EQUILIBRIA FOR INVERSIONS INDUCED BY X RAYS IN ISOGENIC STRAINS OF *DROSOPHILA PSEUDOOBSCURA*[1]

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NATURAL populations of many species of Drosophila show chromosomal polymorphisms, maintained presumably by superior fitness of the heterokaryotypes. Because inversions suppress the recombination in structurally heterozygous chromosomes, they may keep together the coadapted gene complexes inside, and in the vicinity of the inverted sections. This view is supported by the fact that in experimental populations frequency equilibria are regularly established between gene arrangements of similar geographic origin, but not between the same gene arrangements if the chromosomes are derived from geographically different populations (DOBZHANSKY 1948; DOBZHANSKY and PAVLOVSKY 1953). On the other hand, the inversions may produce position effects. The breakage points in naturally occurring inversions are not randomly distributed over the chromosomes, and clustering and coincidence of the breakage points in different inversions is not uncommon (BERNSTEIN and GOLDSCHMIDT 1961; KUNZE-MÜHL and SPERLICH 1955). A case is known in *Drosophila subobscura* in which spontaneous and a X-ray induced inversion shared one of their breakage points (SPERLICH 1959). If an inversion has no selective advantage when it appears, its chances of spreading and becoming established in a population are very small. A favorable position effect could, however, maintain a new gene arrangement, and permit it to accumulate a gene complex coadapted with other gene arrangements in the same populations. The experiments described in this report were made in the hope that they may throw light on the problem of the dynamics of inversion polymorphisms in Drosophila populations.

MATERIALS AND METHODS

Strains of *D. pseudoobscura* isogenic for second and third chromosomes derived from the population of Mather, California, were prepared by MR. B. SPASSKY. The isogenic strains selected for the present experiment showed near-normal viabilities, as could be estimated from the segregation ratio in the F_3 generation of the isogenisation cross. Their third chromosomes had ST (Standard) gene orders. For the extraction of the isogenic lines and for the preparation of the experimental populations, a strain containing the marker genes *Ba*/Δ for the second and *L*/Sc for the third chromosome was used (for further details see SPASSKY, DOBZHANSKY and ANDERSON 1965). The possibility of recombination during the process of extraction is remote.

Chromosome II: The marked chromosome "Ba" contains the marker gene Bare (*Ba*), the

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recessives upturned (*up*) and glassy (*gl*), and an inversion which as far as known suppresses recombination almost entirely (less than 1% crossing over between *Ba* and *gl*). Corresponding to the chromosome map of TAN (1937), *up* is located at the locus *O* close to the chromocenter and *gl* at the locus 83.3 not far from the free end. Any possible single crossing over between the inversions and these recessives can be noticed by the appearance of *up* or *gl* phenotypes among the progeny. All the lines used in the present experiment never showed any of these mutants. There remains a region on the free end of the second chromosome (from *gl* to the end) which was not covered by any mutant gene. Here crossing over could not be detected by the technique used. As will be seen later, crossing over in that region was of minor importance for the present experiment.

Chromosome III: The marked chromosome III contained the dominant Lobe (*L*), the recessive orange (*or*) and the inversion complex Santa Cruz. Since all extracted wild chromosomes had the ST-gene order, crossing over was prevented by the complex Santa Cruz inversion (see DOBZHANSKY and STURTEVANT 1938). The remaining region was marked by the recessive *or*. The mutant gene *or* is located close to the chromocenter and no unmarked region remains.

Males of the isogenic strains were exposed either to three X-ray doses of 3000r with intervals of three days between the exposures, or to two doses of 4500r with the same interval. The radiation facilities consisted of dual and opposed X-ray tubes run at 184 kv and 30 ma. The output was 2183r per min.

Males that had received 9000r X rays were mated to females of the constitution *Ba*/+, *L*/+ (*Ba* and *L* mark the second and the third chromosomes). The normal chromosomes (+,+) of the females were of the same origin and isogenic to the male chromosomes. In the offspring $+^{rad}$/*Ba*,$+^{rad}$/*L* males were taken and crossed to +/+,+/+ females of the isogenic strain. In the next generation, the $+^{rad}$/+,$+^{rad}$/+ males were crossed individually to +/+,+/+ isogenic females. A total of 407 crosses were made. After the females had deposited eggs, the flies were transferred into fresh bottles in order to provide replicates. Two larvae in the first bottle were used for the examination of the salivary chromosomes. If any inversions in either the second or the third chromosome were detected, the culture was used for a population cage experiment. By examining only two larvae in each culture some of the induced aberrations were undoubtedly overlooked. Therefore, inversions which were strongly deleterious in heterozygous condition had little chance to be detected. Also, the different aberrations had to pass meiosis twice before detection and those which caused complications during meiosis were probably eliminated.

In several cultures single or complex inversions were found. Those containing inversions in the fourth chromosome were discarded. In 12 cultures inversions were found either in the second or in the third chromosomes (11 cultures) or in both (one culture). From these 12 cultures, each present in two replicas, 12 different population cages were started. The initial population size was at least 150 flies, and it increased very rapidly in the very first generation. The initial frequency of the inverted gene arrangement was expected to be 25%. Food vials were changed on alternate days. Egg samples were taken after 1, 2, 3, 6 and 12 months in all the cages, and larvae were used for the salivary gland studies. All cultures and all populations were kept in a constant temperature room at 25°C.

RESULTS

The irradiated males isogenic for the second and third chromosomes were mated to females containing identical second and third normal chromosomes. In the F_2 generation a total of 407 single-male cultures were prepared. Of these, 88 cultures proved sterile. Among the remaining 319 cultures, 23 different inversions (single or combined in complexes) and 16 translocations were found. The inversion and autosomal translocation break points were distributed as shown in Table 1.

The number of inversion breaks in the third chromosome is rather high in

TABLE 1

*Distribution of the break points in the autosomes**

	Chromosome		
	Second	Third	Fourth
Inversions	8	20	17
Translocations	12	9	8
Total	20	29	25

* Two inversions appearing as a double inversion in the fourth chromosome have only three break points; for the three X-autosome translocations only the autosomal break points are listed.

comparison to the number of second chromosome breakpoints. In the map of the salivary gland chromosomes of *D. pseudoobscura* by TAN (1937) the relative lengths of the chromosomes II:III:IV are 41.5 : 26.5 : 32.5. If one compares the expected and observed distribution of inversion breaks between the three autosomes by means of chi-square, the distribution is not at random (for inversion break points $x^2 = 11.98$, df $= 2$, P $= 0.01$). This may be due to the fact that four of the ten third-chromosome inversions have their breaks only in the euchromatic region (see Table 2). As shown by BAUER (1936) the heterochromatic regions of different chromosomes are different in length. The second chromosome has less heterochromatin than the third chromosome.

The location of the breaks in the second and third chromosomes was determined carefully (Table 2), using for the third chromosome the chromosome map of DOBZHANSKY and STURTEVANT (1938) and for the second chromosome that of TAN (1937). With two exceptions there were only single inversions. Inversion IV was a complex of two overlapping inversions. Inversion V formed in heterozygous condition usually a single loop, often having a connection with the proximal

TABLE 2

Location of the break points in the third and second chromosome inversions

	Inversion No.	Proximal break point	Distal break point	Coincidence with naturally occurring breaks
Third chromosome	I	Chromocenter	80 C	
"	II	65 B	79 B/C	
"	III	70 C/D	74 B	
"	IV a	75 B	79 B/C	
"	IV b	69 B	76 B/C	AR 76 B/C
"	V	Chromocenter	67 C	
"	VI	71 A	77 B	
"	VII	Chromocenter	79/80	Hypothetical 79/80
"	VIII	Chromocenter	73/74	Sequoia I 73/74
Second chromosome	IX	48/49	54	
"	X	43	58	
"	XI	50/51	55	
Second chromosome	XII_{II}	54	56/57	
Third chromosome	XII_{III}	64 C	76 B/C	AR 76 B/C

end of the second chromosome. It is probable that this inversion was combined with a translocation between the heterochromatic regions of the second and third chromosomes. In culture XII there were two different inversions, one in the second chromosome (inversion XII_{II}) and one in the third (inversion XII_{III}).

It can be seen in Table 2 that coincidences between natural and X-ray induced break points are fairly frequent. HELFER (1941) localized 63 different X-ray induced breaks in the third chromosome of *D. pseudoobscura* and compared it with naturally occurring break points. There were one certain and five probable coincidences. In the present experiment no attempt was made to prove the coincidence with certainty and cases of similarity may be included.

The 12 cultures containing X-ray induced inversions were used to start 12 different population cages. The initial frequency of the inverted gene arrangement (Inv) was 25% because the last cross made before the flies were transferred into the population cages was: +/+, +/+ female × +/+^{rad}, +/+^{rad} male. Samples were taken monthly in the beginning. If the inverted gene arrangement decreased rapidly in frequency during the first half year the population cage was discarded. If not, further samples were taken. Unfortunately three of the 12 cages (V,XI and XII) were contaminated with *D. melanogaster*. The data for these cages were of little value after the contamination occurred.

There remain nine population cages which were transferred from New York to Vienna in January, 1965. The flies were kept in bottles for one generation, but afterwards were transferred to population cages. The food used in Vienna was somewhat different from that used in New York and the population size was smaller in Vienna than in New York.

Table 3 gives the gametic frequency of the inverted chromosomes. In the first sample 100 chromosomes, and in all following samples 300 chromosomes from each population were examined.

TABLE 3

Frequency of the inverted chromosomes in the population cages

Cage	July 1964	August 1964	September 1964	December 1964	May 1965
I	9.0	9.7	9.0	6.0	...
II	32.0	10.0	3.7	0.0	...
III	23.0	16.7	19.7	2.0	...
IV	19.0	15.0	14.7	24.7	5.0
V	13.0	11.7	7.0	*	...
VI	20.0	13.3	13.3	8.0	0.3
VII	18.0	11.3	11.0	0.0	...
VIII	16.0	20.7	20.0	29.3	19.0
IX	7.0	12.3	10.7	14.7	14.0
X	13.0	18.3	10.0	4.0	2.0
XI	16.0	19.7	0.0	*	...
XII_{II}	27.0	19.7	15.7	*	...
XII_{III}	29.0	20.7	15.3	*	...

* Time of contamination.

The data in Table 3 indicate that most X-ray induced inversions are being eliminated. However, in populations VIII and IX equilibria seem to be established. Since it is rather easy to distinguish the standard from the inversion homozygotes in the third chromosome, homozygous Inv/Inv individuals were looked for. The same was done with less certainty for the second chromosome. There were homozygotes Inv/Inv in the populations, I,II,XI and XII, but none were found in the other cages. At least for the cages with high inversion frequencies, it is clear that the inversion homozygotes should have been encountered. Among 650 larvae examined in each cage, assuming a frequency of 20% for the new gene arrangements, 26 larvae were expected to be homozygous for the inverted gene arrangement. Their absence means that they were lethal, or at any rate semilethal. Taking this into consideration, the data in Table 3 show that at least in cages VIII and IX the new inversions were heterotic. The data on cage XII are not valid because of the contamination. There remains cage IV, in which the new inversion had a frequency of 24.7% in December 1964, decreasing to 5.0% in May 1965. This might have been caused by environmental changes after this cage was transferred to Vienna.

In Table 4 the frequencies of the inverted chromosomes VIII and IX are compared with the expected frequency of a completely recessive lethal chromosome, under the assumption that there were two generations a month in the cages.

By May 1965, the differences between the expected and the observed frequencies became very clear. At least in two cages equilibria are established owing to the heterosis of the X-ray induced inversion. Assuming that the frequency of inverted chromosomes in the last sample is the equilibrium frequency, the coefficient of selection can be calculated. In the case of lethality of one of the homozygotes s_2 becomes 1 and s_1 for the other homozygote can be found by the formula: $s_1 = \hat{q}/(1 - \hat{q})$, and the variance of the coefficient of selection can be calculated by: $V_{(s)} = (ds/d\hat{q})^2 V_{\hat{q}} = [1/(1 - \hat{q})^2]^2 V_{\hat{q}}$. Taking $\hat{q}$ as 0.190 for cage VIII and 0.140 for cage IX and a total number of 300 chromosomes examined, s becomes 0.235 ± 0.035 and 0.163 ± 0.027 respectively. Both values are highly significant above 0. In the present case it might be more convenient to express the selective advantage of heterozygotes as the superiority of heterozygotes relative to the fit-

TABLE 4

*Comparison of the frequencies of inverted chromosomes in cages VIII and IX with the frequency expected if the chromosome is lethal when homozygous**

Sample	Theoretical	Cage VIII	Cage IX
Initial 1964	25.0	25.0	25.0
July 1964	16.7	16.0	7.0
August 1964	12.5	20.7	12.3
September 1964	10.0	20.0	10.7
December 1964	6.3	29.3	14.7
May 1965	4.5	19.0	14.0

* The expected frequency was calculated by the formula: $q_n = 1/[1/(q_0+n)]$. Since q_0 is 0.25 it can be written: $q_n = 1/(4+n)$.

ness of the isogenic homozygotes. Taking the fitness of the original homozygous karyotype as 1, the advantage of the heterozygotes becomes 1.307 for cage VIII and 1.195 for cage IX.

DISCUSSION

Some of the X-ray induced inversions have been fairly rapidly eliminated by natural selection in the experimental populations. They were evidently deleterious to their carriers, probably both in homozygous and in heterozygous condition (see especially inversion II, Table 3). Other inversions were apparently lethal or semilethal when homozygous, and perhaps close to neutral when heterozygous (inversion X). Most interesting are the two inversions (VIII and IX), which were lethal when homozygous, but in spite of this have established equilibria in the populations. In his as yet unpublished doctoral dissertation VANN (1966) describes his experiments on *Drosophila melanogaster*, the results of which are most interesting to compare with mine on *D. pseudoobscura* (the generous permission of DR. VANN to quote his unpublished results is gratefully acknowledged). Using several highly inbred lines, VANN induced inversions with X rays. Experimental populations were then established to follow the frequency changes of the inversions. On the genetic background of the inbred lines in which they were induced, most inversions were rapidly reduced in frequency or eliminated. In the populations derived from intercrossing different lines, the inversions were frequently retained. Similar results were obtained by CARSON (1960) in irradiated inbred and outbred populations of *D. melanogaster*.

The problem which must now be faced is how to interpret the fact that at least two inversions in my experiments exhibited heterosis on ostensibly homozygous genetic background. (As shown above, recombination during the process of extraction can be neglected for the third chromosome. For the second chromosome, however, it can not be excluded for a region near the tip of the chromosome. This region is not included in inversion IX but is a considerable distance from it. Therefore the heterosis of inversion IX can not be due to an introduced segment.) One possibility is that the inversion heterokaryotypes were here heterozygous for some genes which underwent mutation simultaneously with the appearance of the inversions. The inverted chromosomes differed, then, from the original ones in one or more point mutations in addition to the inversions. Indeed, WALLACE (1958, 1963) has demonstrated in *D. melanogaster*, that the average effect of the radiation-induced mutations is heterotic if they arise on a homozygous genetic background. These mutations are, however, deleterious when homozygous, and neutral or deleterious on heterozygous genetic backgrounds. MUKAI, CHIGUSA and YOSHIKAWA (1964) showed the same to be true of the spontaneous polygenic mutations on homozygous backgrounds in *D. melanogaster*, and CRENSHAW (1965) obtained similar results in an inbred strain of the beetle Tribolium. It may, then, be that the "heterotic" inversions were *per se* neutral or nearly so, but that they carried one or more heterotic gene changes. On the other hand, it is also possible that the inversion of a block of genes in a chromosome may cause a position effect. This position effect may be favorable in single dose, but unfavor-

able or even lethal in double dose. Inversions with favorable position effects may be multiplied by natural selection. The two heterotic inversions in my experiments are far from "ideal" however, since they are lethal in homokaryotypes although heterotic in heterokaryotypes. A third possible explanation is that semilethal or viability- or fertility-reducing mutants may have occurred in the noninverted chromosomes. As a result the heterozygote superiority could be due to a balanced lethal condition. This is rather improbable since the isogenic lines have been kept in mass cultures. The data now available still do not permit consideration of either hypothesis as proven or as ruled out.

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SUMMARY

Strains made isogenic for their second and third chromosomes were irradiated; nine inversions (one of them double) were induced in chromosome III and four in chromosome II. Experimental populations were made carrying the induced inversions, with initial gametic frequencies of 25%. In six of the populations the induced inversions were eliminated, or were close to elimination, within a year; three populations were lost by contamination (including one with inversions both in the second and in the third chromosomes); and in two remaining populations the inversions established balanced equilibria. These two heterotic inversions are the more remarkable since they are lethal, or at least semilethal, in homokaryotypes. Induced heterotic mutants, as well as heterotic position effect, could explain these heterotic inversions.

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