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

Thesis by

Robert George Helfer

In Partial Fulfillment

of the

Requirements for the Degree of

Doctor of Philosophy

California Institute of Technology

Pasadena, California

1940

ACKNOWLEDGMENTS

To Professor Theodosius Dobzhansky, under whose direction the present investigation was pursued, the author wishes to express his sincere appreciation for the many helpful suggestions and criticisms received in directing this research and in the preparation of the thesis.

To Professors A. H. Sturtevant, T. H. Morgan, J. Schultz, L. J. Stadler, B. P. Kaufmann, S. Emerson, and E. G. Anderson the author wishes to express his thanks for their aid and suggestions, and to Professor C. A. G. Wiersma for his kindness in allowing the author to divide his time between physiological and genetical research.

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Introduction

The gene structure and the gene arrangement are, as a rule, transmitted unchanged from generation to generation. However, mutations altering the genes and variations altering the gene arrangement in the chromosomes do occur from time to time. The different classes of chromosomal variations have been known for approximately twenty years. The first deficiency was discovered in Drosophila melanogaster by Bridges in 1917, the first duplication and translocation by Bridges in 1919 and 1923 respectively, the first inversion by Sturtevant in 1926. These chromosomal aberrations arose spontaneously in the cultures, or (in the case of inversions) were detected in laboratory strains. The discovery of Muller (1928) that gene mutations as well as chromosomal changes can be induced by X-ray treatments gave a great impetus to the study of these phenomena. Instead of waiting for mutations to arise spontaneously, the investigator is able to speed up their occurrence in carefully controlled experiments. To be sure, the known X-ray treatments merely increase the rates of occurrence of the same changes which arise, very much less frequently, without any treatment. At present no methods are available which will induce only definite desired changes, leaving the rest unaffected. A start in this direction has been made by Stadler (1940), who finds that treating maize pollen

grains with ultra-violet radiation gives rise to many apparent gene mutations and deficiencies but to relatively very few major translocations and inversions.

For some time mutations and chromosomal variations were known chiefly as laboratory products, and some biologists (such as Osborn, 1927) doubted that they occurred at all, or at least very often, in nature. A series of investigators, among which Dubinin and his collaborators (1934), Timofeeff-Ressovsky (1927), and Dobzhansky and Sturtevant (1938) may be mentioned, have shown that both mutant genes and chromosomal aberrations are extremely common in wild populations of the species of Drosophila studied in this respect. The naturally occurring chromosomal aberrations are chiefly inversions and perhaps deficiencies while translocations are very rare. This is not surprising, however, since individuals heterozygous for translocations are less fertile than the normal type and are eliminated by natural selection. On the other hand, heterozygosis for inversions does not decrease fertility, or decreases it to a much smaller extent than heterozygosis for translocations (Sturtevant and Beadle, 1936).

The discovery of the induction of chromosomal aberrations by X-ray, as well as that of the occurrence of these aberrations in nature, has raised many new problems. According to Dobzhansky and Sturtevant (1938) the third chromosome of Drosophila pseudoobscura shows, in nature, considerably more variation than the other chromosomes. In other species (Drosophila azteca, Dobzhansky and Sokoloff, 1936; Drosophila algonquin, Miller, 1939) several, and

possibly even all, chromosomes may be variable. It remains unknown whether the third chromosome in Drosophila pseudoobscura is more breakable than the rest, or whether its greater variability in nature is due to some process which eliminates the breaks in the chromosomes other than the third. Dobzhansky and Sturtevant (1938) have also found that most of the gene arrangements in the third chromosome of Drosophila pseudoobscura are related to each other as overlapping inversions, and therefore their phylogenetic relationships can be established. Each of the gene arrangements existing in nature is supposed to have arisen from a definite source and probably has arisen only once. Since some of the gene arrangements are very widely distributed geographically and are represented by enormous numbers of individuals in nature, this raised the question of how and why a gene arrangement once produced can become so frequent. The inferences of Dobzhansky and Sturtevant are, however, based on the assumption that the chromosome is equally or nearly equally, breakable at any one point, and that therefore a repeated origin of the same inversion is not very probable. These assumptions may be tested by observing a sample of newly arisen breaks and comparing their distributions among chromosomes and within chromosomes with that of the breaks found in natural chromosomal aberrations. For this purpose, the induction of breaks by X-ray treatments furnishes a convenient tool, provided, of course, that the induced and the naturally occurring breaks are alike in their distribution. This last proviso cannot be tested directly, but it seems reasonable enough to serve at least as a working hypothesis.

As to the mechanism of the origin of chromosomal aberrations, whether induced or natural, there seems to be no general agreement among the investigators. Two hypotheses have been advanced, namely the so-called "contact hypothesis" (Serebrovsky, 1929) and the "breakage first" hypothesis (Stadler, 1932). Without going into a detailed explanation of the nature of these hypotheses at this point, one may say that the discrimination between them on the basis of experimental evidence has proven to be very difficult, since most of the observable phenomena can be accounted for on either hypothesis. The investigations of Catcheside (1938), Sax (1940), Muller (1938), Bauer, Demerec and Kaufmann (1938), and Bauer (1939) did, however, attempt to bring to light certain phenomena which could serve to elucidate this problem. In the course of the present investigation, certain observations were made which also might be interpreted as favoring breakage first as opposed to the contact hypothesis.

The work to be described here was done while the author was a post-graduate student at the Biology Department of the California Institute of Technology.

Material and Method

Males from the "Texas" strain were treated with X-ray 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 females per culture. The parents were allowed to remain together for three days, after which time the males were removed. This was done in order to insure that the females were impregnated only with sperm which had been mature at the time of treatment. The females were transferred to fresh bottles every three days. The cultures were kept at 17° C., to make the resulting larvae favorable for cytological study.

The larvae of the F_1 generation were dissected from day to day as they matured. The salivary glands were placed in salt cellars with acetocarmine for about four to six minutes. The stained glands were made into permanent slides, using the techniques described by Bauer and Bridges (D.I.S. No. 6, 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 (X 90) and oculars (X 15).

The Chromosome Aberrations Observed

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 involving only breakages between heterochromatic regions of two or more chromosomes were probably overlooked. The different classes of aberrations found in the modified individuals are shown in Table I.

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 liable 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 II. In this table the two limbs of the X-chromosome (XR and XL) are treated as separate chromosomes.

Table II shows that the numbers 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, 8 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 are approximately equal, the dot (chromosome V) is at most one-tenth of the length of the rod-shaped ones, and the two arms of the X are equally long and slightly longer than the rod-shaped autosomes. The Y-chromosome in the strain used (Texas) is relatively very short (Dobzhansky, 1932) 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, and 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 susceptibility 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. In the prophases of nerve cells Bauer (1936) has made some rough estimates. According to him, the second chromosome is practically entirely euchromatic, and has only very little heterochromatin near the spindle attachment. The third, fourth, and 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 1/3 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. With respect to these maps, the ratios of the lengths of the euchromatic portions of the II, XR, IV, III, XL, and dot, are approximately 10: 10: 8: 7: 5: 0.5 respectively. No measurements for the heterochromatic portions can be made from the published data.

Table III shows the number of breaks observed in the euchromatic regions of the chromosomes. The expected numbers of breaks are computed on the basis of the relative lengths of these chromosomes, as indicated above. 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 observed and expected values do not show a very close fit. The deviation is especially large for the two arms of the X-chromosome. Tables IV and V show the same data as Table III recalculated separately for the females and for the males respectively. Here the agreement between the observed and the expected is very satisfactory, except that chromosome five seems to have more breaks than due it. It must be noted, however, that the determination of the length of this chromosome compared to that of the others is only approximate. If the X- and Y-chromosomes are disregarded, the frequency of the breaks in the euchromatic regions of the autosomes appears to be very nearly proportional to their relative lengths (Table VI).

The breaks in the heterochromatic regions are definitely more common in the third and X-chromosomes than in the second and fourth chromosomes (Table II). 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). Combining the hetero- and euchromatic breaks, the data shown in Table VII are obtained. Similar data for females only are shown in Table VIII, and for males only in Table IX. The agreement between the observed and the expected figures in these tables is a satisfactory one. A similar comparison for the autosomes only is shown in Table X; here the agreement between the observed and the expected is also a satisfactory one.

It is justifiable to conclude that the induced breaks are distributed among the chromosomes more or less in proportion to the length of the latter 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 pseudoobscura is decidedly more variable than all the rest. Seventeen different gene arrangements in the third chromosome are described in the paper just referred to, and Dobzhansky informs me that three more have been found since the time of publication. Contrasted to this, only three arrangements are known in the right limb of the X, two in the left limb of the X, six in the second, and only one in the fourth chromosome. The statistical validity of this discrepancy is beyond doubt. One must conclude that although the frequency of breaks is proportional to the chromosomal lengths, the retention of the modified chromosomes in nature is determined by factors other than the likelihood of their origin. One may note here, that the proportionality of the number of induced breaks to the chromosome length, established by these data, is paralleled in the other species studied in this respect, namely Drosophila melanogaster. Bauer, Demerec, and Kaufmann (1938) and Bauer (1939) have demonstrated very convincingly that no chromosome limb stands out among the rest due to its too high or too low breakability.

The problem of the relative frequency of the induced breaks in the hetero- and euchromatic regions is not definitely settled. Bauer, Demerec, and Kaufmann (1938) have established the

fact that the frequency of breakage in the heterochromatin is definitely higher than might be expected from its length in the salivary gland cells. The authors just referred to, as well as Bauer (1939), come to the conclusion that the distribution of breaks in the hetero- and euchromatin is proportional to their lengths as seen in mitotic chromosomes, or even that the heterochromatin is more breakable. 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, Tables VII, VIII, IX, and X seem to show that the numbers of the hetero- and euchromatic 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 in the preceding chapter, 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 nineteen sections and sixty-eight subsections. All the induced breaks have been localized to sections and subsections, and pains were taken 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 and subsection is shown in Table XI. 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; consequently their numbers are shown in Table XI on the dividing lines, and for statistical analysis are counted as 1/2 or 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. The expected as well as the observed values are shown side by side in Table XII; from the differences between these the χ^2 values for each section may be computed (Table XII). These χ^2's each 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 is probably a rather significant heterogeneity. To determine more accurately what region or regions are responsible for this heterogeneity, 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 described above, there is no certain indication that any one portion of the chromosome is more or less liable to break than the rest. To be sure, the possibility can not 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 XII). 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 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 this paper. The data for the natural breaks are shown in Tables XI and XIII. Certain differences between the distribution of the induced and natural breaks are apparent at once. Among the 73 induced breaks in the third chromosome, 10 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 (5 in each), and no breaks were observed in sections 66, 67, and 73. The number of induced breaks in section 76 is below, while in section 66 it is above, the expectation. The number of natural breaks found in the proximal 1/5 of the chromosome is very low (2). The number of natural breaks found in the distal 1/5 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. The breakage points in these inversions are shown in Table XIV. A comparison of the induced and naturally occurring inversions can be made. 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 are identical or even similar to any of the natural ones with respect to the position of their 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 any two of these breaks have 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 quite exactly established (section 69C is one of the most difficult ones for observation). The natural inversion Cowichan has both of 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 might be expected if the distribution of breaks were random. It is of interest to inquire whether a similar phenomenon is observed in Drosophila pseudoobscura. If 2, 3, 4, or more chromosome breaks are induced in the same gamete, their distribution among the

different chromosomes might be calculated with the aid of the simple formula $(a + b + c + d + e)^n$. In this formula the letters a, b, c, d, 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 the dot (V), may be assumed to be equal. The error incurred due to this assumption will be too small to be of any significance in the calculation.

Cases in which two breaks were observed in the modified gamete may be considered first. If the distribution of the breaks were at random, in 20% of the cases they would have occurred in the same chromosome limb, and in 80% in different limbs. 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 XV 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 can be made for the gametes in which 3, 4, or more breaks were recovered (Table XV). 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.

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 Kaufman (1938) and Bauer (1939) for Drosophila melanogaster.

The Reunion of the Broken Ends of Chromosomes

In cells in which several breakages have been induced, the resulting fragments may reunite in a variety of ways. Let us consider, for example, cells with four breaks. The eight fragments may reunite to form two independent inversions or translocations. In this case the rearrangements may be symbolically designated as 2,2 meaning that two independent interchanges are present. On the other hand, the fragment of chromosome A may unite with the fragment of B, another fragment of B with C, C with D, etc. Such rearrangements are symbolized as 4. Since the rearrangements may be visualized as resulting from breakage of four strands united at the same point, the relative frequencies of the 2,2 and 4 classes of aberrations among the cells with four breaks is of interest.

An analysis of the aberrations obtained in the X-ray experiments from the above point of view is presented in Table XVI, in which the expected frequencies of the various types are also indicated. The calculations of these expectancies were made, following Bauer, Demerec, and Kaufmann (1938), by a simple trial and error method. Let us again consider the cells with four breaks. The original chromosomes are AB CD EF and GH. The fragments may reunite in 2,2 combinations in the following three ways: AD, BC, EH, FG, and AF, BE, CH, DG, and AH, BG, CF, ED. Six kinds of progressive reunions are possible, namely AD, CF, EH, GB, and AF, CH, ED, GB, and AH, CF, ED, GB, and AD, CH, ED, GB, and AF, CB, ED, GF, and AH, CB, ED, GF. Thus, in case the reunion of the

broken ends of the chromosomes is at random, the relative frequencies of the 2,2 and 4 combinations will be as 3:6. Now the breaks may be distributed as 2,1,1 (i.e. one chromosome contains two breaks and two other chromosomes contain one each). It may be shown by a method similar to the above that with a random reunion, the frequencies of the 2,2 and 4 combinations must be as 3:14. For the remaining types (2,2 and 3,1 and 4) the ratios will be 3:22. The ratios computed above must now be multiplied by the expectancy of each type indicated in Table XV. Thus the final expected ratio for the 2,2 and 4 interchanges turns out to be 300:1432. Since 19 aberrations involving four breaks each were observed in Drosophila pseudoobscura, the expected frequencies of the 2,2 and 4 cases are 3.98:15.02 (Table XVI). The difference between observation and expectation is very striking and fully significant statistically. The 2,2 reunion is much more frequent and the 4 reunion is much less frequent than would be expected if the reunion were random. Our data for five-break cases are too small for a statistical analysis, but here also the 2,3 reunion is much more frequent than expected on chance. This confirms the similar results obtained for Drosophila melanogaster by the authors quoted above.

Number of Breaks per Sperm

The number of aberrant sperms in which 2, 3, 4, 5, and 6 breaks were observed is shown in Table XVII. From these data it is easy to compute the average number of breaks per changed spermatozoan. 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 meaning of this difference may be established only by further investigation.

Mosaics

In the course of the present study several interesting types of chromosomal aberrations were noticed. The first group to be described is that of mosaics. Mosaic mutations (fractionals) have been known for a long time, but chromosome mosaicism is relatively seldom observed. L. V. Morgan (1939) has described a spontaneous translocation in Drosophila melanogaster which arose in only a part of the body of the fly, and was observed in some cells of its salivary glands, but not in others. Lewitsky and Araratian (1931) have observed different chromosome configurations in different cells of the root tips of Crepis treated with X-ray.

Unexpectedly enough, some of the slides in the present investigation proved to have two or more sorts of cells, some

showing a certain chromosomal aberration and others being normal. It follows that the larvae from which these slides were made were mosaics in which some cells were unchanged while others were modified by X-ray treatment. A suspicion arose that this might be due to contamination. Several facts rule out this supposition. As already stated above, the salivary glands of each larva were stained in a separate container. The slides were so made that each one had but one set of glands. Finally, a mosaic was found in which different cells contained different aberrant chromosomal types which were, however, related to each other. A description of two mosaics has already been published (Helfer, 1940). This description need be but briefly reviewed here. One of these two mosaics contained four different types of tissues.

"An analysis was made of each of the two glands. In one gland, a total of 42 cells proved to be satisfactorily analyzable; the precise status of 8 cells was in doubt and the rest were not clear enough to attempt a classification. The four types of cells are as follows. The first and by far the most frequent type, observed in 58% (24 out of 42) cells examined, departs from normal in having a translocation between the third and probably the Y-chromosomes .... In terms of the maps published by Dobzhansky and Tan, the third chromosome is broken in section 80, between the first and the second dark discs distal to the 'bulb.' As the Y-chromosome in salivary glands is not a distinct body, being simply a part of the heterochromatic chromocenter, it is impossible to determine the position of the break in this chromosome. The second type of cells (11% of the total analyzed)

contains a translocation involving the third and the fourth chromosomes. The third is broken at about the middle of section 66, and the fourth is broken in section 97, the major part of the third being exchanged for the distal end of the fourth chromosome. The third type of cell (19%) is a combination of the preceding two, i.e., the III-Y translocation is present together with the III-IV one. Finally, the fourth type (11%) are normal cells, apparently free from any cytologically detectable abnormality. The second gland of this set contained 15 analyzable cells, 12 doubtful ones and the rest too poor for classification. Again the most frequent type of cell was that having the III-Y translocation (11 out of 15). There was only one clear-cut example of the III-IV translocation, none of the III-Y, III-IV; and three examples of normal third chromosomes ....

"The second mosaic pair of salivary glands contained only two types of tissue. The aberrant tissue consisted of an inversion in the second chromosome for the proximal part of region 43 to the distal part of region 45. The other type of tissue was normal. In one of the two glands 32 out of 48 analyzable cells contained the aberration; in the other gland, out of 36 analyzable cells, 15 were aberrant."

The mechanism of the origin of these two mosaics has been discussed in the publication quoted (Helfer, 1940). It will suffice to mention here that the first of the two mosaics strongly supports the "breakage first" hypothesis of Stadler. The other mosaic can be interpreted equally well by any one of several mechanisms.

Since the publication of the above report, five more mosaics have been found. The first and most interesting of these was a mosaic involving three types of tissue. Some cells contained normal chromosomes; others had an inversion in the fourth chromosome; while, finally, one cell showed an inversion in the fourth chromosome which differed from the other inversion observed. Unfortunately, the slide showing this mosaic was not of the best, as the two salivary glands were not entirely separate from each other and, further, only 19 cells were found to be satisfactorily analyzable. Among these, four cells contained normal chromosomes. In 15 cells an inversion extending almost from the boundary between sections 83 and 84 to the proximal part of section 89 in the fourth chromosome was present. A single cell contained an inversion in the fourth chromosome with the proximal break apparently identical with that of the inversion just described (i.e., on the boundary of sections 83 and 84). The second break lay, however, much more distally, namely in the proximal part of section 99 near the border of section 98.

Several possible explanations of the origin of this mosaic may be suggested. It may have arisen from an egg fertilized by three spermatozoa. One of these spermatozoa would have had to be normal and the two others to have included inversions in the fourth chromosome. This explanation is improbable, however, since the two inversions apparently have one of the breakage points in common. It is very doubtful that two spermatozoa fertilizing the same egg might, by coincidence, have identical breaks. Another possibility is that two inversions arose spontaneously during the

cleavage of the developing egg. This is improbable since spontaneous origin of inversions is extremely rare, and moreover, one would still have to assume that the two independently arising inversions had one break in common. The third hypothesis, and the one which seems to fit the data best, is that the X-ray treatment broke or injured the fourth chromosome at three loci (sections 83, 89, and 99), but that the broken ends did not recombine immediately. If the zygote containing this broken chromosome could have undergone one normal division, the two different inversions could have arisen in the two cells resulting from the next division of one of the daughter cells: the other daughter cell giving rise to normal tissue. This explanation seems to be the most logical one and finds its support in the fact that it is also the explanation which best accounts for the origin of the above mentioned four-tissue mosaic.

The remaining four mosaics observed consisted of two types of tissue only (i.e. normal and aberrant). The aberrant tissue of the first of these involved a translocation between XR and IV. Two others contained II-III translocations. The aberrant tissue of the last mosaic observed consisted of a III-Y translocation. In these last four larvae it is not possible to suggest any one method to account for their origin which would be more likely than several others. It is safe to say, however, they are not merely contaminations resulting from faulty technique.

A Rearrangement involving Maternal as well as Paternal Chromosomes

Another interesting type of aberration observed is one which can be explained only on the assumption that two homologous strands of the same chromosome are involved. The fact which affords the evidence for this assumption is the presence of two "trifurcations" (Figure 1A and 1B). A "trifurcation" is a configuration wherein three chromosome strands meet at one point to form a Y-shaped figure (Figure 2A and 2B). Such a configuration in the salivary chromosomes is possible only if two homologous chromosomes are involved. In this case, it is derived as the result of a deletion in one homologue of the second chromosome, and a corresponding duplication of the deleted section in the other homologue of the same chromosome accompanied by a large inversion.

There is a total of six breaks in the rearrangement, of which five are in the second (three in one homologue and two in the other) and one is in the fourth chromosome. The mechanism by which such an aberration may have occurred is best explained by one of the two following hypotheses. The first of these, and the one which seems more probable, is one assuming that the two homologues involved are paternal and maternal respectively (i.e. the one containing more breaks is presumably the paternal one). The paternal homologue is involved in a translocation with 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 α β. 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'. α β 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 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 (FG) might have become deleted from this maternal strand and transferred to the paternal strand at H'; then the two most distant 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). According to him, 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, as 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 can not be disregarded as the evidence available does not distinguish between them.

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 (Figure 3A). 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 diagram is given in Figure 3B.

Branched Chromosome

A rearrangement has been observed involving an attachment to the side of one of the chromosomes which is best explained on the basis of a branch. In this aberration the fourth chromosome is broken in region 93 (distal), the left limb of the X in the proximal part of region 16, and the second chromosome in region 52. The tip of the fourth chromosome is translocated to the base of the left limb of X; the tip of the left limb of X (in duplicate) is inserted into the side of the second chromosome; the remaining base of the fourth chromosome is left without a free end.

It has been suggested that the rearrangement is not a true branch but a reverse duplication analogous to dominant eyeless (Bridges, 1935). That this is not the case, however, is shown in Figure 4A and 4B, wherein the two tips of the XL branch lie separate from each other. Although Kossikov and Muller (1935) have pointed out the improbability of such a branch being able to propagate itself, on the basis of the present evidence, there seems to be no explanation which may better account for it.

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 concerned with the mechanisms of the origin of chromosomal variations, particularly under the influence of X-rays. These two aspects will be discussed separately.

Dobzhansky and Sturtevant (1938) have found that natural populations of Drosophila pseudoobscura show a large amount of variation in their chromosome structure. In most of the localities from which population samples were studied the populations proved to be heterogeneous. Some individuals have gene arrangements of one type, others of a different type. With the exception of the variability of the Y-chromosome (Dobzhansky, 1935, 1937) which appears to be due to duplications or deficiencies, all of the other known variations may be accounted for by inversions of chromosome segments. Homozygotes and heterozygotes are found for each gene arrangement and their relative frequencies are in accord with the expectation based on the formula $q^2 + 2q(1-q) + (1-q)^2$, where q and (1-q) are the frequencies of each of the gene arrangements involved (Dobzhansky and Queal, 1938a). The fact that the predictions based on this formula (known as Hardy's formula) are borne out by observations shows that the presence of inversions does not split the population into non-interbreeding groups, each characterized by the possession of a definite gene arrangement. The natural populations are panmictic.

A peculiar fact discovered by Dobzhansky and Sturtevant is, that in Drosophila pseudoobscura, the different chromosomes vary by no means to the same degree. The third chromosome is by far the most variable one and 17 gene arrangements were discovered in it. This compares with three in XR, two in XL, six in II, and one in IV. So far, no variation has been detected in the dot (V). Furthermore, the variations in the third chromosome occur throughout the geographic distribution area of Drosophila pseudoobscura with the possible exception of the regions of northern Arizona and New Mexico and southern Utah and Colorado. On the contrary, in most localities, the populations are uniform with respect to the gene arrangement in the second chromosome. The X-chromosome differs in the two races, A and B, into which the species Drosophila pseudoobscura is split (Lancefield, 1929; Tan, 1935) and it varies also in connection with the so-called "Sex-ratio" condition (Sturtevant and Dobzhansky, 1936). On the whole, it seems to be unequivocally established that in nature the third chromosome in this particular species shows a much greater variability than the rest. One of the possibilities that might account for this phenomenon is that the third chromosome, for some reason, is more breakable than the others and thus more prone to give rise to gene rearrangements, particularly inversions. To test this, the distribution of the X-ray induced breaks among the chromosomes of Drosophila pseudoobscura has been studied.

It must be noted here that a similar study made in Drosophila melanogaster by Bauer, Demerec, and Kaufmann (1938)

and by Bauer (1939) has shown that the X-ray induced breaks are distributed among the chromosomes at random, that is in proportion to the length of the latter. The data of Sturtevant (1931) and of Dubinin, Sokolov, and Tiniakov (1936, 1937) indicate, however, that in the wild populations of Drosophila melanogaster the gene arrangement is variable in at least four out of the five long chromosome limbs (i.e. in IIL, IIR, IIIL and IIIR). Nevertheless, the data reported in the present paper fully agree with those of Bauer, Demerec, and Kaufmann; in Drosophila pseudoobscura, as in Drosophila melanogaster, the distribution of breaks is at random and the third chromosome does not show the slightest sign of an increased breakability.

One is forced to look elsewhere for an explanation of the great variability in the nature of the gene arrangement of the third chromosome of Drosophila pseudoobscura. A possible explanation was suggested by Sturtevant and Mather (1938). According to their theory, a slight selection pressure exists in natural populations tending to make the population non-uniform with respect to the gene arrangement. The argument of Sturtevant and Mather is somewhat as follows: A large proportion of the chromosomes in wild populations carry recessive mutant genes deleterious to the viability of the fly (Dubinin and collaborators, 1934; Sturtevant, 1937; Dobzhansky and Queal, 1938b). Now if this population has two gene arrangements in any one chromosome which may be denoted as arrangements M and N, these two arrangements may, due to chance, differ with respect to the deleterious recessive

genes they carry. Since recessive genes are eliminated by natural selection only when they exist in homozygous condition, the homozygotes MM and NN will be eliminated somewhat more frequently than the heterozygotes MN; consequently, the two gene arrangements will tend to become equally frequent. Of course, some deleterious recessives will occur in both arrangements but these will have no effect one way or the other on the frequencies of these gene arrangements. The factor just described will tend to make all the chromosomes of the species (with the possible exception of the X-chromosomes) non-uniform for gene arrangements in natural populations. Sturtevant and Mather point out, however, that the appearance of inversions has also a disadvantageous consequence, namely the suppression of crossing over; hence it limits the formation of new combinations of genes desirable for preservation of the evolutionary "flexibility." To quote them, "The two effects, recombination-reduction and heterosis, are in opposition, and their balance will determine the fate of inversions arising in a wild population." The chromosome which, by chance, develops an inversion first will be the one to become variable with respect to the gene arrangement, while other chromosomes will be kept more uniform to preserve the advantage of free recombination.

This theory of Sturtevant and Mather gives a rather satisfactory account of the conditions found in Drosophila pseudoobscura. It is somewhat contradicted by the fact that in other species of Drosophila (e.g. Drosophila melanogaster, see above; Drosophila azteca, Dobzhansky and Sokoloff, 1939; Drosophila

algonquin, Miller, 1939) several chromosomes appear to be about equally variable. The available knowledge of the mechanics of wild populations is too meager, however, and it is probable that unknown factors are at play which influence the survival of chromosome variations in different species.

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 to each other mostly as overlapping inversions. Overlapping inversions are those in which one of the breaks of the second inversion falls within, and another outside of the region affected by the first inversion (for example, 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). According to Dobzhansky and Sturtevant, any two gene arrangements differing in two overlapping inversions most likely did not arise directly from each other, but through an intermediate step, which in this case 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. Such a phylogeny actually has been established for the gene arrangements found in the third chromosome of Drosophila pseudoobscura.

The above mentioned theory of Dobzhansky and Sturtevant implies, however, that a repeated origin of the same gene arrangement is unlikely, because it would require that a chromosome break repeatedly in the same two or more identical places. If A E D C B F G H and A E D G F B C H have arisen from A B C D E F G H

not through each other, but independently, the chromosome must have been broken repeatedly between A and B and simultaneously between E and F. Assuming that a chromosome is equally likely to break at any locus and that there are 500 loci per chromosome, the probability that, by coincidence, the same two breakages will occur twice is, 500^2 or one in 250,000 inversions, which is very small. The likelihood of the same inversion occurring repeatedly would not appear very small, however, if the assumption of equal probability of the breakage of the chromosome at any one locus is not granted. It is possible that 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 breakage at a given point somehow induces the chromosome to break at a specific other point; in other words, it is conceivable that there exists a certain modal length of the chromosome section lying between the correlated breakage loci. Evidently, only an experimental test can discriminate between these possibilities.

The experimental data presented 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 the induced breaks within chromosomes is approximately, though not entirely, random. No chromosome sections have been shown to be immune to breakage and no sections proved to be so breakable as to deserve the designation of "weak points." Above all, there

is no correlation between the relative positions of two or more breaks if they occur in the same chromosome. Considering the relative lengths of the various sections as they appear in salivary gland cells, one observes that the heterochromatic parts show a high concentration of the induced breaks. According to Bauer, the breakability of heterochromatin is proportional to its length as observed in mitotic rather than in salivary chromosomes; according to the data of the present investigation, it is somewhere between what would be expected on the basis of the mitotic and the salivary lengths. In any case, not only is the heterochromatic portion of the third chromosome of Drosophila pseudoobscura infrequently broken in the inversions encountered in nature, but on the contrary, no "natural" breaks are known in the heterochromatin. It looks almost as though for some unknown reason inversions involving the heterochromatin are not retained in natural populations (according to unpublished data of Sturtevant, inversions involving heterochromatin are, however, found in natural populations of Drosophila robusta). The frequency of breaks within the euchromatic portions of the chromosome increases slightly though significantly from the proximal (spindle attachment) to the distal (free) end. As far as the data of Dobzhansky and Sturtevant on the distribution of the natural breaks go, they seem to indicate a similar regularity, although here the statistical validity of this conclusion is open to question.

Within each major subdivision of the chromosome (i.e. the proximal, middle, and distal parts) there is no statistically

significant concentration or scarcity of either the induced or of the natural breaks in any one specific section. Of course the data now available are not sufficient enough in extent to prove that such concentrations and scarcities may not be discovered. At any rate, the sections in which the apparently great or low densities of the induced breaks are observed do not coincide with the sections in which many and few natural breaks happened to lie. More important still, among the induced inversions, not a single one proved to be identical or even similar to any of the naturally occurring inversions. Among the induced breaks in general (i.e. those observed in the translocations as well as inversions) a few proved to lie at the same or at nearly the same loci as some of the observed natural breaks. The number of such coincidences is small, however, and in only a single case is there a reason to suppose that a real coincidence is involved, i.e. that an induced and a natural break have been observed to occur between exactly the same two adjacent discs. Thus, the present data appear to support the assumption made by Dobzhansky and Sturtevant (1938), that there is little probability of a repeated origin of the same inversion to occur in nature. Of course, with the enormous numbers of individuals and the enormous numbers of generations available in nature, such a repeated origin of the same inversion in nature is not altogether excluded; all that is really certain is that the gene arrangements encountered in nature are not transformed into each other at frequent intervals.

The problem of the mechanism of the origin of chromosomal aberrations is a very difficult one. Two alternative hypotheses have been proposed, namely the "contact hypothesis" of Serebrovsky (1929) and the "breakage first" hypothesis of Stadler (1932). According to the contact hypothesis the chromosome breakage and reunion of the resulting fragments into new associations occur simultaneously, being in effect only two aspects of the same process. It is assumed that due to the action of X-ray radiation or due to chance, two or more chromosome threads occasionally may come in contact or in a very close approximation. Exchanges of chromosome sections then occur at the contact points, perhaps due to a process somewhat resembling crossing-over. Consequently, the contact hypothesis is sometimes called the hypothesis of illegitimate crossing-over. The breakage first hypothesis assumes that if chromosomes are broken by some agent, or agents, the resulting broken ends tend to reestablish connections with each other. If the broken ends of the same chromosome become reunited, the old gene arrangement is restored and no chromosome aberration is formed; if, however, broken ends meet which have not been united before breakage, a genetically detectable result is produced.

One might think that the simplest way to discriminate between these hypotheses would be to observe living cells treated by some agent known to induce chromosomal aberrations. Due to the technical difficulties involved in making such observations in the living material this method has not been used, and all the work done to date consists in detecting either genetically or cytologically

the changes produced by irradiation in fixed and stained material. From the kinds of changes observed, inferences are made as to the probable nature of the mechanism that could have produced them (Catcheside, 1938a, 1938b; Bauer, Demerec, and Kaufmann, 1938; Sax, 1937, 1938, 1939, 1940; Muller, 1938; Bauer, 1939; Eberhardt, 1939; and others). Unfortunately, it so happens that both the contact and the breakage first hypotheses lead to almost the same predictions with respect to the consequences of the treatment that can be observed by the methods employed. It has proven to be exceedingly difficult to devise experimental procedures that would permit a discrimination between the two. Only a few results of this type have been obtained. Bauer (1939) has shown that the relative frequency of complex rearrangements (i.e. of rearrangements involving numerous exchanges within a single "contact point") increases with increasing amount of X-ray treatment. This seems to favor the breakage first hypothesis. According to the contact hypothesis, a complex rearrangement arises if a "knot" in which many chromosome threads are associated is "hit" by X-ray. The frequency of such "knots" will be independent, of course, of the amount of treatment applied, provided that the treatment is administered at the same stage of the life cycle. According to the breakage first hypothesis, however, complex rearrangements arise if many broken chromosome ends are available; thus, the stronger the treatment the more numerous the broken ends, and the greater the chance for complex aberrations.

Sax (1939, 1940) has observed the changes induced in the plant Tradescantia after different time intervals after the X-ray treatment. He found that the number of the chromosomal observations observed depends not only upon the amount of treatment but also upon the manner in which it is administered. The same amount of treatment in terms of r-units is more effective if given within a short time than if given in several portions with intermittent periods of rest. The contact hypothesis would lead one to expect that continuity or intermittency of the treatment should make no difference (one may recall that continuous and intermittent treatments produce the same numbers of gene mutations (Timofeeff-Ressovsky, 1937). On the other hand, if the breakage first hypothesis is true, and if the chromosome fragments retain the ability of becoming re-attached only for a certain length of time, then the results of Sax might be expected. Other results of Sax, such as the dependence of the number of chromosomal aberrations observed with a given treatment upon the environment--particularly temperature, may be considered due to be more favorable to the breakage-first, rather than to the contact hypothesis.

Some of the results of the present study seem to give further support to the breakage first hypothesis. This is particularly true for the mosaic chromosomal aberrations, i.e. for cases where individuals coming in the F_1 generation from treated father and untreated mother were found to have two, three, or even four kinds of tissue with different chromosome configurations.

The appearance of such mosaics is fully compatible with the breakage first hypothesis, provided the breaks, or the weak points in the chromosomes induced at the time of the treatment 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 maternal and the paternal chromosomes may be similarly interpreted. These mosaics are very difficult to explain from the standpoint of the contact hypothesis. Of course, one could suppose that the mosaics were not induced by the X-ray treatment at all, but arose spontaneously during the cleavage divisions. This is not altogether impossible, but very improbable since the spontaneous origin of chromosomal aberrations in somatic tissues is extremely rare. Thus far a single case of this sort has been conclusively established in Drosophila melanogaster (L. V. Morgan, 1939). In the material of the present study there are, however, mosaics among which one has three, and one four, kinds of tissue. For these last mosaics one would be forced to suppose that spontaneous changes have, by chance, taken place in at least two consecutive cleavage divisions—a possibility which is obviously very remote.

Another way to reconcile the occurrence of the mosaics with the contact hypothesis is to suppose that at the time of treatment the chromosomes of the fly are not in the form of single threads but are split once (double), or even twice (quadruple). Such a supposition is not out of the question, since certain investigators, for example, Nebel (1936, 1937a, 1937b), believe

they have observed cytologically that chromosomes are double or quadruple in telophases of cell divisions. This is definitely denied by other investigators (Darlington, 1937). Without going into a consideration of these very involved problems, one may point out that, even granting that chromosomes are split in spermatozoa, it remains difficult to reconcile the observed mosaics with the contact hypothesis. The mosaic showing four types of tissue has cells of the following kinds: (1) with normal chromosomes, (2) with a III-Y translocation, (3) with a III-IV translocation, and (4) with a rearrangement combining the III-Y and III-IV translocations. Not only would one have to suppose in this case that the chromosomes were quadripartite at the time of treatment, but also that a phenomenon akin to a somatic crossing-over involving sister chromosome stands had taken place. The mosaic containing three kinds of tissue would require not only the supposition of quadripartite chromosomes, but an additional one that two of the four strands were, by chance, broken at exactly the same locus.

In conclusion, a few remarks may be made regarding the "unorthodox" chromosome aberrations encountered in the material of the present study, namely the one involving the 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 or attachments to free ends of other chromosomes) at first was interpreted as an argument in

favor of the contact hypothesis. According to the breakage first hypothesis, single breaks must be produced, and in fact the frequency of two break aberrations must be equal to the square of the frequency of single breaks. Muller (1938) has thus concluded that single breaks do occur but none of them are recovered as stable chromosome rearrangements since they are inviable. According to this view the free ends of each chromosome or "telomeres," are endowed with special properties not present in genes occupying the interstitial positions. A chromosome without a telomere is inviable, and non-terminal genes do not acquire telomere properties. On this assumption, the three terminal deficiencies described by Demerec and Hoover (1936) must be in reality two break cases in which the subterminal breaks are so close to the telomere that they escape detection by microscopical observations.

The "telomere" is supposed to be ultra-microscopic in size, and therefore, the theory cannot be tested cytologically. There is, however, some indirect evidence that seems to argue against the hypothesis of the telomere. Among Stadler's deficiencies induced by ultraviolet radiation, a majority appear to be terminal (Stadler, 1940); if they are not terminal, one would have to suppose that ultraviolet radiation is specific for the induction of breakages very near to the telomeres. The results obtained by McClintock (1938a, 1938b) may also have some bearing on their problem. She has studied cytologically the behavior of a ring chromosome in maize. When rings are broken,

due to formation of bridges resulting from crossing-over between the strands, the broken ends reunite to form new rings. She has also found that in plants which are heterozygous for two inversions there is an absence of bridges at the second meiotic division. It may be mentioned in passing that in chromosomes heterozygous for an inversion a cross-over within the inversion will result in the formation of a chromatin bridge at anaphase. Such an absence of bridges in the second meiotic division of the above case may be explained on the assumption that the broken ends of the two chromosomes resulting from the break in the first meiotic bridges are too far away to fuse. The result which makes this explanation doubtful is the fact that 4-strand crossing-over in an individual heterozygous for an inversion also gives negative results in this respect (although again there would be two bridges formed at the resulting anaphase). The alternative hypothesis is that the chromatids of the chromosomes are double at anaphase and that the ends of these sister chromatids unite. Such an occurrence would lead to a formation of a bridge in first mitotic division in the microspore. This is what McClintock obtains. This property of reunion of sister chromatids of broken ends may be the reason why such aberrations as terminal deficiencies are rare. If such a phenomenon occurred it would lead to the formation of mitotic bridges. Breakage of such bridges is apt to be very unequal and thus to lead to inviable cells, the end result being its elimination. More recently, McClintock (1939) found that under certain

conditions the broken chromosome ends may become stabilized. This being the case the telomere hypothesis is unnecessary. In any case, even if the terminal portion of the chromosome under normal conditions is essential for the survival of the chromosome in question, it is possible that terminal genes may, by a kind of mutation, acquire the properties of interstitial genes, and vice versa. The rarity of terminal deficiencies, inversions, and other aberrations may be due to the fact that they can be formed only if mutations producing a new telomere or abolishing an old one arise in a cell which has other chromosome breakages with which exchanges can be effected. The observation depicted in Figure 3 may belong to this class. As closely as can be ascertained by microscopic examination, chromosome II is broken only once, and its terminal portion inserted, in an interstitial position, into chromosome IV.

Due to considerations which are similar in principle to those that have led to the telomere hypothesis, Kossikov and Muller (1935) came to the conclusion that no attachments of chromosome fragments to the sides of other chromosomes, forming branched structures, may occur. Yet an apparent side attachment of a diploid tip of XL to chromosome II has been observed in these data (Figure 4). This case resembles the duplication known as Dominant Eyeless in Drosophila melanogaster. According to Bridges (1935), the Dominant Eyeless represents, however, an intercalation of two homologous sections of an unknown origin fused at the tip, so that the side attachment is in reality only

an apparent one. A similar explanation may possibly apply also to the case described here for Drosophila pseudoobscura, although it is contradicted by the observation that in some cells the free ends of XL may flare apart, as though not attached to each other. To suppose that this is due to an artifact, such as pressure of the cover slip, seems very improbable. The question, evidently, remains an open one.

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 the lengths of the latter.

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 observations 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 involving a terminal attachment, and another showing what appears to be a branched chromosome, are described.

TABLE I.

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

Two break cases

Inversions in Chromosome	Number of Cases	Translocations involving Chromosomes	Number of Cases
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 in Chromosomes		III-IV	3
		III-V	1
IV	1	III-XR	2
		III-XL	3
Deletions in Chromosomes		III-Y	2
		IV-V	1
II	1	IV-XR	7
III	1	XR-XL	1
IV	1		
XR	1		

(cont. on next page)

TABLE I (continued)

Three break cases

Translocations involving more than Two Chromosomes	Number of Cases	Inversions in Chromosomes	Number of Cases
II-XL-XR	1	II (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		

(cont. on next page)

TABLE I (continued)

Four break cases

Translocations involving more than Two Chromosomes	Number of Cases	Inversions and Interchromosomal Translocations between Chromosomes	Number of Cases
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		
II-IV and II-III	1		
II-XR and XR-XL	1		
III-II and IV-XL	1		
II-IV and IV-II	1	Inter- and Intrachromosomal Translocations	
		Intrachromosomal in XR, Interchromosomal III-XR	1

(cont. on next page)

TABLE I (continued)

Five break cases

Translocations involving more than Two Chromosomes	Number of Cases	Inversions and Interchromosomal Translocations of Chromosomes	Number of Cases
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

Complex Translocation involving exchanges and Transfer of Interstitial Section of One Chromosome into Another		Inversions and Intrachromosomal Translocations of Chromosomes	
Tr. II-Y, Section of IV into Y	1	Inv. II, Intrachromosomal Tr. IV	1
Tr. II-XR, Section of IV into XL	1		
Tr. XL-IV, Section of XR into XR	1		

Six break cases

Complex Translocation involving Maternal and Paternal Chromosomes	Number of Cases
II-II-II-II and II-IV	1

Complex Translocation involving Deletion and Inversion of Chromosomes	
Del. III, Tr. III-IV, Del. and Inv. II	1

TABLE II

Total number of chromosome breaks observed (females and males combined).

Chromosome	II	III	IV	XR	XL	Dot	Y
Euchromatic breaks	106	64	81	36	15	8	0
Heterochromatic breaks	2	10	3	4	4	0	14
Total	108	74	84	40	19	8	14

TABLE III

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

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	106	87.830	+18.170	3.759	0.10-0.05
XR	10	68.256	87.830	-19.574	4.362	0.05-0.02
IV	8	81	70.264	+10.736	1.640	0.20
III	7	64	61.481	+ 2.519	0.103	0.80-0.70
XL	5	28.440	43.915	-15.475	5.453	0.02
Dot	0.5	8	4.392	+ 3.608	2.964	0.10-0.05

TABLE IV

Distribution of breaks in the euchromatic regions of the chromosomes (female data only).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	47	42.222	+4.778	0.541	0.50-0.30
XR	10	36	42.222	-6.222	0.917	0.50-0.30
IV	8	40	33.776	+6.224	1.147	0.30-0.20
III	7	27	29.554	-2.554	0.221	0.20-0.10
XL	5	15	21.110	-6.110	1.768	0.20-0.10
Dot	0.5	6	2.111	+3.889	7.165	0.01

TABLE V

Distribution of breaks in the euchromatic regions of the chromosomes (data for males only).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	59	54.510	+4.490	0.370	0.70-0.50
IV	8	41	43.608	-2.608	0.156	0.70-0.50
III	7	37	38.157	-1.157	0.035	0.90-0.80
Dot	0.5	2	2.726	-0.726	0.193	0.70-0.50
Y	0	0	0	0	0	0

TABLE VI

Distribution of breaks in the euchromatic regions of the autosomes (combined data for both females and males).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	106	101.570	+4.430	0.193	0.2-0.1
IV	8	81	81.256	-0.256	0.008	0.98
III	7	64	71.099	-7.099	0.709	0.5-0.3
Dot	0.5	8	5.079	+2.921	1.680	0.20

TABLE VII

Distribution of breaks in the heterochromatic and euchromatic regions of the chromosomes (combined data for both sexes).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	108	95.275	+12.725	1.700	0.20-0.10
XR	10	75.840	95.275	-19.435	3.965	0.05-0.02
IV	8	84	76.220	+7.780	0.794	0.50-0.30
III	7	74	66.693	+7.307	0.800	0.50-0.30
XL	5	36.024	47.638	-11.614	2.831	0.10-0.05
Dot	0.5	8	4.764	+3.236	2.198	0.20-0.10

TABLE VIII

Distribution of breaks in the heterochromatic and euchromatic regions of the chromosomes (female data only).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	48	46.173	+1.827	0.072	0.80-0.70
XR	10	40	46.173	-6.173	0.825	0.50-0.30
IV	8	43	36.938	+6.062	0.995	0.50-0.30
III	7	31	32.321	-1.321	0.054	0.90-0.80
XL	5	19	23.087	-4.087	0.724	0.80-0.70
Dot	0.5	6	2.309	+3.691	5.900	0.02-0.01

TABLE IX

Distribution of breaks in the heterochromatic and euchromatic regions of the chromosomes (data for males only).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	60	61.540	-1.540	0.039	0.90-0.80
IV	8	41	49.232	-8.232	1.376	0.30-0.20
III	7	43	43.078	-0.078	0.00014	0.99
Dot	0.5	2	3.077	-1.077	0.377	0.70-0.50
Y	0.5	14	3.077	+10.923	38.776	0.01

TABLE X

Distribution of breaks in the heterochromatic and euchromatic regions of the autosomes (combined data for both females and males).

Chromosome	Ratio	Number of Breaks Observed	Expected	Deviation	χ^2	Probability
II	10	108	107.450	+0.550	0.003	0.95
IV	8	84	85.960	-1.960	0.045	0.90-0.80
III	7	74	75.215	-1.215	0.020	0.90
Dot	0.5	8	5.373	+2.627	1.284	0.30-0.20

TABLE XI

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

Section	Subsection	Number of Induced Breaks	Number of Discs	Number of Natural Breaks
Hetero-chromatin	---	10	---	
63	---	0	40	
64	1-A; 1-B/C	2	25	1
65	1-C	1	24	1
66	3-B; 4-C	7	45	
67	1-B; 1-C	2	39	
68	2-A	2	37	2
69	1-B; 2-C; 1-E	4	45	3
70	2-A; 4-C; 1-D	7	39	3
71	1-A	1	21	2
72	2-A; 3-C	5	28	1
(72/73)		1		
73	2-B	2	26	
74	1-A	1	22	1
75	1-A; 2-B; 2-B/C; 1-C	6	26	2
(75/76)		1		
76		0	27	5
(76/77)		1		
77	3-B	3	13	2
(77/78)		1		1
78	2-A; 1-C	3	23	2
79	1-A; 2-B; 2-C; 1-C/D; 1-D	7	29	5
80	1-B; 3-C	4	21	1
81	1-A; 1-B	2	24	2
Total		73	554	38

TABLE XII

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
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

TABLE XIII

Distribution of breaks in natural inversions.

1	64C - 69D	(Cuernavaca)
2	65C - 75C	(Pikes Peak)
3	68D - 74B	(Tree Line)
4	68C - 79A	(Santa Cruz)
5	69C - 76C	(Oaxaca)
6	69C - 79A	(Estes Park)
7	70B - 76B	(Arrowhead)
8	70D - 78A	(Chiricahua I)
9	70C - 79B	(Texas)
10	70/71 - 73/74	(Sequoia I)
11	70/71 - 77/78	(Klamath)
12	71C - 79D	(Cowichan)
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)

TABLE XIV

Breakage points in the induced inversions in the third chromosome (combined data for both sexes).

(1) Heterochromatic region - 65C

(2) Heterochromatic region - 70C

(3) Heterochromatic region - 75B/C

(4) Heterochromatic region - 81A

(5) 64B - 75/76

(6) 66B/C - 77B

(7) 69B - 77/78

(8) 70A - 72/73

(9) 70A - 74A

(10) 72A - 75B

(11) 75B - 78A

(12) 78C - 80C

TABLE XV

Distribution of multiple breaks among the chromosomes.

Number of Breaks	Distribution	Number of Cases			Per Cent		χ^2	Probability
				Total	Observed	Expected		
2	1,1	35	22	57	61.95	80	3.744	0.05
	2	20	15	35	38.04	20	14.976	0.01
3	1,1,1	2	1	3	30.0	48		
	2,1	0	5	5	50.0	48		
	3	1	1	2	20.0	4		
4	2,1,1	4	6	10	52.63	57.6		
	1,1,1,1	3	1	4	21.05	19.2		
	3,1	3	1	4	21.05	12.8		
	2,2	1	0	1	5.26	9.6		
	4	0	0	0		0.8		
5	2,1,1,1	2	2	4				
	2,2,1	0	1	1				
	3,2	1	1	2				
	3,1,1	1	0	1				
	4,1	1	0	1				
6	2,2,1,1							
	3,1,1,1							
	3,2,1	0	1	1				
	4,1,1							
	5,1	0	1	1				

TABLE XVI

Distribution of the types of rearrangements (combined data for both sexes).

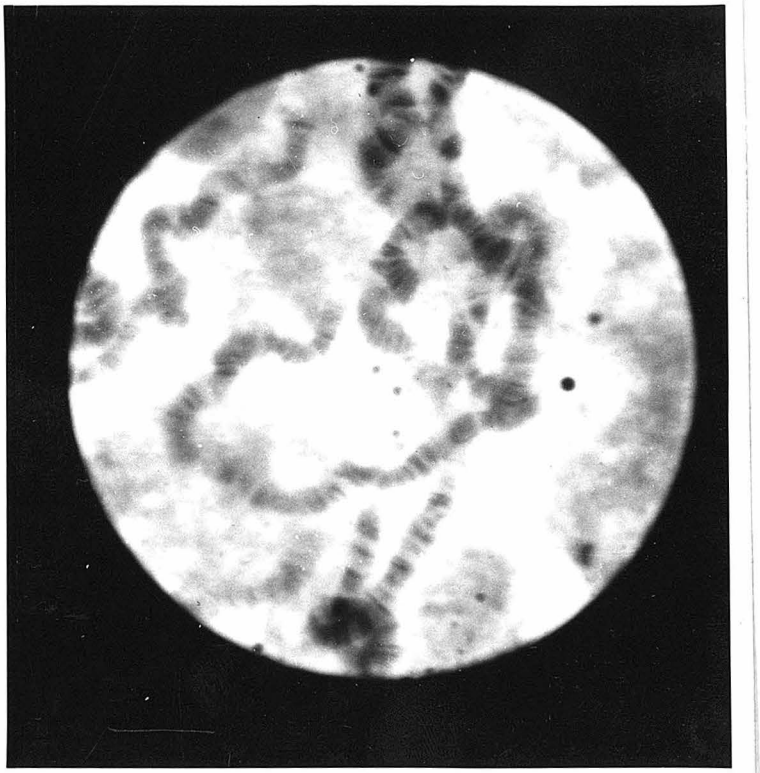
Number of Breaks	Combination of Breaks	Total Observed	Total Expected	χ^2	Probability
	2 & 2	18	3.98	49.387	0.01
4					
	4	1	15.02	13.087	0.01
	2 & 3	7			
5					
	5	2			

TABLE XVII

Distribution of the frequency of breaks per changed sperm (combined data for both sexes).

Number of Breaks	Number of Sperms	M ± m
2	92	
3	10	
		2.63 ± 0.13
4	19	
5	9	
6	2	

A



B

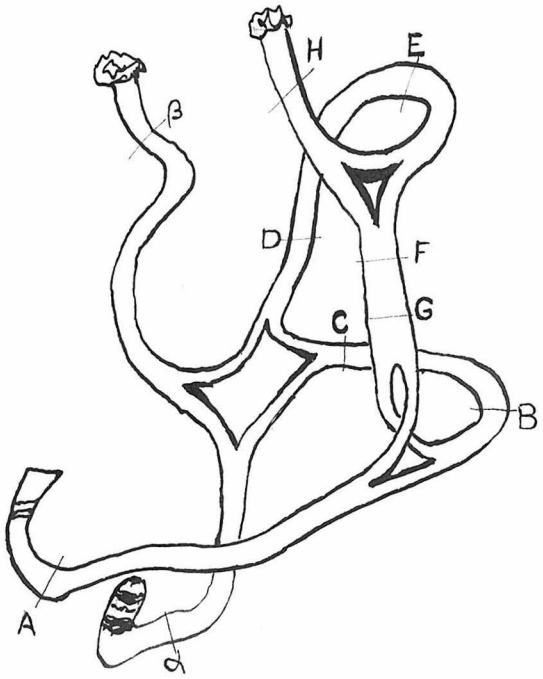
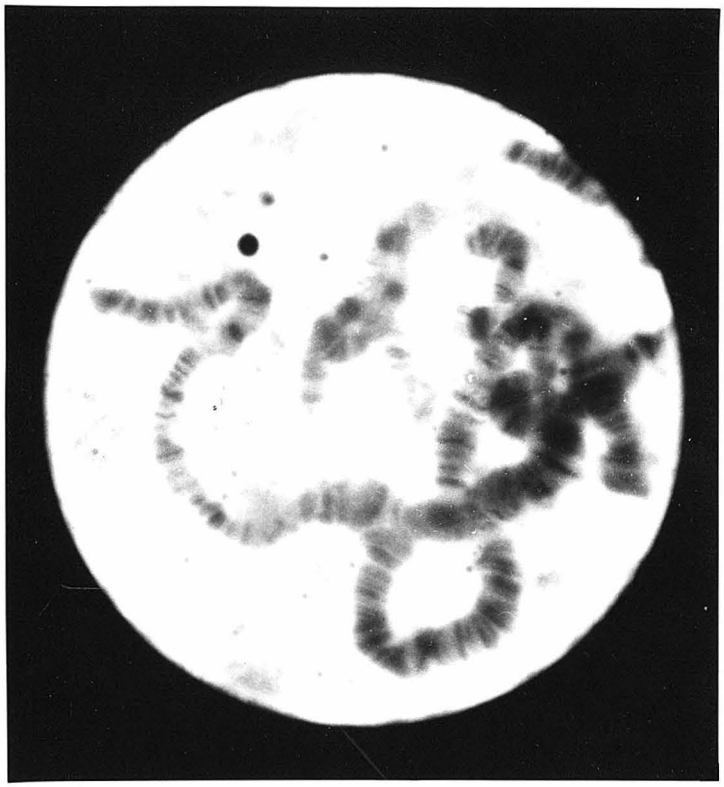


Figure 1.- A microphotograph with an accompanying diagram of the aberration involving maternal as well as paternal chromosomes.

A



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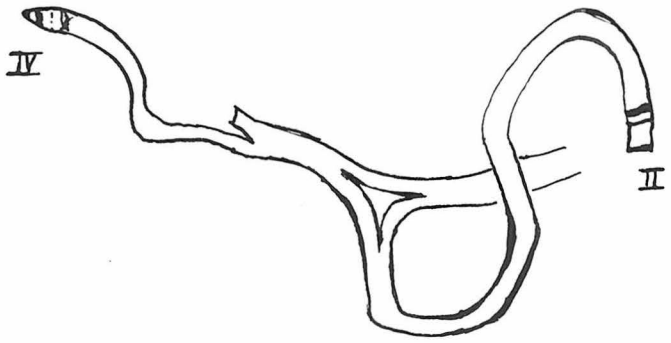
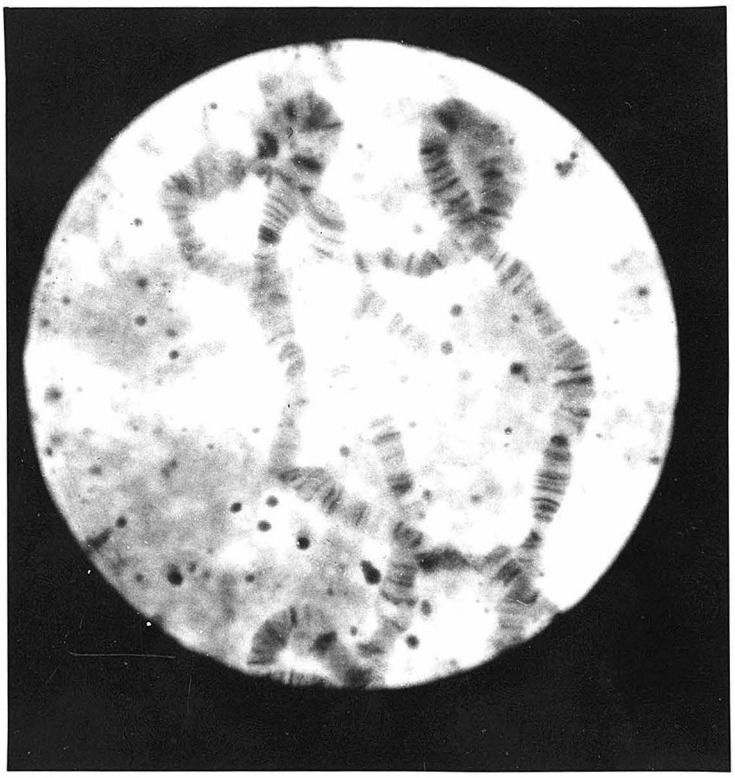


Figure 2- A microphotograph with an accompanying diagram of a "trifurcation" contained in the rearrangement involving maternal as well as paternal chromosomes.

A



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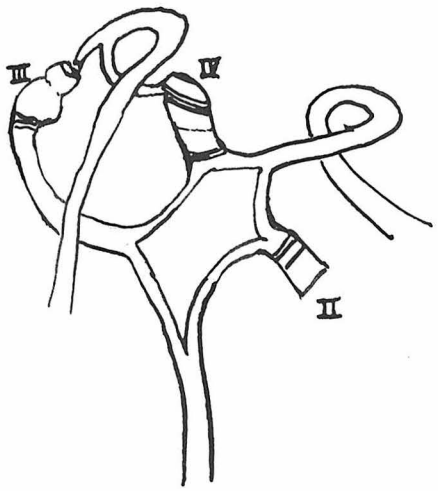
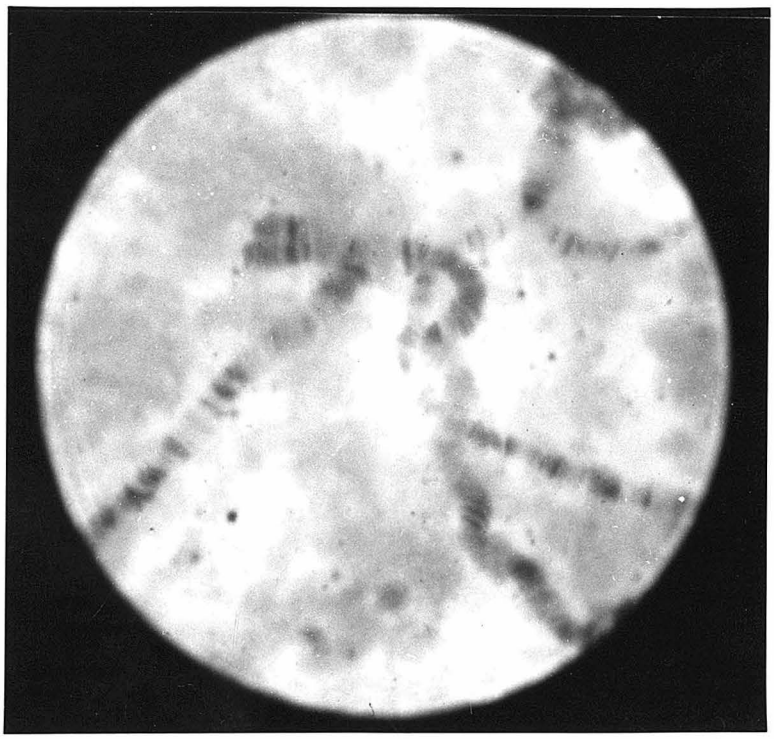


Figure 3 - A microphotograph with an accompanying diagram of the aberration involving the intercalation of the distal end of III into a break in IV.

A



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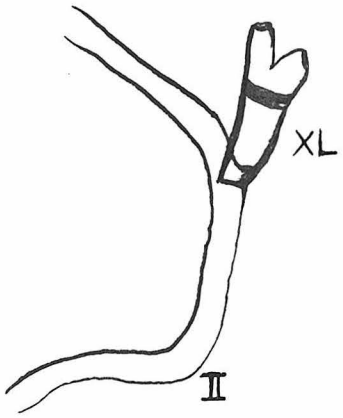

Figure 4 - A microphotograph with an accompanying diagram of the side attachment of II.

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Reprinted from the Proceedings of the NATIONAL ACADEMY OF SCIENCES,
Vol. 26, No. 1, pp. 3–7. January, 1940.

TWO X-RAY INDUCED MOSAICS IN DROSOPHILA PSEUDOÖBSCURA

BY R. G. HELFER

W. G. KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES, CALIFORNIA INSTITUTE OF TECHNOLOGY

Communicated December 4, 1939

The mechanism of the origin of chromosomal aberrations is still an open question. The so-called contact hypothesis, advanced originally by Serebrovsky,[1] assumes that translocations and other gene rearrangements are formed due to chance union of chromosomes accompanied by the development of new associations between genes, somewhat in the manner of "illegitimate" crossing-over. According to this view, the breakage of the original chromosomes and the reattachment of the resulting fragments occur practically simultaneously. The alternative hypothesis assumes that chromosomes are broken first, and that some time may elapse before the points of fracture either reunite to restore the original situation or form new attachments.[2,3] Unfortunately, the problem is such that critical evidence has been difficult to obtain.[4, 5, 6, 7] The mosaic translocations described below may possibly shed some light on the question.

Normal males of race *A* of *Drosophila pseudoöbscura* were treated with x-ray (5000) units, and outcrossed to normal untreated females. The

salivary glands of the F_1 larvae were taken, stained in aceto-carmine, and permanent smear preparations were made with the aid of the usual technique. Each slide contained only the two glands of a single individual. In the course of study of these slides, two very remarkable aberrant sets of glands were found. Instead of having the customary single type of tissue, either completely normal or having all cells containing the same aberration, these two sets of glands were mosaics of more than one kind of tissue. Several facts show that this result cannot be due to contamination (i.e., mixing the glands of several individuals in the same slide). In the first place each slide contains two and only two glands; in these particular slides the two glands lie separately. Secondly, both glands of each set are of the same sex. Thirdly, and this is the main argument, both glands of each set contain mixtures of tissues, the same cytological condition being observed in some cells of either gland.

The more complex of the two sets of mosaic glands apparently contains four different tissues. An analysis was made of each of the glands of this mosaic. In one gland, a total of 42 cells proved to be satisfactorily analyzable; the precise status of 8 cells was in doubt and the rest were not clear enough to attempt a classification. The four types of cells are as follows. The first, and by far the most frequent type, observed in 58% (24 out of 42) cells examined, departs from normal in having a translocation between the third and probably the *Y*-chromosomes (Fig. 1*a*). In terms of the maps published by Dobzhansky and Tan,[8] the third chromosome is broken in section 80, between the first and the second dark discs distal to the "bulb." As the *Y*-chromosome in salivary glands is not a distinct body, being simply a part of the heterochromatic chromocenter, it is impossible to determine the position of the break in this chromosome. The second type of cells (11% of the total analyzed) contains a translocation involving the third and the fourth chromosomes (Fig. 1*b*). The third is broken at about the middle of section 66, and the fourth is broken in section 97, the major part of the third being exchanged for the distal end of the fourth chromosome. The third type of cell (19%) is a combination of the preceding two, i.e., the III–*Y* translocation is present together with the III–IV one (Fig. 1*c*). Finally, the fourth type (11%) are normal cells, apparently free from any cytologically detectable abnormality. The second gland of this set contained 15 analyzable cells, 12 doubtful ones and the rest too poor for classification. Again the most frequent type of cell was that having the III–*Y* translocation (11 out of 15). There was only one clear-cut example of the III–IV translocation, none of the III–*Y*, III–IV; and three examples of normal third chromosomes.

Several mechanisms which may produce such a mosaic may be suggested. If one were to suppose that two sperm fertilized a single egg and that each one had one chromosome aberration in it, a mosaic individual

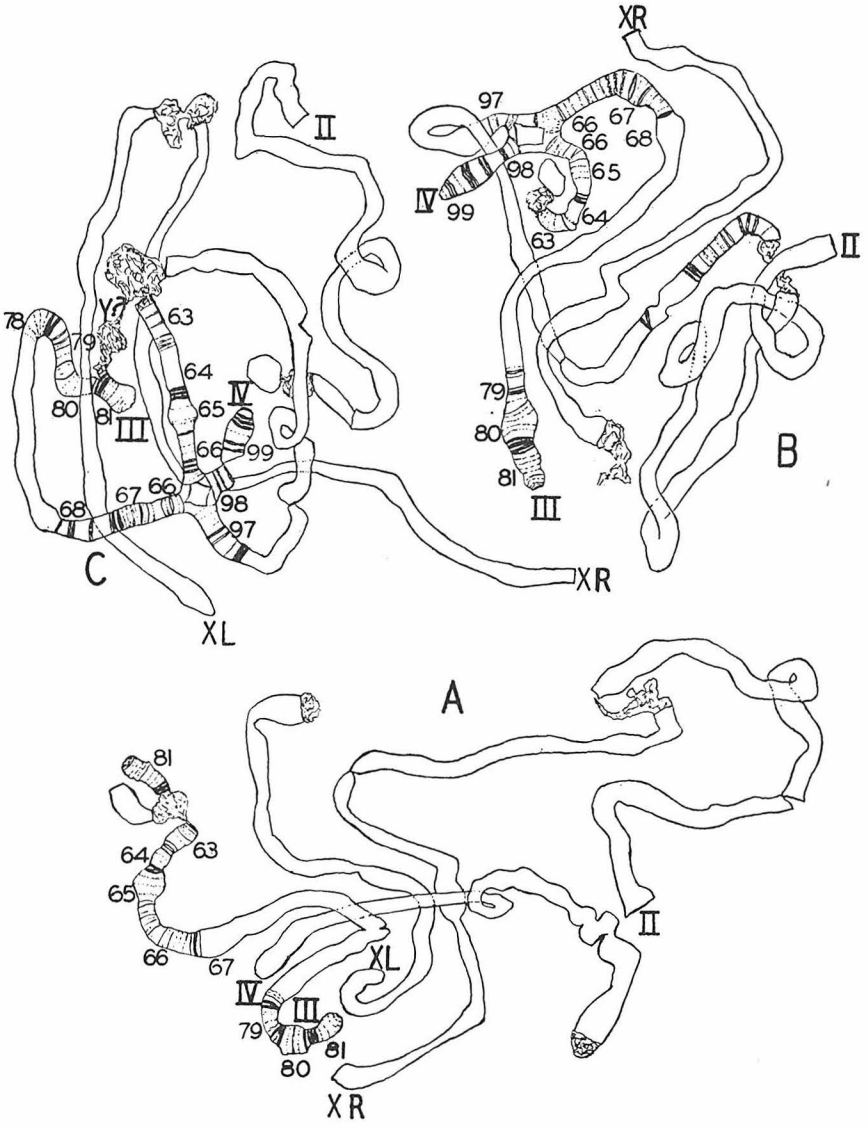

FIGURE 1

Three types of aberrant tissue found in the salivary glands of an F_1 male offspring from a cross between x-rayed males to normal females. *A*—translocation involving the tip of III and the *Y*-chromosome, *B*—translocation between the base of III and the tip of IV, *C*—combination of translocations *A* and *B*.

would result. Such an individual might have two different types of salivary gland tissue. That it is possible for both nuclei of the first cleavage division to be incorporated in the salivary gland tissue is supported by recent work of Kaufmann.[9] The points in the present evidence which automatically rule out this hypothesis are that not two but four types of tissues are present, and that one of these contains an aberration combining the properties of the two other aberrant types (Figs. 1*a* and 1*b*). Another possibility is that x-rays as such had no effect on the sperm, but that during the course of the development three types of the aberrant tissues arose spontaneously. A spontaneous translocation has been described in an individual of *Drosophila melanogaster* that has not been treated with x-ray, and this individual has been a mosaic of normal and aberrant tissue.[10] Since spontaneous chromosomal changes are relatively very rare, to suppose that so rare an event takes place three times in the development of a single individual is, however, too improbable. Still another possibility is that one translocation took place due to the irradiation (for example the III–*Y* translocation), and then another (the III–IV translocation) occurred spontaneously after fertilization in a part of the modified tissue. This view is also ruled out because of two securely established facts, namely the presence of cells with the III–IV but without the III–*Y* translocation, and of the apparently normal cells containing neither translocation. A somatic crossing-over would have to be invoked to produce these additional types.

The fourth possibility is one which assumes that the chromosomes in the sperm are in the four-strand stage. Were such the case a workable hypothesis could be developed to account for the formation of a four-tissue mosaic. For example, if one supposed that the breaks induced by the x-ray at any level effect only two of the four strands present, and if in the third chromosome two of the strands are broken in region 80, whereas the other two are broken in region 66, a cross-over occurring between one strand broken at 80 and one broken at 66 would result in one unbroken normal strand, one broken both at 80 and at 66, one broken at 80 and the fourth broken at 66. Reattachment of broken parts might occur before the chromosomes went into the first cleavage spindle. Then, all these suppositions granted, the segregation in the first and the second cleavages must be such that each of the resulting four nuclei contains one of the four types of cells found. The main weakness of this hypothesis is the assumption of crossing-over among the four strands of a chromosome of a haploid group.

The fifth possibility, and the one which seems most probable to the author on the basis of the available evidence, is that the breakage of the chromosomes due to x-rays need not occur at the time of the treatment but may be delayed for one or more cell generations. Let it be assumed that the action of the x-rays has weakened, or actually broken, the third chromosome in two places, in sections 80 and in 66, the fourth chromosome in sec-

tion 97 and the *Y*-chromosome at an undetermined point. Such a sperm has fertilized a normal egg. During the process of the chromosome splitting in the first two cleavage divisions the weaknesses or the breaks in the chromosomes have persisted. In one of the resulting cells the broken ends have become reunited to restore the original gene arrangements, thus giving rise to cells with normal chromosomes. In one of these normal cells, before the weaknesses have become healed, an exchange has occurred between the third chromosome and the *Y*-chromosome. This would give rise to the III–*Y* aberration. In another cell, or cells, an exchange has taken place between the fragments of the third and the fourth chromosomes which is later followed by an exchange between the third and the *Y*-chromosomes. Thus the four types of tissue have arisen containing a III–IV translocation, a III–*Y* translocation and a combination of the two.

The rather involved character of the above explanation must be admitted, but it seems to be the one that best fits the observed facts. It must be noted, however, that it is not entirely unprecedented. Indeed, Lewitsky and Araratian[11] have described a mosaic translocation in a root of a Crepis seed treated with x-ray, in which some cells were normal, others contained a translocation involving certain chromosomes and still others had the chromosomes further modified, with the first modification being preserved. Lewitsky and Araratian's observations, as well as the facts presented in this article, constitute evidence in favor of the view that breakage, or "weakening" of the chromosomes due to irradiation with x-rays precedes the reattachment and formation of aberrations.

The second mosaic pair of salivary glands contained only two types of tissue. The aberrant tissue consisted of an inversion in the second chromosome from the proximal part of region 43 to the distal part of region 45. The other type of tissue was normal. In one of the two glands 32 out of 48 analyzable cells contained the aberration, in the other gland, out of 36 analyzable cells, 15 were aberrant. This mosaic is not critical as an evidence for the "breakage first" hypothesis, since any one of several mechanisms might have produced it.

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DOMINANCE MODIFIERS OF SCUTE IN DROSOPHILA PSEUDOOBSCURA

R. G. HELFER
California Institute of Technology, Pasadena, California

Reprinted from GENETICS **24**: 278–301, March, 1939

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R. G. HELFER
California Institute of Technology, Pasadena, California

Received November 28, 1938

INTRODUCTION

THE question of the nature and origin of dominance has been much discussed in recent years. Two of the best motivated theories have been advanced by FISHER (1928a, 1928b, 1929, 1931, 1934) and by WRIGHT (1929a, 1929b, 1934a, 1934b) and HALDANE (1930, 1932, 1933) respectively. Views essentially similar to WRIGHT'S and HALDANE'S were expressed also by MULLER (1932) and PLUNKETT (1932). FISHER'S contention is that dominance has evolved as a result of the action of many small modifying genes. He believes that mutations arising for the first time in the history of the species are, as a rule, neither dominant nor recessive. It is the action of specific groups of modifying genes, built up as a result of a long selection process, which determines the expression of a mutant gene in heterozygous condition. WRIGHT and HALDANE suppose that evolution of the dominance of a gene is a function of the physiological action of this gene itself. They contend that genes act to produce a character by means of enzymes; if any link in the chain of reactions producing some character is defective in any way, the net result will be disastrous to the end-product. Therefore, those genes which produce an excess of enzyme which would be great enough to withstand adverse changes in the environment, would be selected because of their own survival value. In such a way the wild genotype would gradually become dominant to the frequently recurring mutations.

It was hoped that some experimental evidence bearing on the above problems might be obtained in *Drosophila pseudoobscura*. It is known that *D. pseudoobscura* exists in two races which have been designated as A and B; furthermore many wild strains which have been obtained from different parts of the geographical area of the species are available. From some preliminary investigations of PROFESSOR A. H. STURTEVANT (unpublished), it was known that various strains of both race A and race B differ in their reactions to the race A semidominant mutation Scute. Accordingly, at the suggestion of PROFESSOR STURTEVANT, it was decided to investigate in more detail the manifestation of the gene Scute in heterozygotes with various different strains of race A as well as race B.

MATERIAL AND METHOD

Various wild strains of race A and of race B of *Drosophila pseudoobscura* were obtained through the courtesy of PROFESSOR TH. DOBZHANSKY.

Strains from widely separated areas were taken, so as to increase the probability of obtaining widely different sets of modifying genes, and to see if any relation existed between the geographical origin of a strain and its reaction to the degree of dominance of Scute. These wild strains were crossed to the standard mutant stock, *orange Scute purple*. These mutants, *or*, *Sc*, and *pr*, all lie in the third chromosome. This standard testing stock belongs to race A. As Scute is more or less dominant, the first generation offspring were counted in all cases. Reciprocal matings were made for the first few series of crosses. Later this was deemed no longer necessary and it was discontinued. It may be mentioned that the gene Scute of *D. pseudoobscura* is not homologous to that of *D. melanogaster*. The *D. pseudoobscura* mutation affects all the bristles on the head, thorax, and scutellum in a rather haphazard fashion, cases of asymmetry being frequent. Also, it is located in the third chromosome, whereas the scute of *D. melanogaster* is a sex-linked recessive affecting various bristles in a definite pattern. For the purposes of the present investigation, however, only the four dorsocentral and four scutellar bristles were counted. The usual procedure was to note the number present, making no attempt to distinguish between them.

The temperature was maintained as constant as possible by keeping the cultures in an incubator designed by BRIDGES (1932). The temperature was maintained at 24°C. In order that all external variations be minimized as completely as possible, the flies from which the eggs were to be obtained were transferred to fresh bottles every 24 hours. Only flies from bottles containing between 25 and 150 individuals were used for counts. Most of the bottles produced about 50 to 100 flies. This rule was rigidly adhered to in order to remove any influences that under- and over-population might have on bristle formation. Flies were examined within one or two days after hatching to lessen the chance of any error induced by possible oversight of broken-off bristles; older flies often lose many bristles in this way, and, due to the extremely dark thorax of *D. pseudoobscura*, a certain amount of misclassification may occur.

DOMINANCE OF SCUTE IN HYBRIDS WITH VARIOUS STRAINS

A summary of the results of bristle counts in the hybrids between *or Sc pr* and various wild strains is presented in tables 1 and 2. The name of each strain indicates the geographical locality in which its wild ancestor was collected. (For further information regarding the origin of these strains the papers by DOBZHANSKY 1935, 1937, and DOBZHANSKY and STURTEVANT 1938, may be consulted.) The tables show the mean bristle numbers with their standard errors ($M \pm m$), and the numbers of individuals studied (n) to obtain these means. It is quite evident that the

degree of the dominance of Scute is not alike in different hybrids. The hybrids between *or Sc pr* and Merritt-2, for example, have 3.01 and 3.23 bristles present in females and males respectively, while the *or Sc pr*×Henshaw-3 hybrids have only 1.21 bristles in females and 1.35 bristles in the males (table 2, first experiment). Thus, Scute is more nearly dominant in hybrids with Henshaw-3 than in those with Merritt-2. Other strains give results intermediate to those observed when Henshaw and Merritt are used, the variation apparently being continuous. The numbers of bristles present in female and in male hybrids from a given cross are, on the whole, constant. The males, however, tend to have somewhat more bristles than do females. The minor discrepancies observed are mostly within the limits of the sampling errors.

Since the bristle number is a character that seems to be highly sensitive to culture conditions, repeated tests were made using in part the same strains (tables 1 and 2). The results show that the differences observed are undoubtedly real and not accidental; thus, in every instance the hybrids with Merritt have more bristles than those with Henshaw. An attempt to find a correlation between the degree of the dominance of Scute in the hybrids with a given strain and the geographical origin of the latter proved unsuccessful. For example, some of the strains from British Columbia, which is the extreme northern part of the distribution of *D. pseudoobscura*, gave some weak (Merritt-2, Nakusp-3), and others strong (Kamloops, Mara-3) dominance of Scute. Race A and B show no consistent differences in this respect (compare Grand Canyon-3 and Henshaw-3, Lassen-8 and Crater Lake-2). We can not, of course, exclude the possibility that the populations of either race inhabiting some small areas may give a consistently high or consistently low degree of dominance in hybrids with Scute, but there is certainly no pronounced geographical trend for the species or a race as a whole in this respect. Such geographical trends have been observed, however, for some other variable genetic characters in *D. pseudoobscura* (sex-ratio, STURTEVANT and DOBZHANSKY 1936; the gene arrangement in the third chromosome, DOBZHANSKY and STURTEVANT 1938; the "strength," DOBZHANSKY, unpublished, and others).

Reciprocal crosses (*or Sc pr* ♀ ×wild ♂ and wild ♀ ×*or Sc pr* ♂) were arranged for some strains. The comparison of the results of such reciprocal crosses might be of interest as a method for the detection of sex-linked genes modifying the dominance of Scute. Indeed, the male hybrids from the *or Sc pr* ♀ ×wild ♂ crosses have always the X chromosome of the *or Sc pr* strain and the Y chromosomes of the wild ones; those from the reciprocal crosses have different X chromosomes depending upon the wild strain used, and the Y chromosome of the *or Sc pr* parent. Comparison of table 1 with table 2 shows that in the wild ♀ ×*or Sc pr* ♂ cross, the

TABLE 1

Mean number of bristles present in F_1 offspring from the cross of or Sc pr ♀ × wild strain ♂.

STRAIN	RACE	FEMALES		MALES	
		M ± m	n	M ± m	n
Experiment 1 11/29/36–12/2/36					
Merritt-2	A	3.01 ± 0.08	306	3.23 ± 0.10	334
Nakusp-3	A	2.51 ± 0.10	212	2.04 ± 0.10	180
Chelan-10	A	1.72 ± 0.12	96	1.38 ± 0.11	111
Campbell-3	B	1.57 ± 0.11	115	1.58 ± 0.14	80
Chelan-2	A	1.54 ± 0.10	205	1.63 ± 0.08	251
Chelan-7	A	1.34 ± 0.10	133	1.16 ± 0.10	126
150-Mile House-5	B	1.29 ± 0.11	76	1.71 ± 0.12	99
Sisters-9	B	1.26 ± 0.14	53	1.31 ± 0.14	61
La Push-4	B	1.23 ± 0.09	167	1.41 ± 0.11	134
Henshaw-3	A	1.21 ± 0.06	266	1.35 ± 0.08	228
Experiment 2 1/20/37–1/28/37					
Grand Canyon-3	A	2.60 ± 0.12	157	2.31 ± 0.14	132
Merritt-2	A	2.29 ± 0.10	221	2.62 ± 0.11	156
Lassen-8	B	2.07 ± 0.10	123	1.72 ± 0.11	118
La Grande-2	A	1.94 ± 0.09	193	1.97 ± 0.10	158
Cuernavaca-2	A	1.82 ± 0.12	193	2.70 ± 0.18	100
Oaxaca-4	A	1.76 ± 0.09	199	1.72 ± 0.09	187
Taos-1	A	1.65 ± 0.09	203	1.71 ± 0.11	139
Campbell-3	B	1.63 ± 0.11	102	1.34 ± 0.11	122
Big Horn-6	A	1.55 ± 0.07	224	1.39 ± 0.09	171
Sequoia-15	A	1.54 ± 0.09	171	1.36 ± 0.10	112
Oaxaca-5	A	1.53 ± 0.09	154	1.76 ± 0.10	131
Estes Park-1	A	1.18 ± 0.10	143	1.31 ± 0.10	113
Sequoia-8	B	1.14 ± 0.10	140	1.37 ± 0.11	134
Julian W-5	A	1.13 ± 0.08	183	1.08 ± 0.08	186
Santa Lucia-11	B	1.02 ± 0.08	143	1.18 ± 0.09	134
Crater Lake-2	B	0.97 ± 0.12	66	1.31 ± 0.13	63
Henshaw-3	A	0.96 ± 0.08	139	1.46 ± 0.10	125
Experiment 3 3/16/37–3/25/37					
Chiricahua-2	A	4.31 ± 0.11	165	4.72 ± 0.15	114
Grand Canyon-3	A	3.01 ± 0.21	93	2.18 ± 0.19	105
Julian E-6	A	2.57 ± 0.12	166	2.64 ± 0.11	165
Merritt-2	A	2.30 ± 0.11	169	2.45 ± 0.14	137
Arrowhead	A	2.29 ± 0.19	116	2.45 ± 0.18	126
Dollar Lake-2	A	2.09 ± 0.08	236	2.07 ± 0.10	139
Barton Flats-9	A	2.06 ± 0.10	161	1.67 ± 0.10	152
Magdalena-2	A	1.74 ± 0.10	151	1.53 ± 0.10	133
Kamloops	A	1.72 ± 0.11	111	1.74 ± 0.11	110
Black Hills-5	A	1.71 ± 0.10	142	1.85 ± 0.09	150
Big Bear-2	A	1.70 ± 0.10	148	1.73 ± 0.11	115
Mara-3	A	1.48 ± 0.12	116	1.05 ± 0.10	116
Durango-3	A	1.46 ± 0.08	227	1.39 ± 0.11	97
San Gabriel-7	A	1.44 ± 0.11	140	1.52 ± 0.10	139
Skaha-8	A	1.43 ± 0.08	177	1.73 ± 0.11	133
Henshaw-3	A	1.43 ± 0.09	171	1.51 ± 0.10	136
Zion-5	A	1.34 ± 0.09	157	1.34 ± 0.09	149
Guadelupe-5	A	1.31 ± 0.08	164	1.19 ± 0.08	147
Skaha-2	A	1.26 ± 0.09	149	1.31 ± 0.10	141
Pavilion	A	0.99 ± 0.09	126	0.83 ± 0.08	108

male hybrids have consistently higher mean bristle numbers than the female hybrids; in the reciprocal crosses (table 1) the numbers of bristles

TABLE 2

Mean number of bristles present in F_1 offspring from wild-type females× or Sc pr males.

STRAIN	RACE	FEMALES		MALES	
		M±m	n	M±m	n
Experiment 1 12/19/36–12/24/36					
Chelan-10	A	2.69±0.11	151	5.96±0.16	75
Merritt-2	A	2.55±0.09	231	3.44±0.14	176
Nakusp-3	A	2.52±0.12	151	4.25±0.14	126
Oaxaca-4	A	2.28±0.14	130	3.28±0.19	87
Campbell-4	B	2.05±0.12	157	2.83±0.12	115
Chelan-2	A	2.00±0.08	212	2.86±0.10	193
Texas	A	1.96±0.16	98	3.31±0.16	67
Sequoia-8	B	1.78±0.11	114	2.96±0.16	73
Chelan-7	A	1.60±0.12	132	3.60±0.15	102
150-Mile House-5	B	1.52±0.08	199	2.90±0.13	107
La Push-4	B	1.52±0.09	165	3.78±0.13	156
Henshaw-3	A	1.22±0.09	103	2.62±0.20	63
Experiment 2 3/7/37–3/10/37					
Grand Canyon-3	A	3.26±0.15	132	5.19±0.15	113
Merritt-2	A	2.95±0.13	139	3.94±0.14	116
La Grande-2	A	2.35±0.10	168	3.25±0.12	143
Oaxaca-5	A	1.58±0.10	165	3.15±0.13	137
Julian W-5	A	1.47±0.13	84	2.62±0.24	69
Estes Park-1	A	1.43±0.10	156	2.23±0.16	126
Henshaw-3	A	1.26±0.09	136	2.13±0.11	126
Taos-1	A	1.25±0.10	120	2.04±0.12	121
Sequoia-15	A	1.25±0.10	102	2.51±0.13	115
Cuernavaca-2	A	1.19±0.09	153	2.42±0.13	164
Big Horn-6	A	1.11±0.12	79	2.20±0.14	81

in females are, as a rule, only slightly lower than in the males. It appears either that the Y chromosome of the *or Sc pr* strain has modifiers lessening the dominance of Scute, or that the X chromosome of the same strain has genes enhancing its dominance. The effects of the presumed modifiers located in the X or Y chromosomes of various wild strains might express themselves in shifting the positions of males in the tables with respect to that of the females. It can be seen that in tables 1 and 2 the strains are arranged according to the diminishing bristle numbers in the females; on the whole the seriation of the number of bristles in the males coincides with that for the females, indicating that the dominance modifiers are either absent in the X and Y chromosomes of the wild strains, or that their relative strengths in these chromosomes and in the autosomes are alike. In a few instances this is not so, however. For example, in the *or Sc pr*

♀ ×wild ♂ crosses (table 1), the hybrids with the 150-Mile House strain show an equal or smaller number of bristles in the females than the hybrids in which the Campbell-3, Chelan-2, and Chelan-7 strains took part Yet, the cross *or Sc pr* ♀ ×150-Mile House-5 ♂ produces males with more bristles than similar crosses to Campbell and Chelan. Similar "shifts" are observed for Cuernavaca-2 (second experiment) and for Mara-3 and Skaha-8 (third experiment, table 1). This suggests that modifiers in the Y chromosome may be the cause. Among the wild-type ♀ ×*or Sc pr* ♂ crosses, "shifts" are observed for Nakusp-3 and La Push-4 hybrids (table 2), indicating dominance modifiers in the X chromosome.

The validity of the above conclusions is nevertheless questionable due to the high sensitivity of the bristle number to environmental influences. As a control we may use comparisons of the bristle numbers in the female hybrids in reciprocal crosses. The genetic constitution of females obtained in reciprocal crosses is evidently alike. Nevertheless, in the *or Sc pr* ♀ ×wild ♂ crosses, the strains Cuernavaca-2 and Big Horn-6 gave rather high, and in the reciprocal crosses low, bristle numbers (compare tables 1 and 2). Repeated tests were made for these strains, the reciprocal crosses being arranged simultaneously. As shown in table 3, this time no difference between the crosses was observed, although the dominance of Scute in Cuernavaca-2 hybrids was lower than in those with Big Horn-6.

TABLE 3

Repeated reciprocal comparisons giving mean number of bristles present in F_1 females.

STRAIN	RACE	*or Sc pr* ♀ ×+♂		+♀ ×*or Sc pr* ♂	
		M±n	n	M±n	n
Grand Canyon-3	A			2.84±0.15	149
Merritt-2	A			2.66±0.11	196
Cuernavaca-2	A	2.10±0.14	117	2.31±0.11	156
Big Horn-6	A	1.34±0.10	110	1.59±0.09	162
Henshaw-3	A	1.15±0.09	120	1.85±0.10	147

A perusal of the data (tables 1, 2 and 3) also shows that F_1 females from the cross *or Sc pr* ♀ ×wild strain ♂ (table 1), tend to have fewer bristles than do the F_1 females from the reciprocal cross (table 2). As these females differ only in the source of their cytoplasm this may be explained as due to a maternal effect. Since the data are not comprehensive enough this conclusion can not be regarded as established at present.

IS THERE A VARIETY OF WILD-TYPE ALLELES OF SCUTE?

The experiments discussed above show conclusively that the degree of the dominance of Scute in hybrids with different wild strains is variable.

One may now inquire what causes are responsible for these variations. Theoretically, three possibilities present themselves. First, the wild-type alleles of Scute found in different wild strains may not all be alike, some possibly being more and others less recessive with respect to the mutant gene Scute. Second, the wild strains tested may carry at loci other than Scute different modifying genes which influence the manifestation of the latter in heterozygotes. These modifiers may be present in the third as well as in other chromosomes. The third possibility is that the phenomena observed are due to the presence of a variety of wild-type alleles at the Scute locus as well as to the dominance modifiers at other loci. The experiments to be described below were designed to discriminate between these possibilities.

To reveal the presence of different wild-type alleles of Scute, strains must be obtained that differ only in the origin of their Scute locus, and that are otherwise isogenic. Strictly speaking, this requirement is unattainable since there is no technique available that would permit the creation of completely isogenic strains. One may, however, try to approach this condition as closely as possible. The Merritt-2 and Henshaw-3 strains are known to give different numbers of bristles if crossed to the *or Sc pr* strain. Merritt-2 and Henshaw-3 were outcrossed, therefore, to a strain homozygous for the third chromosome recessive genes orange (*or*), polychaete (*po*), purple (*pr*), and crossveinless (*cv*). Polychaete is a recessive allele of Scute. The *or*, *pr* and *cv* genes lie in the same chromosome to the left and to the right of the *Sc* locus respectively. The hybrid females were back-crossed to *or po pr cv* males, and crossover chromosomes were obtained which had the middle part (carrying the wild-type allele of *Sc* and *po*) from the Merritt or Henshaw strain, and the remainder from *or po pr cv* strain. Four more back-crosses to the *or po pr cv* strain were carried out to make it probable that in the resulting strains all the Merritt and Henshaw chromosomes were replaced by the homologues from the *or po pr cv* strain. Finally, the *or* $+^{sc}$ (Henshaw) *pr cv*/*or po pr cv* and *or* $+^{sc}$ (Merritt) *pr cv*/*or po pr cv* males were crossed to *or Sc pr* females. A scheme of these crosses is represented in figure 1.

Two classes of flies appear in the offspring: those heterozygous for Scute and the $+^{sc}$ alleles of Merritt and Henshaw respectively, and those carrying *Sc* and *po*. They can be distinguished because of the fact that *Sc*/*po* flies have rough eyes. The results of the bristle counts are summarized in table 4. One may see that the numbers of bristles present in $+^{sc}$ (Merritt)/*Sc* and $+^{sc}$ (Henshaw)/*Sc* flies of either sex are alike. This shows that there is no appreciable difference between the wild-type alleles of *Sc* present in the Merritt-2 and Henshaw-3 strains.

Another experiment, similar in principle to the one just described, was

carried out to compare the wild-type alleles of Scute present in race A and race B. A strain obtained from PROFESSOR TH. DOBZHANSKY had one race-A third chromosome carrying *Sc* and *pr* on an otherwise pure race-B background (figure 2). This strain was maintained by crossing *Sc pr*

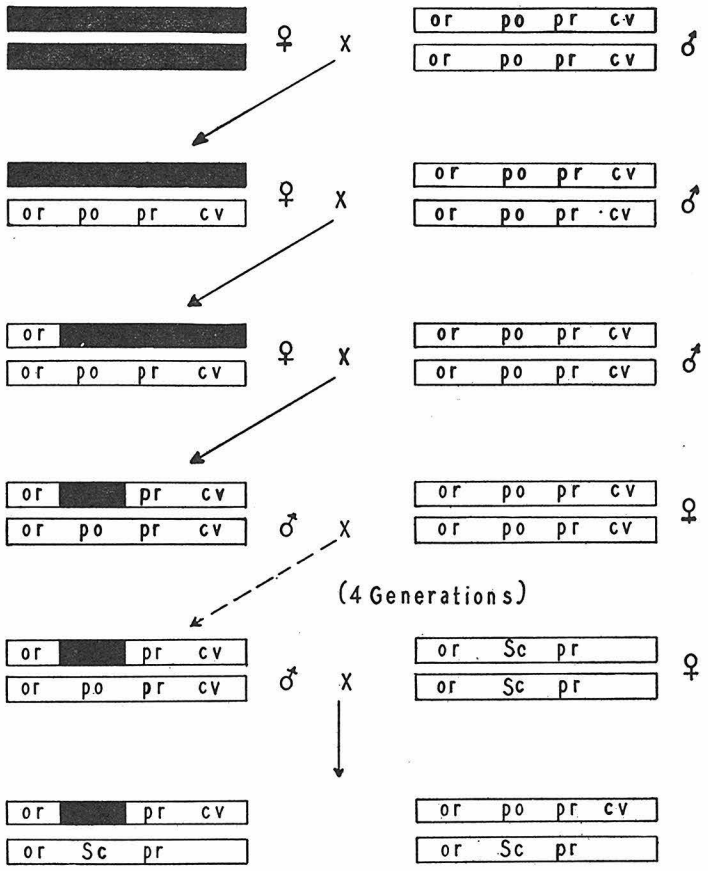

FIGURE 1.—Diagram of the third chromosomes of *D. pseudoobscura* showing method of testing for the possible differences between the Scute loci of Merritt and Henshaw strains. Black = Merritt or Henshaw chromosomes. White = neutral strain.

(race A)/*or* (race B) females to homozygous *or* (race B) males in every generation. Since the third chromosomes of race A and B differ in an inverted section (TAN 1935), the frequency of crossing over between the two chromosomes is very low, and the presence of the marking genes permits keeping the third chromosome of race A intact. The number of bristles present in flies of this strain was very high, namely 5.77 ± 0.08 (table 5,a). A crossover was obtained which appeared wild-type in phenotype (figure 2), but which must have had all race-B chromosomes except

TABLE 4

Mean number of bristles present in or Sc pr ♀ × or +Henshaw pr cv/or po pr cv ♂ and or Sc pr ♀ × or +Merritt pr cv/or po pr cv ♂ crosses.

TYPE OF OFFSPRING	FEMALES		MALES	
	M ± m	n	M ± m	n
(a) +Merritt/*Sc*	1.16±0.09	159	1.39±0.12	93
(b) +Henshaw/*Sc*	1.21±0.10	137	1.43±0.12	86
(a′) *po*/*Sc*	2.83±0.09	148	2.06±0.11	95
(b′) *po*/*Sc*	3.03±0.09	160	2.22±0.15	87

for a short section of the third chromosome containing the wild-type allele of orange coming from race A. This crossover fly (a male) was outcrossed to pure race A females homozygous for orange and purple. A non-orange female was selected in the next generation and again crossed to *or pr* race-A males. This was repeated for two more generations. As a

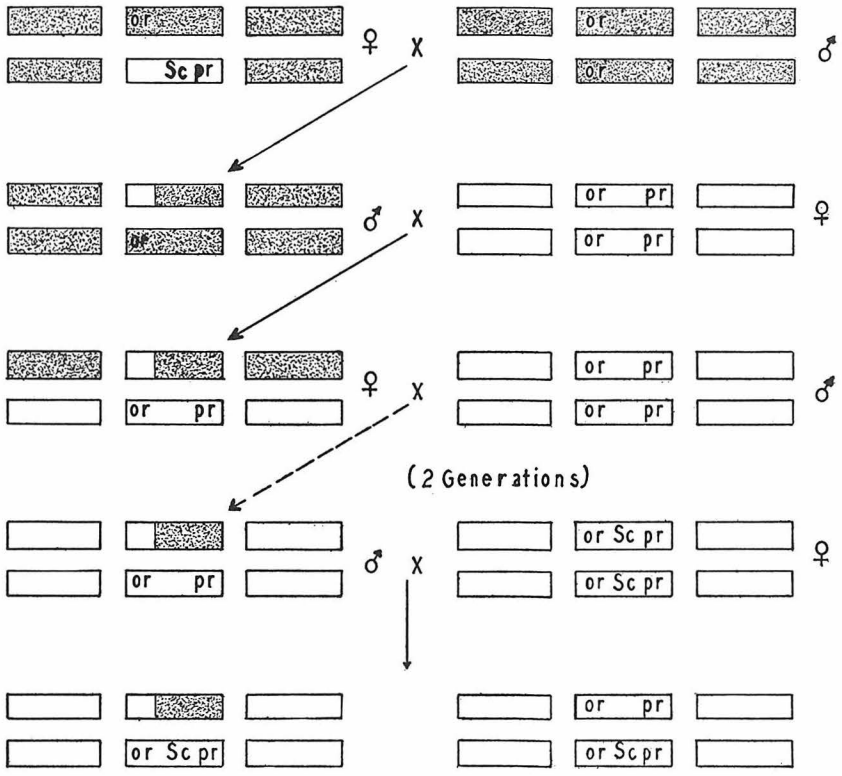

FIGURE 2.—Diagram showing method used to determine the possible differences between the Scute locus of race A and race B of *D. pseudoobscura*. Stippled = race-B autosomes. White = race-A autosomes.

result of this procedure, flies were obtained that had race-A chromosomes except for a section of the third containing the wild-type alleles of Scute and purple (figure 2). Males of this constitution were then crossed to *or Sc pr* race-A females. Two types of flies appear in the offspring, namely *or Sc pr*, and *Sc*. Both types are heterozygous for *Sc*, but the first of them has only race-A chromosomes while the second carries a wild-type allele of Scute from race B. The number of bristles present in these flies is shown in table 5 under (b) and (c) respectively. These numbers are not significantly different, showing that the wild-type alleles of Scute present in the particular race-A and race-B strains studied are virtually alike.

Two other sets of figures are also included in table 5. Under (a) is shown the number of bristles present in the heterozygous Scute flies from the original strain of DOBZHANSKY (see above, also the upper line of figure 2). Under (d) is shown the number of bristles present in the F_1 hybrids from the cross of *or Sc pr* race A ♀ ×orange race B ♂. The *or* race-B strain used here is the same one as that applied by DOBZHANSKY in his crosses. The number of bristles in (a) and (d) are very different indeed, suggesting that the orange race-B strain carries recessive modifiers tending to suppress the effect of Scute. Obviously, these modifiers must be located in chromosomes other than the third.

TABLE 5

Mean number of bristles present in flies carrying race A and race B wild-type alleles of Scute respectively.

STRAIN	FEMALES		MALES	
	M±n	n	M±n	n
(a) *Sc pr/or*	5.77±0.08	273	5.73±0.08	267
(b) *or Sc pr/+B⁺+*	2.45±0.13	383	3.26±0.17	244
(c) *or Sc pr/or A⁺ pr*	2.59±0.13	348	3.01±0.16	186
(d) *Sc/or B*	1.41±0.13	139	1.26±0.17	93

DOMINANCE MODIFIERS IN THE X CHROMOSOME

An experiment was arranged to test the possible differences in the X chromosomes of two widely divergent strains, Merritt and Henshaw. The strain Merritt-2 is known to give high numbers of bristles in hybrids with *or Sc pr*, while the Henshaw-3 strain gives low bristle counts in the same hybrids. Reciprocal crosses of Merritt ♀ ×Henshaw♂ and Henshaw ♀ ×Merritt ♂ were made. The males obtained in the offspring of the first of these crosses carry an X chromosome of the Merritt strain and a Henshaw Y. The males from the second cross have a Henshaw X and a Merritt Y chromosome. As far as the autosomes are concerned, the two types of males are alike. Now, these males were outcrossed to *or Sc pr*

females, and bristle counts were made in the offspring (table 6). If the genes modifying the expression of Scute located in the X chromosome were dominant to those in the *or Sc pr* X chromosome, the daughters of the (Merritt ♀ ×Henshaw ♂) males must have more bristles than the daughters of the (Henshaw ♀ ×Merritt ♂) fathers. Table 6 shows that this is certainly not the case. A similar experiment was arranged using the strains Big Horn-6 (low) and Grand Canyon-3 (high bristle numbers). The results obtained (table 6) may be interpreted as indicating the presence of some modifiers in the X chromosome dominant to those in the X chromosome of the *or Sc pr* strain, but in any event the difference observed here is much smaller than that found in *or Sc pr* × Grand Canyon and *or Sc pr* × Big Horn crosses (tables 1 and 2).

TABLE 6

Mean number of bristles present in the offspring of the following four crosses.

CROSS	FEMALES		MALES	
	M±m	n	M±m	n
or Sc pr ♀ ×(Merritt-2 ♀ /Henshaw-3 ♂)♂	4.73±0.11	525	5.45±0.19	145
or Sc pr ♀ ×(Henshaw-3 ♀ /Merritt-2 ♂)♂	5.03±0.11	417	5.15±0.20	118
or Sc pr ♀ ×(Grand Canyon-3 ♀ /Big Horn-6 ♂)♂	4.05±0.13	387	3.43±0.22	138
or Sc pr ♀ ×(Big Horn-6 ♀ /Grand Canyon-3 ♂)♂	3.54±0.11	487	3.40±0.21	123

DOMINANCE MODIFIERS IN THE AUTOSOMES

By the process of elimination of other possibilities we are forced to conclude that the variation of the bristle numbers observed in hybrids between *or Sc pr* and different wild strains is due to the presence of dominance modifiers in the autosomes of these strains. The experimental procedure employed to test the validity of this conclusion is as follows (figure 3). The mutant genes Smoky (*Sm*), orange (*or*), and Curly (*Cy*) were used as markers for the second, third, and fourth chromosomes respectively. Merritt-2 and Henshaw-3 females were crossed to *Sm or Cy* males. Sons showing *Sm* and *Cy* (and heterozygous for orange) were selected in the offspring and outcrossed to *or Sc pr* females. In the next generation, eight classes of flies appear showing all the combinations of the marking genes *Sm*, *or*, and *Cy*. The presence or absence of these markers show which, if any, of the autosomes coming from the Merritt and Henshaw strains are carried by the fly in question. Thus, a *Sc/Sm or Cy* fly carries no chromosomes from the strain to be tested; a *Sc/or Cy* fly carries only the second chromosome; a *Sc/Sm* fly has the third and the fourth chromosomes of Merritt or Henshaw, etc. (see figure 3). A summary of mean bristle counts in all types of flies obtained in these crosses is given in table 8, for females and males separately.

The experiment is thus reduced to comparisons of the effects of individual chromosomes of the Merritt and Henshaw strains with those of their homologues in the *or Sc pr* strain. For example, if the second chromosome of the Merritt strain carries modifiers increasing the number of

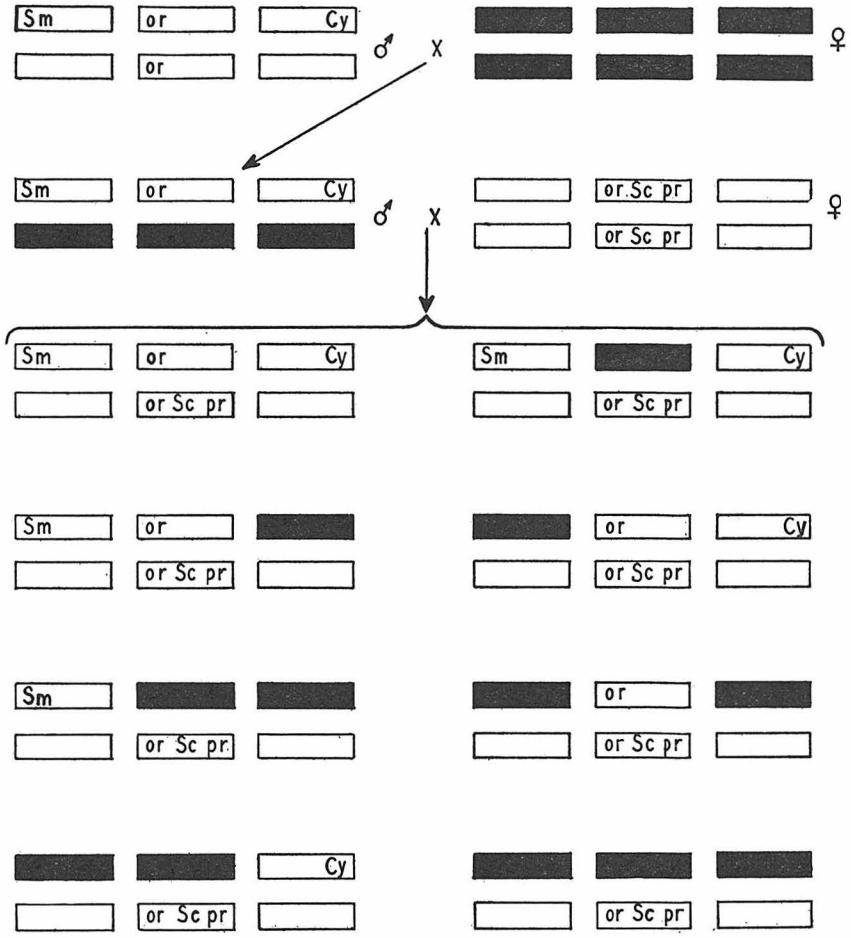

FIGURE 3.—Diagram showing method used to test the individual autosomes of wild strains for dominance modifiers of Scute. Black = autosomes of wild strain tested. White = autosomes of *or Sc pr* standard testing strain.

bristles in the flies heterozygous for Scute, then the flies manifesting Smoky should have fewer bristles than those free of this gene. If such modifiers are present in the third or fourth chromosome, similar differences should be observed between orange and non-orange, and between Curly and non-Curly flies. The presence of modifiers of the dominance of Scute is clearly shown when one examines the mean number of bristles present

in the different classes of offspring obtained in the crosses *or Sc pr* ♀ ×*Sm or Cy*/Merritt ♂, *Sm or Cy*/Henshaw ♂, *Sm or Cy*/Grand Canyon ♂, and *Sm or Cy*/Big Horn ♂ (table 8). For example, in the offspring of the cross of *or Sc pr* ♀ ×*Sm or Cy*/Merritt ♂, the classes can be divided into two main groups, those having high mean bristle numbers, and those having low ones. Upon closer examination, one finds that all classes having low means contain the fourth chromosome marker *Cy*, irrespective of what other markers may or may not be present. On the other hand, all classes not marked by *Cy*, that is, containing the Merritt fourth chromosome, have high means. In other words, the gene, or genes, present in the Merritt strain which tend to suppress the dominance of Scute, and thus to produce many bristles in the Merritt/Scute hybrids, lie in the fourth chromosome of the Merritt strain.

TABLE 7

Mean number of bristles in homozygous Scute flies of the standard or Sc pr strain.

FEMALES				MALES			
M	±	m	n	M	±	m	n
1.357	±	0.09	168	1.98	±	0.16	68

In the cross of *or Sc pr* ♀ ×*Sm or Cy*/Henshaw ♂ the differences in the means of the various classes are hardly significant. One can not show any definite trend for any of the chromosomes. This may be due to the fact that heterozygous Scute in *or Sc pr*/Henshaw hybrids is completely dominant in the sense that heterozygous Scute and homozygous Scute would be alike in this background (compare tables 1 and 2 with table 7). Moreover, the modifiers in the *Sm*, *or*, and *Cy* chromosomes of the standard strain do not differ significantly from those in their respective homologues in the Henshaw strain. The slight differences in means of the various classes may be due to the presence of weakly opposing modifiers in the *Sm or Cy* chromosomes, or to the interactions of various combinations of these chromosomes with those of Henshaw.

An analysis of the Grand Canyon strain (table 8) shows that each of the autosomes of this strain contains some modifiers tending to suppress the dominance of Scute. The sum total of these modifiers suppresses Scute to such an extent that it has a mean number of bristles equal to that of Merritt. On the other hand, Big Horn (table 8) which gives a low mean, and does not have any important modifiers which differ from those of the *Sm or Cy* chromosome in suppressing the manifestation of Scute, may even have some actually enhancing Scute. These are located in the fourth

TABLE 8

Mean number of bristles present in offspring of or Sc pr ♀ × Sm or Cy/Merritt, Sm or Cy/Henshaw, Sm or Cy/Grand Canyon, Sm or Cy/Big Horn males.

	FEMALES		MALES	
	M±m	n	M±m	n
Cross to Merritt				
wild type	3.12±0.13	249	3.63±0.16	139
Sm	3.14±0.18	94	3.99±0.22	68
or	2.82±0.13	199	3.15±0.19	119
Cy	1.23±0.13	196	2.34±0.22	76
Sm or	2.97±0.22	66	3.46±0.25	44
or Cy	1.21±0.13	165	1.90±0.22	86
Sm Cy	1.60±0.15	131	1.87±0.17	106
Sm or Cy	1.58±0.23	72	1.65±0.19	63
Cross to Henshaw				
wild type	1.21±0.12	188	1.57±0.17	108
Sm	1.10±0.13	156	1.29±0.15	78
or	0.95±0.10	206	1.21±0.15	116
Cy	1.16±0.11	169	1.55±0.21	74
Sm or	1.33±0.19	91	1.28±0.20	32
or Cy	1.22±0.13	150	1.58±0.18	79
Sm Cy	1.29±0.11	187	1.71±0.18	91
Sm or Cy	1.61±0.16	114	1.58±0.20	62
Cross to Grand Canyon				
wild type	2.82±0.16	197	2.91±0.20	126
Sm	2.06±0.14	205	1.82±0.22	91
or	1.94±0.16	161	2.36±0.22	91
Cy	2.10±0.15	165	1.35±0.20	72
Sm or	1.76±0.15	132	1.75±0.28	49
or Cy	1.69±0.14	146	1.64±0.20	97
Sm Cy	1.78±0.15	208	1.74±0.16	106
Sm or Cy	1.57±0.16	121	1.48±0.20	67
Cross to big Horn				
wild type	1.43±0.12	166	1.80±0.14	157
Sm	1.58±0.15	160	1.85±0.26	61
or	1.57±0.13	186	1.40±0.14	137
Cy	2.01±0.16	169	1.99±0.18	102
Sm or	1.04±0.14	92	1.76±0.35	33
or Cy	1.76±0.15	173	1.62±0.19	107
Sm Cy	1.87±0.17	160	1.90±0.24	62
Sm or Cy	1.86±0.21	106	2.15±0.30	53

chromosome. This is shown by the fact that all classes having the marker *Cy* from the *Sm or Cy* strain have a higher mean number of bristles than do those having the Big Horn fourth chromosome. Thus the fourth chromosome of the Big Horn strain differs from that of the *Sm or Cy* strain in modifiers increasing the dominant effect of Scute.

TABLE 9

The χ^2 values for one degree of freedom for the comparisons of bristle numbers in the classes of flies shown in table 8 (further explanation in text).

CROSS	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
or Sc pr ♀ ×	Females	0.3336	3.3244	498.6649
Sm or Cy/Merritt ♂	Males	6.3511	15.3373	221.6777
or Sc pr ♀ ×	Females	11.1117	0.1853	10.2736
Sm or Cy/Henshaw ♂	Males	0.0003	0.6229	5.7379
or Sc pr ♀ ×	Females	28.7180	41.0079	33.1835
Sm or Cy/Grand Canyon ♂	Males	25.6705	6.0986	69.5099
or Sc pr ♀ ×	Females	0.8731	3.5270	40.8005
Sm or Cy/Big Horn ♂	Males	6.1116	7.7835	5.8999

TABLE 10

The deviation χ^2 values for one degree of freedom for comparisons of various combinations of the bristle numbers in the classes of flies shown in table 8.

COMBINATION OF BRISTLE NUMBERS	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
Merritt and Henshaw	Female	0.0425	2.1838	230.7472
	Male	4.9129	12.5665	100.2239
Grand Canyon and Big Horn	Female	16.8496	39.5555	0.0399
	Male	2.5467	14.5746	17.5445
Merritt and Big Horn	Female	2.5245	8.7078	150.2030
	Male	1.5832	24.6504	84.0996
Grand Canyon and Henshaw	Female	0.9086	26.3722	7.2538
	Male	12.8913	6.0955	21.0547
Merritt and Grand Canyon	Female	18.8365	33.9440	386.1411
	Male	34.4637	19.0469	278.1108
Henshaw and Big Horn	Female	2.1068	0.9153	48.6509
	Male	1.8823	6.6120	11.1457

Unfortunately, the data given in table 8 enable us to compare the various wild strains only to the standard one (*Sm or Cy*). In order to detect the similarities and differences between the effects of homologous chromosomes of the wild strains themselves (Merritt vs. Henshaw, for example)

it is necessary to use a more comprehensive statistical technique. According to the advice of DR. K. MATHER, the testing of the significance of the differences observed was made with the aid of two-by-two contingency tables, and the χ^2 values were obtained according to the formula: $\chi^2=(a_1a_4-a_2a_3)^2n/(a_1+a_2)(a_3+a_4)(a_2+a_3)(a_2+a_4)$ (FISHER 1928c). In this formula, a_1 is the total number of bristles present, and a_2 is the total number of bristles absent in a given class of flies (for example, in non-Curly females of the Merritt cross); a_3 and a_4 are the total numbers of bristles present and absent respectively in the flies of the class to be compared with the first (for example, in the Curly females from the same cross): n is equal to the sum of a_1, a_2, a_3, a_4. The resulting value of the χ^2 is a measure of the significance of the differences observed (FISHER 1928c). The χ^2 values for various comparisons made are presented in table 9. These values are for one degree of freedom. Looking up the probable significance of these values in FISHER'S table, the χ^2 of 2.706 has a probability of being a chance occurrence of one in ten, and a χ^2 of 6.635 of only once in one hundred trials. An examination of the females from the *Sm or Cy*/Merritt cross shows that the χ^2 value of the Curly-non-Curly comparison is the only one which is significant (table 9). Thus Merritt females differ from those of the standard testing strain in the fourth chromosome only. This is but a confirmation of the datum for Merritt given in table 8. In the female offspring of the *Sm or Cy*/Henshaw cross, the χ^2 values of *Sm* non-Smoky and Curly non-Curly are both significant. This indicates that there are modifiers in the second and fourth chromosomes of the Henshaw strain which suppress the dominance of Scute. The third chromosome is neutral. In the *Sm or Cy*/Grand Canyon cross the χ^2 values for all three classes of comparisons are significant. Thus in each of the autosomes tested there are factors suppressing the effects of Scute. The only significant deviation from the standard strain in the *Sm or Cy*/Big Horn cross is in the fourth chromosome. The above analysis shows that in two cases out of four examined, the modifiers affecting the dominance of Scute are located entirely in the fourth chromosome, in one case they are in the second and fourth chromosomes, and in only one case do we find modifiers in the third chromosome as well.

The data for males agree in general with those for the females. The main difference between the *Sm or Cy*/Merritt males and the standard strains is, as in the females, due to the fourth chromosome. The second and third chromosomes also show significant effects. These are not great enough, however, to account for the large suppression of Scute in hybrids with Merritt. The results for the *Sm or Cy*/Grand Canyon males are entirely in accord with those for the females; that is, the χ^2 values for the second, third and fourth chromosomes are all significant. An examination

of *Sm or Cy*/Big Horn males gives results slightly different from those of the females. In the males each chromosome has a significant modifying effect, whereas in the females, the only deviation from the standard strain is in the fourth chromosome.

A final test was made to compare the differences between the respective autosomal homologues of the four tested strains themselves. In order to do this, the number of bristles present in the corresponding classes of any two strains (Merritt and Henshaw, for example) were added together, and these combined values were used as single ones. Two-by-two contingency tables were constructed from these values and χ^2 were obtained in the same manner as above. These are deviation χ^2's and are given in table 10. They are subtracted from the total χ^2 values obtained by adding together the separately calculated ones from the two strains being tested (Merritt and Henshaw for example). The resulting heterogeneity χ^2 is an indication of the direct difference between the autosomes of the strains tested.

Unfortunately, the method first devised by DR. MATHER and used in this paper is not absolutely accurate. Since this paper was written, a more accurate method has been devised by him. The method used (the subtraction method) is accurate only when none of the χ^2 values is significant. In such cases where the deviation χ^2 is significant, the heterogeneity χ^2 is underestimated. Furthermore, discrepancies in the value of p, a measure of the proportion of bristle places where there are actually bristles present, further exaggerates the error. The method used counts these values in the classes tested to be the same. Unfortunately, this is not always true. Therefore, in a few extreme cases the deviation χ^2 is much overestimated and appears to give negative values for the heterogeneity χ^2. In spite of this, the analysis is accurate enough to indicate whether there is or is not a significant difference between the two chromosomes compared.

The results of the heterogeneity χ^2 values are given in table 11. As has been shown already (table 9), Merritt deviates very significantly from the females of the standard strain in the fourth chromosome only. Henshaw females differ from this strain in the second as well as in the fourth chromosome. One might surmise that Merritt females would differ directly from Henshaw females in these two autosomes, the second and fourth, and in this example, this is the case (table 11).

Grand Canyon females differ very significantly from the standard strain in all three autosomes. Big Horn females deviate from it in the fourth chromosome only (table 8). One can not definitely state in this case just how dissimilar these two strains are. It is safe to assume that their second and third chromosomes are not alike, but the fourth chromosomes might be similar if both differ from the standard strain in the same direction. On

the other hand, they might deviate from each other more than either one does from the standard strain, due to the fact that they differ in opposite directions. The latter possibility proves to be the case. Thus, the fourth chromosomes of these two strains are even less alike when compared to each other than if they are compared to the homologues in the *Sm or Cy* strain. The second and third chromosomes do not deviate as much from each other (table 11) as either does from the standard strain. This is due to both of them differing from the standard in the same direction.

Merritt and Big Horn females differ from the standard strain in their fourth chromosomes. Although the signs of the differences are alike, their fourth chromosomes are still very significantly dissimilar from each other (table 11). Here we have a very special case. Due to the overestimation of the deviation χ^2, the heterogeneity χ^2 value for the second and third chromosomes seems to be negative. Although this is not really the case, it is safe to assume that there is no significant difference between these two chromosomes.

Grand Canyon females and Henshaw females are unlike in all three tested autosomes. It is interesting to note, however, that the second and fourth chromosomes of these two strains deviate from the standard strain in opposite directions (table 11). The third chromosomes of these two strains differ from the standard strain in the same direction but they are still significantly dissimilar to each other. Although Grand Canyon also differs significantly from Merritt in all three autosomes, both of them deviate from the standard strain in the same direction as is shown by results given in table 11.

Henshaw and Big Horn females differ in their second autosomes (table 11). This is in accord with expectations (see table 9). It is especially interesting to note that although each strain varies significantly from the standard strain in the fourth chromosome, this difference disappears on direct comparison. In short, the fourth chromosomes of the Henshaw strain are not significantly different from those of the Big Horn strain. The third chromosomes are also alike. This example is a good one to show how insecure it is to compare two strains to each other through the medium of a standard third strain. The data for the males (table 11) on the whole lead to the same conclusions as those for the females.

PATTERN EFFECTS

As shown above, different strains of *D. pseudoobscura* produce hybrids with Scute having different numbers of bristles (tables 1 and 2). The question arises whether the Scute pattern is, or is not, changed in these strains, that is, whether any pattern effects are produced. The decrease of the total number of bristles in hybrids with one strain as compared with another

may be due to every bristle being removed proportionately more frequently in the first than in the second (lack of pattern effects); or else, a certain bristle, or set of bristles, may be affected disproportionately in different genotypes. The strains Merritt and Henshaw were studied in detail to detect the possible pattern effects. The four dorsocentral and the four scutellar bristles were counted in the hybrids between these

TABLE 11

The heterogeneity χ^2 values for one degree of freedom obtained by subtracting the deviation χ^2 from the total χ^2 (further explanation in text).

COMBINATION	SEX OF OFFSPRING	COMPARISON		
		SMOKY-NON-SMOKY	ORANGE-NON-ORANGE	CURLY-NON-CURLY
Merritt and Henshaw	Female	11.3928	1.4259	278.1914
	Male	1.3585	3.3937	127.1917
Grand Canyon and Big Horn	Female	12.7415	4.9794	73.9441
	Male	29.2354	−0.6025	57.8653
Merritt and Big Horn	Female	−1.2178	−1.8564	389.2624
	Male	10.8795	−1.5396	143.4780
Grand Canyon and Henshaw	Female	38.9212	14.8210	36.2033
	Male	12.7795	0.6260	54.1931
Merritt and Grand Canyon	Female	10.2152	10.3883	145.7073
	Male	−2.4421	2.2890	13.0768
Henshaw and Big Horn	Female	9.8780	2.7970	2.4232
	Male	4.2296	1.6944	0.4921

strains and Scute, the presence or absence of the anterior and the posterior dorsocentral and scutellar bristles being recorded separately for each individual. The data obtained are summarized in table 12.

The data were organized in four-by-four contingency tables, and the probable significance of any differences calculated by means of the χ^2 method. The formula used was $\chi^2 = S(a^2/m) - n$, where a represents the observed values, m represents the expected values (on the assumption that all four clásses are equal), and n is the total number of bristles present. The comparison between Merritt and Henshaw gave a $\chi^2 = 7.82$ for three degrees of freedom. Although the method used slightly underestimates the difference, thus giving a minimum value for the χ^2, the significance of this difference is doubtful, since the probability of such a deviation occurring by chance is once in twenty trials. Merritt compared with the homozygous *or Sc pr* standard strain gave a $\chi^2 = 6.97$ for three degrees of freedom. This

is not significant, as the probability of such a value occurring by chance is between one in ten and one in twenty trials. Henshaw when compared to the *or Sc pr* strain gave a significant χ^2 value of 12.46.

The difference between the actions of Scute in Merritt and Henshaw hybrids is therefore essentially quantitative, the presence of a pattern effect being doubtful. Heterozygous Scute in the Merritt hybrid takes off bristles in much the same manner as does Scute in homozygous condition. There is, however, a perceptible qualitative difference between the action of Scute in Henshaw/*Sc* hybrids and its action in homozygous Scute.

TABLE 12

Number of bristles present in F_1 females of wild-type ♀ × or Sc pr ♂ crosses.

CROSS	DORSOCENTRAL		SCUTELLAR		NO. OF FLIES
	ANTERIOR	POSTERIOR	ANTERIOR	POSTERIOR	
Merritt ♀ × *or Sc pr* ♂	77	128	80	120	139
Henshaw ♀ × *or Sc pr* ♂	39	71	28	45	136
or Sc pr (Homozygous)	33	49	34	65	168

ARE THE DOMINANCE-SUPPRESSING MODIFIERS OF SCUTE SPECIFIC?

The presence of modifiers in various strains of *D. pseudoobscura* tending to suppress the manifestation of the semi-dominant mutation Scute has already been shown (tables 1 and 2). The question has been raised as to whether these modifying genes are of a non-specific nature, tending to increase the number of bristles present in flies in general, or whether they are specific, acting only in the presence of Scute itself. In order to distinguish between these two possibilities the following experiment was undertaken: Virgin females from a strain of race B of *D. pseudoobscura* containing the sex-linked recessives scarlet and scutellar were crossed to males of Merritt-3, Grand Canyon-2, Henshaw-3, and Big Horn-6; the scutellar bristles present in the F_1 males were counted.

The race-B mutant scutellar is sex-linked and recessive, and removes bristles only from the scutellum and the head, whereas the race-A mutant is an autosomal dominant which also removes the large dorsocentral bristles from the thorax. It seems logical to conclude that these two genes act in different ways to produce their effects. If the action of the Scute modifiers is a specific one for this mutant, one should expect no relation between the seriation of the same group of strains in hybrids with Scute as compared with their seriation in hybrids with scutellar. On the other hand, if these modifiers are of a general nature, acting solely to produce extra bristles, then one would expect to obtain parallel seriations from such an experiment.

The results of the experiment (table 13) are in accord with the assumption that the modifiers are specific. Merritt-2 and Grand Canyon-3 which act alike, producing high mean bristle numbers in hybrids with Scute (tables 1 and 2), are very significantly different from each other in their action on scutellar. Merritt has a high number of bristles in scutellar hybrids with it, but Henshaw-3 and Big Horn-6 (which have low mean numbers of bristles in Scute hybrids), also have mean bristle numbers of the same magnitude as Merritt. Furthermore, Grand Canyon has the lowest mean bristle number of the group tested with scutellar, yet in Scute hybrids it usually has one of the highest bristle numbers. Thus the modifiers tending to suppress the dominance of Scute in heterozygous form appear to be specific.

TABLE 13

Mean number of bristles present in F_1 males from cross of scutellar scarlet ♀ × Merritt-2, Grand Canyon-3, Henshaw-3 and Big Horn-6 ♂s.

STRAIN	n	M±m
Grand Canyon-3	242	0.56±0.04
Merritt-2	89	1.18±0.08
Henshaw-3	121	1.06±0.08
Big Horn-6	97	0.95±0.08

DISCUSSION

The purpose of the present work has been to collect experimental evidence bearing on the problem, of the nature and origin of dominance. As stated in the introduction, two distinct theories have been advanced in this field by FISHER (1928a, b, 1931, 1934) and by WRIGHT (1929a, b, 1934a, b,) and HALDANE (1930, 1932, 1933) respectively. FISHER'S theory attributes the development of dominance to a selection of specific modifying genes which tend to make each mutant heterozygote virtually indistinguishable from the wild-type. The theories of WRIGHT-HALDANE postulate rather a multiplicity of wild-type alleles which in connection with the rest of the genetic system, tend to make most of the frequently recurring mutations recessive.

The facts at hand show that the manifestation of Scute in hybrids with different wild strains of *D. pseudoobscura* is variable. Some strains such as Henshaw-3 and Big Horn-6, produce heterozygotes in which Scute is almost completely dominant; others, for example Merritt-2 and Grand Canyon-3, make the Scute heterozygotes decidedly intermediate between homozygous Scute and homozygous wild-type. Although no strain was found making Scute completely recessive, it is quite obvious that the species *D. pseudoobscura* is not uniform in its reaction to Scute. The variability observed shows no clear geographical trend; it looks as though

differences in the reaction to Scute may be found between strains from any region. If the evolutionary process be supposed to lead towards making the Scute mutation eventually recessive, this process is by no means completed.

The genetic causation of the variability in the degree of the dominance of Scute has been studied in four strains of race A and in one strain of race B of *D. pseudoobscura*. In every instance this variability proved to be due to modifying genes located in various chromosomes, more or less at random. There seems to be no tendency for such modifiers to be concentrated in the third chromosome which carries the locus of Scute itself. Moreover, there is no evidence, in the few cases studied, of the existence of a variety of wild-type alleles of Scute. Finally, evidence was obtained showing that the action of these modifiers appears to be specific for the mutant Scute. Therefore, our data, as far as they go, are in accord with expectations based on FISHER'S theory. On the other hand, they can not be construed as in any way contradictory to the alternative theory of WRIGHT-HALDANE. It is possible that the development of dominance is due to a selection of wild-type alleles of the mutant genes themselves as well as to that of modifiers at other loci. Moreover, presence of such modifiers is not in itself a proof that the dominance of the wild-type condition over the mutant will eventually be attained by their selection.

ACKNOWLEDGMENTS

I wish to express my appreciation for the suggestions and aid of PROFESSOR TH. DOBZHANSKY and PROFESSOR A. H. STURTEVANT who made this work possible, and to DR. K. MATHER for his help in the statistical treatment of the data and particularly of those in the Smoky-orange-Curly, and in the pattern-effect experiments.

SUMMARY

1. A study was made of various strains of race A and B of *D. pseudoobscura*, and their reactions to the semi-dominant mutation Scute. It was found that some strains gave high bristle numbers in hybrids with Scute (Merritt and Grand Canyon), others gave low bristle numbers (Henshaw and Big Horn), and still others were intermediate (tables 1 and 2).

2. No relation was found between the degree of dominance of Scute in hybrids with a given strain and the geographical origin of the latter.

3. In tables 1 and 2, the females of the various strains are arranged in descending order with respect to the number of bristles present. In general, the males agree with this seriation. On occasion, they do not. Those cases where the male disagrees with the female seriation suggest the presence of modifiers in the Y chromosomes of the wild strains.

4. The existence of modifiers located in the X chromosome is suggested in those crosses of wild-type ♀ × *or Sc pr* ♂ where the male offspring disagree with the normal female seriation.

5. Several strains were analyzed to see if wild-type alleles of Scute of different strengths were the reason, or at least a part of the reason, for the different behavior of Scute in hybrids with these strains. No difference was found between the wild-type alleles of Scute present in race A and race B strains, which had been made isogenic except for a region of the chromosome around the locus of Scute. In another test, the widely unlike strains of race A, Merritt and Henshaw, proved to show no difference as far as their Scute loci were concerned.

6. A more complete analysis of two of the strains with high bristle numbers (Merritt and Grand Canyon), and two strains with low bristle numbers (Henshaw and Big Horn), shows that the main differences in their reactions to Scute are due to the presence of modifying genes in the autosomes (table 11). In one case (Merritt) the modifiers are mainly in the fourth chromosome, in another (Grand Canyon) they are found scattered in all of the autosomes tested. In Henshaw there are no very strong modifiers, although some weak ones exist in the second and fourth chromosomes. Big Horn has modifiers in the fourth chromosome, and some rather weak ones in the third.

7. A study was made to see if the pattern in which Scute removes the bristles is affected in hybrids with Merritt and Henshaw. It was found that the Scute pattern was not significantly altered in hybrids with Merritt, but it was in hybrids with Henshaw.

8. A final test was made to determine the degree of specificity of these Scute modifiers. It was found that the effects produced seemed to be specific for this mutant, and were not general modifiers tending to increase the number of bristles per se.

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