. C., *Z. Zellforsch. u. mikroscop. Anat.,* **26,** 439–461 (1937).

[8] Sturtevant, A. H., Carnegie Inst. Washington, Publ. 421, 1–27 (1931).

[9] Dobzhansky, Th., *Am. Nat.,* **65,** 214–232 (1931).

[10] Sturtevant, A. H., and Beadle, G. W., *Genetics,* **21,** 554–604 (1936).