A COMPARISON OF X-RAY INDUCED AND NATURALLY OCCURRING CHROMOSOMAL VARIATIONS IN DROSOPHILA PSEUDOOBSCURA

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INTRODUCTION

THE mechanisms of the origin of chromosomal aberrations remain rather obscure. Two hypotheses have been advanced, namely the so-called "contact" (SEREBROVSKY 1929) and the "breakage first" (STADLER 1932) hypotheses. Without considering them in detail, one may say that the discrimination between them has proven to be very difficult, since most observable phenomena can be accounted for on either basis (CATCHESIDE 1938a, b, MULLER 1938, BAUER, DEMEREC, and KAUFMANN 1938, BAUER 1939, SAX 1940, and others). The discovery that chromosomal aberrations are not only laboratory products but commonly occur in natural populations as well has raised a variety of new problems. Thus in Drosophila, translocations and inversions are very common among induced aberrations, while only inversions are found in natural populations (DOBZHANSKY and STURTEVANT 1938). In *Drosophila pseudoobscura* the third chromosome is, in nature, far more variable than the rest, while in other species several or all chromosomes are about equally variable (*Drosophila melanogaster*, DUBININ and collaborators 1934; *Drosophila azteca*, DOBZHANSKY and SOKOLOFF 1939; *Drosophila algonquin*, MILLER 1939). DOBZHANSKY and STURTEVANT (1938) have raised the questions as to whether or not the chromosomes are equally breakable at any point and whether or not it is necessary to assume that every one of the naturally occurring chromosomal variants (particularly inversions) has arisen from a definite source and arisen only once. The investigation to be reported in the following pages has been started in the hope of securing data that would have a bearing on some of the above problems.

MATERIAL AND METHOD

Males from the "Texas" strain were treated with X-rays and crossed to females from the orange purple strain of Race A of *Drosophila pseudoobscura*. Both strains are known to have the Standard gene arrangement in the third chromosome. The treatment, amounting to 5000 r-units, was administered for sixty minutes from a Westinghouse High Voltage Deep Therapy Tube. The flies were shielded by a 1/16 inch copper plate. From five to seven treated males were mated to from five to eight untreated

TABLE 1

The distribution of the various classes of aberrations observed (raw data).

TWO BREAK CASES

Inversions		Translocations	
Chromosome	Number	Chromosomes	Number
II	10	II–III	15
III	5	II–IV	11
IV	9	II–V	2
XR	5	II–XR	5
XL	1	II–XL	1
		II–Y	3
Duplications		III–IV	3
IV	1	III–V	1
Deletions		III–XR	2
II	1	III–XL	3
III	1	III–Y	2
IV	1	IV–V	1
XR	1	IV–XR	7
		XR–XL	1

THREE BREAK CASES

Translocations		Inversions	
Chromosomes	Number	Chromosomes	Number
II–XL–XR	1	III (tandem)	1
XL–IV–II (branch)	1	IV (mosaic)	1
II–III–IV (intercalation)	1		
Translocations involving transfer of interstitial sections of one chromosome into another		Inversion and translocation	
Section of IV into III	1	Inv. III, Tr. II–III	2
Section of III into Y	1		
Section of II into IV	1		

FOUR BREAK CASES

Translocations		Inversions and translocations	
Chromosomes	Number	Chromosomes	Number
XL–XR–II–V	1	Inv. III, Tr. III–IV	2
III–IV and IV–XR	1	Inv. III, Tr. IV–Y	1
III–IV and III–Y (mosaic)	1	Inv. III, Tr. XR–XL	1
II–III and II–Y	1	Inv. II, Tr. IV–III	1
II–XL and IV–XR	1	Inv. II, Tr. II–XL	1
III–Y and II–IV	1	Inv. II, Tr. II–IV	1
II–III and II–IV	1	Inter- and Intrachromosomal Translocations	
II–IV and II–III	1	Intrachromosomal in XR, Interchromosomal III–XR	1
II–XR and XR–XL	1		
III–II and IV–XL	1		
II–IV and IV–II	1		

FIVE BREAK CASES

Translocations		Inversions and translocations	
Chromosomes	Number	Chromosomes	Number
II–III–Y–II–III	1	Complex Inv. IV, Tr. IV–V	1
III–II–Y–II–V	1	Inv. II, Tr. II–IV, Tr. XR–V	1
		Inv. III, Tr. Section of IV into III	1

TABLE 1—*Continued*

Complex translocation involving exchanges and transfer of interstitial section of one chromosome into another		Inversions and intrachromosomal translocations	
Tr. II–Y, Section IV to Y	1	Inv. II, Intrachromosomal Tr. IV	1
Tr. II–XR, Section IV to XL	1		
Tr. XL–IV, Section XR to XR	1		

SIX BREAK CASES

Complex translocation involving maternal and paternal chromosomes		Complex translocation involving deletion and inversion of chromosomes	
Chromosomes	Number	Chromosome	Number
II–II–II–II and II–IV	1	Del. III, Tr. III–IV, Del. and Inv. II	1

females per culture. In order to insure that the females were impregnated only with sperm which was mature at the time of treatment, the parents were allowed to remain together for three days, after which time the males were removed. The females were transferred to fresh bottles every three days, and the cultures were kept at 17°C.

The larvae of the F_1 generation were dissected from day to day as they matured. The salivary glands were placed in saltcellars with acetocarmine for about four to six minutes. The stained glands were made into permanent slides, using the techniques described by BAUER and BRIDGES (1936). Care was taken to place on each slide only the two glands of a single individual, well separated from each other. In the event one or both glands had become fragmented, each piece was crushed so as to have it occupy a separate area on the slide. These precautions proved to be very important, since they made it possible to establish unequivocally the presence of mosaic aberrations. Observations were made with the aid of a Zeiss oil-immersion objective (× 90) and oculars (× 15).

CHROMOSOME ABERRATIONS

A total of 413 slides, each representing a single F_1 individual, were examined. Among them, 281 slides (138 females and 143 males) were found to contain apparently normal chromosomes. In the remaining 132 slides (74 females and 58 males) chromosomal aberrations were detected. It must be noted that this fraction of the individuals showing aberrations represents only a minimum estimate. Although care was exercised to detect aberrations of any kind, very small changes, such as deficiencies and duplications for single discs or small groups of discs, might have been missed. Likewise translocations between the heterochromatic regions of two or more chromosomes were probably overlooked. The different classes of aberrations found in the modified individuals are shown in table 1.

DISTRIBUTION OF BREAKAGE POINTS AMONG THE CHROMOSOMES

The material obtained may be analyzed in a variety of ways. The first question that arises is whether or not some of the chromosomes are more likely than others to undergo breakage. The total number of induced breaks recorded in all chromosomes is 347. Their distribution in the different chromosomes is shown in table 2. In this table the two limbs of the X chromosome (XR and XL) are treated as separate chromosomes.

TABLE 2

Total number of chromosome breaks observed.

Chromosome		II	III	IV	XR	XL	V	Y
Euchromatic breaks	♀	47	27	40	36	15	6	
	♂	57	37	41			2	0
Total		106	64	81	36	15	8	0
Heterochromatic breaks	♀	1	4	3	4	4	0	
	♂	1	6	0			0	14
Total		2	10	3	4	4	0	14
Grand Total		108	74	84	40	19	8	14

The number of breaks observed in the chromosomes are not alike (108 in II, 74 in III, 84 in IV, 40 and 19 in XR and XL, respectively, eight in V, and 14 in Y). Since, however, the chromosomes of *Drosophila pseudoobscura* are not equal in length, this factor must be taken into account. At metaphase, the three rod-shaped autosomes, II, III and IV, are approximately equal, the dot-shaped chromosome V is at most one-tenth of the length of the rod-shaped ones, and the two equal arms of the X are slightly longer than the rod-shaped autosomes. The Y chromosome in the Texas strain is relatively very short (DOBZHANSKY 1935) being of about the same length as the rod-shaped autosomes. In the salivary gland cells, the relative lengths of the chromosome limbs are decidedly unequal. Here, however, the problem is complicated by the presence of two types of chromatin, namely euchromatin and heterochromatin (PAINTER 1935, BAUER 1936). Since the recent works of KAUFMANN and DEMEREC (1937) and BAUER (1939) have shown that the heterochromatic portions of the chromosomes of *Drosophila melanogaster* may differ in susceptiblity to breakage from the euchromatic portions, it is important to distinguish between the heterochromatic and euchromatic breaks in our material.

No exact measurements of the hetero- and euchromatin in *Drosophila pseudoobscura* have been published. BAUER (1936) has made some rough estimates in the prophase chromosomes of nerve cells. According to him, the second chromosome has only very little heterochromatin near its spindle attachment. The third and fourth chromosomes and the right

limb of the X chromosome have considerably more heterochromatin than the second, while in the left limb of the X at least the proximal one-third of the length is composed of heterochromatin. As to the relative lengths of the euchromatic portions in the salivary gland chromosomes, one may employ the maps published by DOBZHANSKY and TAN (1936) and TAN (1937) as a standard of comparison. In these maps, the ratios of the lengths of the euchromatic portions of the II, XR, IV, III, XL, and V, are approximately 10:10:8:7:5:0.5, respectively.

Table 2 shows the number of breaks observed in the euchromatic regions of the chromosomes. The expected numbers of such breaks are computed on the basis of the relative lengths of the euchromatic portions of these chromosomes. Moreover, the figures for the right and left limb of the X have been corrected for the sex ratio observed among the larvae studied. It is obvious that the female larvae contain the treated X chromosome and no Y chromosome, while the male larvae have only the untreated X chromosome in which no induced breaks are expected and a treated Y chromosome. The correction factor for the sex difference turns out to be

TABLE 3

χ^2's for one degree of freedom calculated from the expected versus the observed distribution of breaks among the chromosomes (on the basis of salivary length ratios).

CHROMOSOME	RELATIVE LENGTHS	EUCHROMATIC REGIONS ONLY			HETEROCHROMATIC AND EUCHROMATIC REGIONS		
		♀♀S	♂♂S	♀♀S AND ♂♂S	♀♀S	♂♂S	♀♀S AND ♂♂S
II	10	0.541	0.370	3.759	0.072	0.039	1.700
XR	10	0.917		4.362	0.825		3.965
IV	8	1.147	0.156	1.640	0.995	1.376	0.794
III	7	0.221	0.035	0.103	0.054	0.0001	0.800
XL	5	1.768		5.453	0.724		2.831
V	0.5	7.165	0.193	2.964	5.900	0.377	2.198
Y	0.5					38.776	

1.896. The observed and expected values do not show a very close fit. The resulting χ^2's are given in table 3. The deviation is especially large for the two arms of the X chromosome. The expected euchromatic breaks may be recalculated separately for the females and for the males, respectively. Here the agreement between the observed and the expected is very satisfactory (table 3), except that chromosome five seems to have more breaks than its due. It must be noted, however, that the determination of the length of this chromosome compared to that of the others is only approximate. The breaks in the heterochromatic regions are definitely more frequent in the third and X chromosomes than in the second and fourth chromosomes (table 2). As far as the second chromosome is concerned,

this might have been expected in view of the scarcity of heterochromatin in this chromosome (see above).

It is justifiable to conclude that the induced breaks are distributed among the chromosomes more or less in proportion to their lengths as seen in the salivary gland cells. In this respect the induced breaks are very different from the naturally occurring ones. According to DOBZHANSKY and STURTEVANT (1938), the third chromosome of *Drosophila pseudo-obscura* is decidedly more variable than all the rest. The present data agree with those of BAUER, DEMEREC, and KAUFMANN (1938), to the extent of showing that the number of breaks in the heterochromatin is much greater than would be expected from its length in the salivary gland cells. No proportionality to the lengths of the mitotic chromosomes is observed; however, table 2 seems to show that the numbers of the hetero- and eu-chromatic breaks combined are not equal in all the rod-shaped autosomes and in the two limbs of the X chromosome, as they would be expected to be in view of the approximate equality of the lengths of these chromosomes at mitosis. These data are perhaps most consistent with the assumption that the frequency of breaks in the heterochromatin is higher than would be expected from its length in the salivary gland cells, but lower than expected from its length in the mitotic prophases.

DISTRIBUTION OF THE INDUCED BREAKS IN THE THIRD CHROMOSOME

As shown above, no chromosome or chromosome limb stands out among the rest with respect to its high or low breakability. This does not mean, however, that certain sections within each chromosome may not be more likely to undergo breakage than others. To test this possibility, the positions of the observed breakage points within the chromosomes must be determined. At present this has been done only for the breaks in the third chromosome, which is of most interest for the purpose of comparison of the distribution of the induced and natural breaks. The map of the third chromosome as seen in salivary gland cells published by DOBZHANSKY and STURTEVANT (1938) was used as a standard. This map is divided into 19 sections and 68 subsections. All the induced breaks have been localized to sections and subsections, and an attempt was made to localize some of the breaks even more exactly, if possible to a disc. Such an exact localization is evidently desirable for those induced breakage points whose position more or less coincides with certain natural breakages.

The number of induced breaks observed in each section is shown in table 4. This table shows also the number of discs recorded for each section. These latter data were supplied by PROFESSOR DOBZHANSKY. Some of the breaks proved to lie at the boundaries between the sections; for statistical analysis these are counted as one-half of a break belonging to each of the

two neighboring sections. The number of breaks per section varies from zero (in section 63) to seven (in sections 66, 70, and 79) and ten (heterochromatin). This fact does not necessarily mean, however, that some sections are inherently more breakable than the rest. In the first place, the number of discs is greater in some sections than in others, and secondly, a certain amount of variation must be expected even if the breaks are distributed perfectly at random. The problem must be examined statistically. On the assumption that the likelihood of breakage is proportional to the number of discs included in any part of the chromosome, one may compute the number of breaks expected to fall in any one section from the observed total of 63. From the differences between the observed and the expected values, the χ^2 values for each section may be computed (table 4). Each

TABLE 4

Distribution of breaks in the euchromatic regions of the third chromosome (combined data for both sexes).

SECTION	NUMBER OF DISCS	NUMBER OF BREAKS OBSERVED	NUMBER OF BREAKS EXPECTED	χ^2	PROBABILITY
Heterochromatin		10			
63	40	0	4.621	4.621	
64	25	2	2.888	0.237	
65	24	1	2.773	1.134	
Total	89	3	10.282	5.157	0.05–0.02
66	45	7	5.199	0.629	
67	39	2	4.505	1.393	
68	37	2	4.274	1.210	
Total	121	11	13.987	0.634	0.5 –0.3
69	45	4	5.199	0.277	
70	39	7	4.505	1.382	
71	21	1	2.426	0.838	
Total	105	12	12.130	0.0014	0.98–0.95
72	28	5.5	3.235	1.586	
73	26	2.5	3.004	0.846	
74	22	1	2.542	0.935	
75	26	6.5	3.004	4.069	
76	27	1	3.119	1.440	
Total	129	16.5	14.903	0.171	0.70–0.60
77	13	4	1.502	4.154	
78	23	3.5	2.657	0.267	
79	29	7	3.350	3.977	
80	21	4	2.426	1.021	
81	24	2	2.773	0.216	
Total	110	20.5	12.708	4.778	0.05–0.02
Grand Total				**30.268**	0.05–0.02
				10.741	0.05–0.02

of these χ^2's have one degree of freedom. Summing them up together, a general heterogeneity χ^2 is obtained which has 18 degrees of freedom. This heterogeneity χ^2 has a value of 30.268, which indicates that the observed heterogeneity may occur by chance in from five to two trials per hundred. This seems a rather significant heterogeneity. To determine more accurately what region or regions are responsible, the chromosome was divided into five more or less equal parts. The most distal of these parts contains 110 discs and includes the sections 77 to 81. The number of breaks expected in this part on the basis of random distribution is 12.7, and the number observed was 20.5. This difference between observation and expectation is 7.8 breaks; the χ^2 (equal to 4.778 for one degree of freedom) has the probability of chance occurrence of 0.05 to 0.02. The next portion includes sections 72 to 76 and contains 129 discs. The difference between the observed and expected numbers of breaks has a χ^2 of 0.171 and is not significant. The third and fourth portions include sections 69 to 71 and 66 to 68, respectively. The number of observed and expected breaks in these portions agree very well. Finally, the most proximal portion of the chromosome contains sections 63 to 65 and has 89 discs. The observed number of breaks in this portion is three, while the expected number is 10.28. The corresponding χ^2 is 5.157; the probability of such or greater deviation occurring by chance is between 0.05 to 0.02. The conclusion can be drawn that, at least in the third chromosome, the frequency of induced breaks is somewhat higher in the distal portion than in the middle and somewhat higher in the middle than in the proximal portion. This observation agrees with those of BAUER, DEMEREC, and KAUFMANN (1938) and BAUER (1939), who found that the distal portions of the chromosomes of *Drosophila melanogaster* are more breakable than the proximal portions.

With the exception of the inequality in the distribution of the induced breaks just described, there is no certain indication that any one portion of the chromosome is more or less likely to break than the rest. To be sure, the possibility cannot be entirely excluded that such inequality exists. Thus, no breaks were observed in section 63, while 4.6 breaks were expected for this section. On the contrary, four breaks were observed in section 77, while only 1.5 breaks were expected (table 4). These deviations are almost statistically significant. It must be noted, however, that section 63 lies next to the heterochromatin, and breakages in this section might have been missed.

A comparison may now be made of the breaks induced in the third chromosome by the X-ray treatment with those observed in the natural chromosomal variations by DOBZHANSKY and STURTEVANT (1938). The loci of these "natural" breaks are recorded in the paper just referred to, and PROFESSOR DOBZHANSKY has supplied more exact data for these, as

well as for certain other natural breaks observed in the third chromosome since the time of publication of his paper. The data for the natural breaks are shown in table 5. Certain differences between the distribution of the induced and natural breaks are apparent at once. Among the 73 induced breaks in the third chromosome (table 4), ten were in the heterochromatin, while among the 38 natural breaks none is heterochromatic. The number of the natural breaks so far recorded is too small for an exact statistical analysis. It may be noted, however, that the greatest numbers of natural breaks were observed in sections 76 and 79 (five in each), and no breaks were observed in sections 66, 67, and 73. The number of induced breaks

TABLE 5

Distribution of breaks in natural and induced inversions.

NATURAL	INDUCED
1. 64C–69D (Cuernavaca)	Heterochromatic region—65C
2. 65C–75C (Pikes Peak)	Heterochromatic region—70C
3. 68D–74B (Tree Line)	Heterochromatic region—75B/C
4. 68C–79A (Santa Cruz)	Heterochromatic region—81A
5. 69C–76C (Oaxaca)	64B–75/76
6. 69C–79A (Estes Park)	66B/C–77B
7. 70B–76B (Arrowhead)	69B–77/78
8. 70D–78A (Chiricahua I)	70A–72/73
9. 70C–79B (Texas)	70A–74A
10. 70/71–73/74 (Sequoia I)	72A–75B
11. 70/71–77/78 (Klamath)	75B–78A
12. 71C–79D (Cowichan)	78C–80C
13. 71C–81A (Ukiah)	
14. 72B–77A/B (Hidalgo)	
15. 75C–80A (Olympic)	
16. 76A–79D/80A (Hypothetical)	
17. 76A–79D (Mammoth)	
18. 76A–78A (Wawona)	
19. 77A/B–81C (Sequoia II)	

in section 76 is below, while in section 66 it is above the expectation. The number of natural breaks found in the proximal one-fifth of the chromosome is very low (two). The number of natural breaks found in the distal one-fifth of the chromosome is relatively high (13). To this extent the distribution of the natural and induced breaks parallel each other.

Another point of comparison of the natural and induced breaks suggests itself. As shown by DOBZHANSKY and STURTEVANT (1938), the different gene arrangements of the third chromosome encountered in nature may be derived from each other by inversions. Twelve different inversions were induced by X-ray treatment. A comparison of the induced and naturally occurring inversions can be made (table 5). As already mentioned above,

none of the latter involve breaks in the heterochromatic region, while among the induced inversions, four have one break in heterochromatin. None of the induced inversions is identical or even similar to any of the natural ones with respect to the position of its breakage points. Five among the natural inversions—namely, Pikes Peak, Arrowhead, Klamath, Ukiah, and Hypothetical—are derived directly from the standard gene arrangement. None of the induced inversions resembles any of these.

An inquiry should also be made to determine if any of the induced breaks (whether observed in inversions or in other types of aberrations) coincide with any of the natural breaks. With 63 induced and 36 natural breaks in the euchromatin of the chromosome, some breaks were bound to occur rather close to each other. To determine whether or not any two of these breaks had actually taken place at the same point, such neighboring breaks were examined very carefully. One of the induced breaks (in subsection 75C) proved to be identical, apparently, with one of the breaks in the natural inversion Pikes Peak. No more coincidences of this sort were established with certainty, although two more cases may be considered as questionable coincidences. An induced break in section 69C may possibly have occurred at the same point as the breaks in Oaxaca and Estes Park, although the location of the latter is not exactly established (section 69C is one of the most difficult ones for observation). The natural inversion Cowichan has both its breaks (75B/C and 79C/D) closely mimicked by two induced breaks observed in different treated individuals. Breaks induced in sections 75C and 81A are similar to, but probably not identical with, the breaks observed in the natural inversions Olympic and Ukiah, respectively.

The conclusion is justified that the loci at which the third chromosome has been observed to be broken in the naturally occurring inversions are not especially breakable under the influence of X-rays. Furthermore, none of the naturally occurring inversions has been reproduced as a result of X-ray treatment.

DISTRIBUTION OF MULTIPLE BREAKS AMONG CHROMOSOME LIMBS

BAUER, DEMEREC, and KAUFMANN (1938) and BAUER (1939) have shown that following X-ray treatment inversions occur more frequently and translocations somewhat less frequently than expected if the distribution of breaks were random. It is of interest to inquire whether a similar phenomenon is observed in *Drosophila pseudoobscura*. If two, three, four or more chromosome breaks are induced in the same gamete, their distribution among the different chromosomes may be calculated with the aid of the simple formula $(a+b+c+d+e)^n$. In this formula the letters a, b, d, c, and e represent the relative lengths or break probabilities in the

five chromosome limbs; and n represents the number of breaks investigated. In order to simplify the calculations, all the chromosome limbs in *Drosophila pseudoobscura*, with the exception of V, may be assumed to be equal. The error incurred due to this assumption will be too small to be of much significance in the calculation.

Cases in which two breaks were observed will be considered first. If the distribution of the breaks were at random they would have occurred in the

TABLE 6

Distribution of multiple breaks among the chromosomes.

NUMBER OF BREAKS	DISTRIBUTION	NUMBER OF CASES		TOTAL	PERCENT OBSERVED	PERCENT EXPECTED	χ^2	PROBABILITY
2	1, 1	35	22	57	61.95	80	3.744	0.05
	2	20	15	35	38.04	20	14.976	0.01
	1, 1, 1	2	1	3	30.0			
3	2, 1	0	5	5	50.0			
	3	1	1	2	20.0			
	2, 1, 1	4	6	10	52.63			
	1, 1, 1, 1	3	1	4	21.05			
4	3, 1	3	1	4	21.05			
	2, 2	1	0	1	5.26			
	4	0	0	0				
	2, 1, 1, 1	2	2	4				
	2, 2, 1	0	1	1				
5	3, 2	1	1	2				
	3, 1, 1	1	0	1				
	4, 1	1	0	1				
	2, 2, 1, 1							
	3, 1, 1, 1							
6	3, 2, 1	0	1	1				
	4, 1, 1							
	5, 1	0	1	1				

same chromosome limb in 20 percent of the cases, and in different limbs in 80 percent. The former are recovered as inversions and the latter as translocations. Hence the relative frequencies of inversions and translocations among the two-break aberrations must be as 1:4. Table 6 shows that this is not at all the case, since 35 inversions and 57 translocations were observed. The difference between the observed and expected figures is statistically very significant.

Similar calculations may be made for the gametes in which three, four, or more breaks occurred (table 6). Since the number of such cases in these data is small, no statistical analysis could be made. Nevertheless, the data,

as far as they go, confirm the conclusion of BAUER, DEMEREC, and KAUFMANN that a disproportionately high number of inversions occur.

THE DISTANCE BETWEEN BREAKAGE POINTS

As shown in the preceding paragraph, the distribution of the induced breaks among the chromosome limbs, as well as their distribution within the third chromosome, may, with certain reservations, be said to be random. Up to now, however, only the distribution of breaks taken one at a time has been considered. The problem has still another aspect. If several breaks occur in a single cell, will they occupy certain predestined positions with respect to each other? In other words, is the distance between breaks in the same or in different chromosomes more or less fixed, or are the breaks independent? BAUER, DEMEREC, and KAUFMANN (1938) have worked out a statistical technique for testing the dependence or independence of the breakage points. Although the data available for the distribution of the induced breaks for the third chromosome of *Drosophila pseudoobscura* are too scanty for a detailed statistical treatment, the calculations made indicate that the positions of the breaks with respect to each other are independent. This is in accord with the results of BAUER, DEMEREC, and KAUFMANN (1938) and BAUER (1939) for *Drosophila melanogaster.*

NUMBER OF BREAKS PER SPERM

The number of aberrant sperms in which two, three, four, five, and six breaks were observed is shown in table 1. From these data the average number of breaks per changed spermatozoan may be computed. This proved to be 2.63 ± 0.13. This value obtained for *Drosophila pseudoobscura* is significantly lower than those obtained by BAUER, DEMEREC, and KAUFMANN (1938) and BAUER (1939) for *Drosophila melanogaster* with a similar amount of X-ray treatment (5000 r-units). These figures are 3.126 ± 0.164 and 3.21 ± 0.11, respectively. The cause of this difference is unknown.

MOSAICS

In the course of the present study several unusual types of chromosomal variations were recorded. The first group to be described is that of mosaics. Mosaic gene mutations (fractionals) have been known for a long time, but only very recently L. V. MORGAN (1939) has described a spontaneous translocation in *Drosophila melanogaster* which was observed in some cells of a salivary gland of an individual but not in others. LEWITSKY and ARARATIAN (1931) have also described what appears to be chromosomal mosaics in Crepis treated with X-ray. Some of the slides in the present investigation proved to have two or more sorts of cells, showing that the

larvae from which these slides were made were mosaics. A suspicion arose that this might be due to contamination, but several facts rule out this possibility.

A description of two of the mosaics has already been published (HELFER 1940). It will suffice here to state that one of them had two kinds of tissue, while the other had four. In the latter, some cells had normal chromosomes; others had a III-Y translocation; others a III-IV translocation; and still others a combination of the III-Y and the III-IV translocations. Since the publication of the above report, five more mosaics have been found. The most interesting one among these involves three types of tissue. Among 19 cells which were found to be satisfactorily analyzable, four cells contained normal chromosomes; in 15 cells an inversion in the fourth chromosome extending from the boundary between sections 83 and 84 to the proximal part of 89 was present; while a single cell had an inversion whose proximal break coincided with that in the inversion just described, while the distal break fell in section 99 near the border of 98. The remaining four mosaics consisted each of two types of tissue, one containing normal and the other aberrant chromosomes. One of the latter involved an XR-IV translocation, and the two others II-III translocations (of course, different ones).

A REARRANGEMENT INVOLVING MATERNAL AS WELL AS PATERNAL CHROMOSOMES

Another interesting type of aberration observed was one which can be explained best on the assumption that two homologous strands of the same chromosome were involved. The fact which affords the evidence for this assumption is the presence of two "trifurcations" (fig. 1A). A "trifurcation" is a configuration wherein three chromosome strands meet at one point to form a Y-shaped figure (fig. 1B). Such a configuration in the salivary chromosomes is possible only if two homologous chromosomes are involved. There is a total of six breaks in the rearrangement which involved the second and fourth chromosomes, of which five are in the second (three in one homologue and two in the other) and one is in the fourth chromosome. In order to trace more clearly the rearrangements observed, the chromosomes are lettered. The normal sequence for the second chromosome is represented as A B C D E F G H and the fourth chromosome as $\alpha\beta$. To distinguish between maternal and paternal strands, the paternal homologues of these two chromosomes are designated by the primes of the above letters. The four resulting strands observed in this rearrangement are A B C D E H, A′ G′ F′ E′ D′ β', $\alpha\,\beta$, and α' C′ B′ G F H′. These four gene sequences may be obtained in three simple steps. Let us assume that the X-rays caused the second chromosome of the sperm to break at three

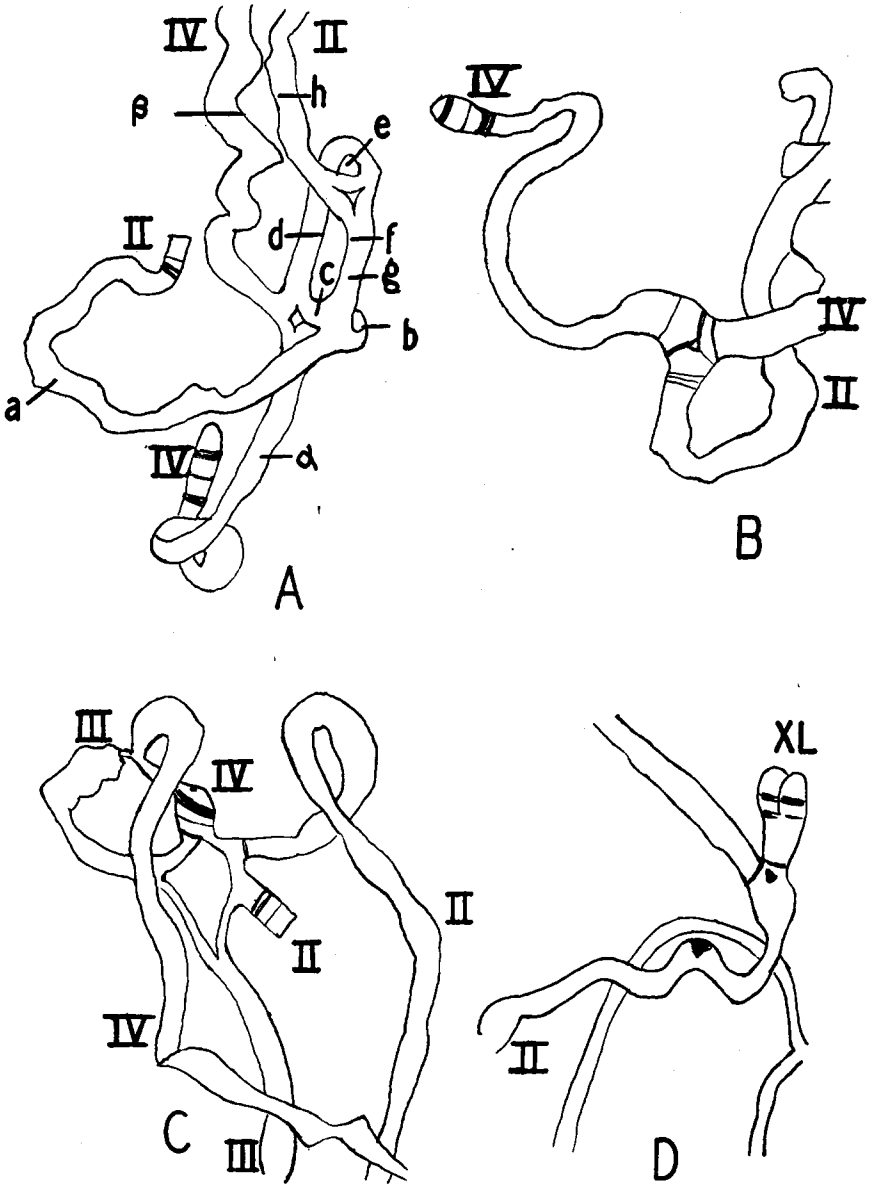

FIGURE 1.—Diagrammatic sketches of four chromosomal aberrations: (A) an aberration involving both maternal and paternal chromosomes, (B) another view of (A) showing a trifurcation, (C) an aberration involving a terminal intercalation, (D) an aberration involving a side attachment.

different loci and the fourth at one locus; these breaks did not take effect immediately. It is possible that during the first mitotic division of the zygote two spontaneous breaks occurred in the maternal homologue of the second chromosome. As a result, a section (F G) might have become deleted from this maternal strand and transferred to the paternal strand at H′; then the two most distal of the three breaks in the paternal strand (between A′ and B′; and G′ and H′) might reunite in inverted order and the medial break (between D′ and C′) reattach to the broken ends of the fourth chromosome. This hypothesis favors the "breakage first" theory of chromosomal rearrangements.

The alternative hypothesis proposed to explain the origin of this aberration was kindly suggested by DR. B. P. KAUFMANN (personal communication). He suggests that the two second chromosome homologues observed in this aberration are both of paternal origin. The main weakness of this hypothesis is its failure to account for the absence of the maternal second chromosome, since according to this hypothesis one should expect the individual to contain the second chromosome in triplicate. Such an individual would be inviable (TAN 1937). It is possible, however, that the maternal second had been eliminated at some very early division leaving the cell with only the two paternal homologues. Although this hypothesis seems less likely, it cannot be disregarded, since as the evidence available does not distinguish between them. It is interesting to note that on either hypothesis the behavior of the untreated maternal second chromosome is abnormal.

TERMINAL INTERCALATION

A very complicated aberration was observed which involved the intercalation of the free end of the third chromosome into a break in the fourth chromosome (fig. 1C). This aberration also contained a duplication for the tip of the fourth chromosome. Such duplications for parts of chromosomes have been observed in *Drosophila melanogaster* (KAUFMANN and BATES 1938) as well as in several other aberrations in the present investigation. In the present rearrangement the third chromosome was broken in the distal part of section 75C. The free end was translocated, in reverse position, into section 98 (distal) of the fourth chromosome; the base was attached to the free end of the second chromosome (broken in section 61); and the base of chromosome two was attached to the duplicated tip of chromosome four.

A BRANCHED CHROMOSOME

A rearrangement has been observed which appears to have produced a branched chromosome. In this aberration chromosome IV is broken in the distal part of section 93, XL is broken in the proximal part of section 16,

and the second chromosome in section 52. The tip of chromosome IV is attached to the basal part of XL; the tip of XL (in duplicate) is inserted sidewise in chromosome II; the remaining basal portion of IV appears to have acquired no fragment of any other chromosome. It is, of course, possible that this rearrangement involves no branched structure, but is a reverse duplication analogous to that apparently present in dominant Eyeless (BRIDGES 1935). Such an interpretation is made difficult by the observation that in some cells, one of which is shown in figure 1D, the two tops of the XL branch are clearly separate. It seems unlikely that this separation is an artifact produced by the pressure of the cover slip.

DISCUSSION

As already stated in the introduction, the results of the present study have a bearing on two groups of problems: those involving the genetic composition of natural populations and those concerning the mechanism of the origin of chromosomal variation, particularly under the influence of X-rays. These two aspects are discussed separately.

DOBZHANSKY and STURTEVANT (1938) have found that natural populations of *Drosophila pseudoobscura* show a large amount of variation in their chromosome structure. With the exception of the variability of the Y chromosome (DOBZHANSKY 1935, 1937) which appears to be due to duplications and deficiencies, all other variations may be accounted for by inversions of chromosome segments. The third chromosome is by far the most variable one, and 17 distinct gene arrangements were discovered in it. This compares with three in XR, two in XL, six in II, and two in IV; no variation has been detected in V. Furthermore, the variations in III occur throughout the distribution area of the species, while in most localities the populations are uniform with respect to the gene arrangement in chromosomes II and IV. The X chromosome differs in the two races, A and B, into which the species is split (LANCEFIELD 1929, TAN 1935) and also varies in connection with the so-called sex-ratio condition (STURTEVANT and DOBZHANSKY 1936).

It is thus unequivocally established that the third chromosome of *Drosophila pseudoobscura* shows in nature a much greater variability than the rest. One of the possibilities that might account for this phenomenon is that the third chromosome is, for some reason, more breakable than others and thus more prone to give rise to gene rearrangements, particularly inversions. In *Drosophila melanogaster* the X-ray induced breaks are distributed among the chromosomes at random (BAUER, DEMEREC, and KAUFMANN 1938, BAUER 1939), but STURTEVANT (1931) and DUBININ, SOKOLOV, and TINIAKOV (1936, 1937) have shown that in the wild populations of this species at least four out of the five long chromosome limbs

(2L, 2R, 3L, and 3R) are variable. Nevertheless, the data reported in the present paper show that in *Drosophila pseudoobscura* the distribution of the induced breaks is also at random, and the third chromosome shows no sign of an increased breakability. One is forced to look somewhere else for an explanation of the great variability of this chromosome in nature. A possible explanation was suggested by STURTEVANT and MATHER (1938), but it cannot be regarded as well established so far.

Another problem raised by the findings of DOBZHANSKY and STURTEVANT (1938) concerns the distribution of breaks within the third chromosome of *Drosophila pseudoobscura*. The various gene arrangements encountered in nature in this chromosome are related as overlapping inversions—that is, inversions of the type A B C D E F G H , A E D C B F G H, and A E D G F B C H. Gene arrangements differing in two overlapping inversions most likely did not arise directly from each other, but through an intermediate step, which would have been of the type A E D C B F G H. This enables one to trace the phylogeny of the gene arrangements encountered in natural populations. This theory implies, furthermore, that a repeated origin of the same gene arrangement is unlikely, because such an event would require that a chromosome break repeatedly in the same two or more identical places. Assuming that the chromosome is equally likely to break at any locus and that there are 500 loci in the chromosome (a conservative estimate), the probability that, by coincidence, the same two breaks will occur repeatedly is 500^2, or one in 250,000 inversions, which is very small. This probability however, need not be so small if the chromosome has certain "weak points" at which the occurrence of breakage is more likely than at other points. Furthermore, it is conceivable that the occurrence of a break at a given locus somehow induces the chromosome to break at a specific other point; in other words, a modal length of the chromosome section lying between correlated breakage loci may exist. These possibilities may be tested experimentally.

The data reported above for the third chromosome of *Drosophila pseudoobscura*, as well as those of BAUER, DEMEREC, and KAUFMANN (1938), and BAUER (1939) for the chromosomes of *Drosophila melanogaster* agree in showing that the distribution of induced breaks within chromosomes is approximately, though not entirely, random. No chromosome sections have been shown to be immune to breakage, and no section proved to be so breakable as to deserve the designation of "weak point." Above all, there is no correlation between the relative positions of two or more breaks if they occur in the same chromosome. The heterochromatic portion shows a high concentration of induced breaks relative to its length in the salivary gland chromosome. No "natural" breaks are known in the heterochromatin indicating that inversions involving heterochromatin are not retained in

natural populations for unknown reasons. The frequency of the induced breaks within the euchromatic portion increases slightly, though significantly, from the proximal to the distal (free) end. The distribution of the natural breaks seems to show a similar regularity, although here the statistical validity of this conclusion is open to question.

The available data do not exclude the possibility that certain points in the chromosome are more breakable than others; all that is really certain is that such points, if they exist, are not concentrated in any specific sections. More important still, none of the induced inversions proved to be identical, or even similar to any of the natural ones. Among the induced breaks in general (that is, those observed in translocations as well as in inversions), a few proved to lie close to the loci of some of the natural breaks. The number of such coincidences, however, is small, and only in a single case is there a reason to suppose that a real identity is involved. Thus, the present data support the assumption of DOBZHANSKY and STURTEVANT (1938) that there is so little probability of a repeated origin of the same inversion in nature that the gene arrangements are not transformed into each other at frequent intervals.

As between the two proposed hypotheses of the origin of chromosomal aberrations—namely, the contact hypothesis of SEREBROVSKY (1929) and the breakage first hypothesis of STADLER (1932), the results of the present study seem to favor the latter. According to the contact hypothesis, the chromosome breakage and the reunion of the resulting fragments occur simultaneously, being in effect only two aspects of the same process. The breakage first hypothesis assumes that if the chromosomes are broken by some agent, or agents, the broken ends tend to reëstablish connections with other broken ends. A time interval may elapse between the breakage and the reattachment. In the seven mosaic chromosomal aberrations described above, individuals coming in the F_1 generation from a treated father and an untreated mother proved to have two, three, and even four kinds of tissues with different chromosome configurations.

The appearance of such mosaics is compatible with the breakage first hypothesis, provided the breaks, or the weak parts in the chromosomes induced by X-ray, may persist long enough to be able to establish new associations after fertilization or after the cleavage division following fertilization. The aberration which seems to involve exchanges between the paternal and the maternal chromosomes may be similarly interpreted. These aberrations are difficult to explain from the standpoint of the contact hypothesis. The supposition that they arose spontaneously during the cleavage divisions, rather than in the X-ray treated sperm, is very improbable, since spontaneous chromosome breakage in somatic tissues is very rare. Furthermore, in the mosaics involving three and four kinds of

tissue one would have to suppose that spontaneous breaks have taken place in at least two consecutive cleavage divisions and twice at the same point in the chromosome—an obviously very remote contingency. Another way to reconcile the occurrence of the mosaics with the contact hypothesis is to suppose that at the time of the treatment the chromosomes were not in the form of single, but in the form of double, or even quadruple threads. Such a supposition is not out of the question, since certain investigators (for example, NEBEL 1936, 1937a, 1937b) claim to have observed cytologically double and quadruple chromosomes at telophases. This is definitely denied by others (for example, DARLINGTON 1937). Without going into consideration of this very controversial question, one may point out that, even granting that chromosomes are double or quadruple in spermatozoa, it remains difficult to reconcile the observed mosaics with the contact hypothesis. Thus the mosaic with four types of tissue (HELFER 1940) would require not only quadripartite chromosomes in a treated spermatozoan but, in addition, a kind of somatic crossing-over between sister strands.

In conclusion, a few remarks may be made regarding the unorthodox chromosomal variants found—namely, the one involving an intercalation of the terminal portion of chromosome III into chromosome IV and the one with an apparent side attachment. The absence, or at least the great rarity, of chromosomal aberrations involving single breaks, such as terminal deficiencies, inversions, and non-reciprocal translocations, leads to the assumption that the free end of each chromosome, the so-called telomere, has special properties not present in the interstitial genes. Therefore, a telomere cannot take the place of an interstitial gene and *vice versa* (MULLER 1938). Certain facts contradictory to this assumption however, are on record. Thus, DEMEREC and HOOVER (1936) have described terminal deficiencies that appear to be authentic, and STADLER (1940) finds that among the deficiencies induced by ultra-violet radiation a majority also seem terminal (compare also the results of MCCLINTOCK 1938a, b, obtained by other methods). It is not claimed that the terminal intercalation and deficiency described here (fig. 1C) are beyond any doubt, although the free end of chromosome III of *Drosophila pseudoobscura* has a structure so characteristic that the removal of even a minute part of it might be expected to be detectable. Furthermore, it would seem that, granting that telomeres differ in some way from interstitial genes, the possibility of the latter acquiring the properties of telomeres, and *vice versa*, by a process akin to mutation cannot be excluded. A similar reasoning applies to the problem of branched chromosomes (KOSSIKOV and MULLER 1935).

SUMMARY

1. The distribution of 347 breaks induced by X-ray treatment in the

chromosomes of *Drosophila pseudoobscura* was studied. The frequencies of the breaks in the different chromosomes are in proportion to their relative lengths.

2. The induced breaks in the third chromosome are not distributed entirely at random. The frequency of the breaks in the heterochromatin as compared with those in the euchromatin is much greater than would be expected on the basis of the lengths of the heterochromatic portions in the salivary gland cells but probably smaller than would be expected on the basis of its length in the mitotic chromosomes. Within the euchromatic portions the frequency of breaks increases slightly from the proximal to the distal end.

3. Aside from the regularity mentioned in the preceding paragraph, the breaks in the third chromosome show no tendency to be concentrated around any "weak points." In any case, a comparison of the induced breaks with those observed in the naturally occurring chromosomal aberrations shows very few coincidences. None of the inversions induced by X-ray treatment proved similar to any of the naturally occurring inversions.

4. The reunion of the chromosome fragments produced by X-ray treatment is not at random, inversions being more and translocations less frequent than expected.

5. Several mosaic chromosomal aberrations are described. An analysis of these aberrations seems to argue in favor of the "breakage first," rather than the "contact" hypothesis of the origin of chromosomal aberrations.

6. The "breakage first" hypothesis is also favored by the observed aberration in which paternal as well as maternal chromosomes seem to be involved.

7. An aberration which appears to involve a terminal attachment, and another showing what appears to be a branched chromosome, are described.

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