INVERSIONS IN THE CHROMOSOMES OF DROSOPHILA PSEUDOOBSCURA*

TH. DOBZHANSKY AND A. H. STURTEVANT
California Institute of Technology, Pasadena, California

Received August 23, 1937

INTRODUCTION

THE linear arrangement of genes within chromosomes is constant from generation to generation in each line of descent. The degree of this stability is comparable to that of the gene structure; genes change by mutation, chromosomes change by the occurrence of chromosomal aberrations. Strains and races of the same species, as well as distinct species, may differ in gene arrangement. One of us (STURTEVANT 1917, 1931) has shown that strains of *Drosophila melanogaster* coming from the same or from distinct geographical localities may differ in having blocks of genes rotated by 180° (inversion). The method of detection of inversions used in these early studies was based chiefly on observations on the strength of linkage in hybrids between different strains, but the laboriousness of this method limits its applicability. The discovery of the salivary gland chromosome method has rendered the task much easier. TAN (1935) in *Drosophila pseudoobscura*, and DUBININ, SOKOLOV and TINIAKOV (1936) in *melanogaster* have observed numerous inversions in hybrids between strains and races. DOBZHANSKY and TAN (1936) and DOBZHANSKY and BAUER (unpublished) have found extensive differences between the gene arrangements in two pairs of species of Drosophila, namely *pseudoobscura* and *miranda*, *athabasca* and *azteca*.

In the present article we shall report the results of comparisons of the gene arrangement in the chromosomes of strains of *D. pseudoobscura* coming from different geographical regions. This species has five pairs of chromosomes; one of them, the third, is especially variable in the gene arrangement. Other chromosomes are relatively more constant, yet some variation has been observed in every chromosome, except the small fifth. Some of the gene arrangements are encountered in populations inhabiting a major part of the species area, while others are more restricted in their distribution. In many localities the population is mixed with respect to the chromosome structure, and inversion heterozygotes are very common in nature. Moreover, as pointed out in our preliminary communication (STURTEVANT and DOBZHANSKY 1936a), a comparison of the different gene arrangements in the same chromosome may, in certain cases, throw light on the historical relationships of these structures, and consequently on the history of the species as a whole.

* The cost of Plate 1 is paid for by the Galton and Meudel Fund.

MATERIAL AND METHODS

In the salivary gland cells of many Diptera, including Drosophila, the homologous chromosomes undergo a very intimate pairing. It is now clear that this pairing is due to a mutual attraction between the homologous loci in the chromosomes rather than between chromosomes as wholes. Two chromosomes containing the same genes arranged in identical linear orders fuse to form a double strand in which homologous loci lie on exactly the same levels. Pairing of chromosome sections having dissimilar disc patterns (and consequently dissimilar genes) is never observed, at least in our species. If the gene arrangements in two chromosomes are not identical, the homologous discs still tend to become associated, forcing the chromosomes to form at times very complex pairing configurations. For example, if two homologous chromosomes have a section inverted, a loop-like configuration appears in the salivary gland cells (fig. 1).

This chromosome pairing furnishes an easy and accurate method for comparison of the gene arrangements in different strains of the same species, or in different species if these can be crossed. A strain is selected the gene arrangement in which is arbitrarily chosen as a standard. Strains to be tested are crossed to the standard one, and the chromosomes are examined in the salivary glands of the larvae of the first generation hybrids. If the strains crossed are identical with respect to gene arrangement, all the chromosomes in the hybrids are represented by paired strands radiating from the chromocenter. If the gene arrangements are different in any respect, some of the chromosomes in the hybrids show abnormal pairing configurations, from the appearance of which the precise nature of the difference can be deduced.

The procedure just outlined has been followed in the present investigation. A strain of race A of *D. pseudoobscura* carrying the third-chromosome recessives orange and purple was chosen as a standard, and the chromosomes in the hybrids between this and various other strains have been examined. As a rule, orange purple females have been crossed to males from the strains to be tested. The presence of the recessive mutant genes has served as a check against the possible non-virginity of the mothers. Tests of the race B strains were usually performed by crossing them to a race B strain from Klamath, California, homozygous for orange. The Klamath strain of race B differs in the gene arrangement from the standard race A strain, but the nature of the difference being known, the results obtained may be translated in terms of the standard race A arrangement.

The known distribution area of *pseudoobscura* extends from the Pacific coast to the Rocky Mountains, the western edge of the prairies, into central Texas, and from central British Columbia to southern Mexico. In

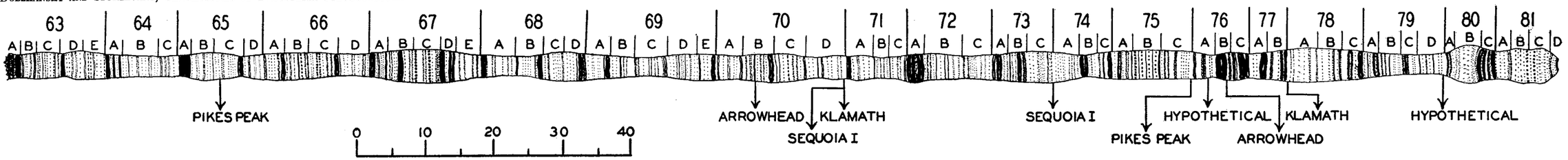
recent years a fairly large collection of strains of this species has been accumulated in this laboratory. The wild ancestors of these strains have been collected out of doors with the aid of traps containing fermenting banana mash. Both females and males come to the traps; a majority of the females are already fertilized by one or more males before coming to the traps. Each laboratory strain is derived from a single wild female. In most localities where collecting has been done several strains have been isolated. The strains are designated by the name of the locality in which their wild ancestors have been collected, and by serial numbers. Thus, "Taos-4" is the strain No. 4 from Taos, New Mexico.

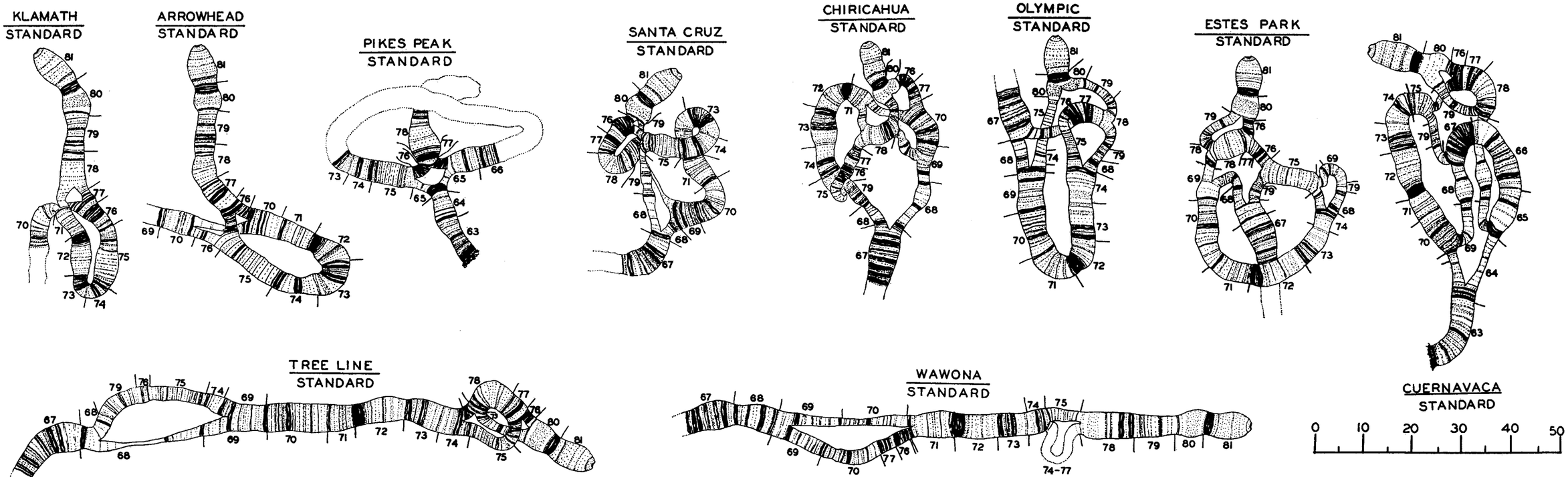
Examination of the chromosomes in the hybrids between the standard strain and strains coming from various geographical localities furnishes qualitative, as well as roughly quantitative, information regarding the kinds of gene arrangements encountered in the populations inhabiting these localities. For a more precise quantitative analysis of wild populations this technique is however unsatisfactory, since the variety of gene arrangements originally present in a given strain may be decreased if this strain is kept in the laboratory for many generations. Therefore, the relative frequencies of the different gene arrangements in some localities have been determined by a different method. Wild males collected out of doors, or single sons of wild females, are outcrossed to the standard orange purple females, and the chromosome configurations are studied in several hybrid larvae from each cross. If the wild fly was a structural heterozygote (for example, if it had one third chromosome differing from the other in gene arrangement), at least two types of hybrid larvae must appear. This has been observed in many instances. Sometimes all hybrid larvae examined are alike, which may be due either to the parents being structurally homozygous, or to the smallness of the sample tested. The following convention was therefore adopted: if three or more larvae from a given cross have been examined and found to be alike, the tested individual is regarded as having been structurally homozygous; if less than three larvae have been examined and only one type was found, only one chromosome is assumed to have been tested. It may be noted that in most cases six or seven larvae were examined from each cross.

Cytological examination has been made mostly on temporary aceto-carmine mounts. This method is unrivalled as a time-saving device, and

EXPLANATION OF PLATE 1

Plate 1.—Above—a map of the standard gene arrangement in the third chromosome of *Drosophila pseudoobscura*, showing the division into the sections and sub-sections, and also the loci of the breakages in some of the inversions recorded in this chromosome. Below—configurations observed in the third chromosome in various inversion heterozygotes. The scales (40 and 50 micra) indicate the magnification used for making the drawings of the standard map and of the other configurations respectively.


63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81
PIKES PEAK
ARROWHEAD
KLAMATH
SEQUOIA I
SEQUOIA I
PIKES PEAK
HYPOTHETICAL
ARROWHEAD
KLAMATH
HYPOTHETICAL
0 10 20 30 40


KLAMATH STANDARD
ARROWHEAD STANDARD
PIKES PEAK STANDARD
SANTA CRUZ STANDARD
CHIRICAHUA STANDARD
OLYMPIC STANDARD
ESTES PARK STANDARD
CUERNAVACA STANDARD
TREE LINE STANDARD
WAWONA STANDARD
74-77
0 10 20 30 40 50

moreover fresh temporary mounts are frequently superior even to the best permanent preparations. The drawings of the chromosomes reproduced below were made with the aid of a camera lucida, under the magnification of 90 × objective, 10 × ocular (Zeiss). The map of the standard third chromosome (Plate 1) is a composite drawing made up of several separate ones representing the given part of the chromosome most clearly. The magnification here is 120 × objective, 10 × ocular. In all the drawings which are not composites the parts of the chromosomes in which the disc patterns were not clear enough in a given preparation are represented by dotted outlines only. In general, cells in which the amount of pairing of homologous chromosome sections was at its maximum were selected for making the drawings.

GEOMETRICAL CONSEQUENCES OF MULTIPLE INVERSIONS

The occurrence of an inversion in a chromosome leads to the emergence of a "new" chromosome having a modified gene arrangement. This "new" chromosome may, in turn, undergo further change due to another inversion, or due to a translocation, deficiency, or to some other chromosome aberration. The occurrence subsequently of several inversions in the same chromosome may, theoretically, lead to particularly interesting results, which should be considered before the data on the gene arrangement in the chromosomes of *D. pseudoobscura* are presented.

Assume that a chromosome has genes arranged in the order ABCDEF. An inversion of the section containing the genes from B to E inclusive gives rise to a chromosome AEDCBF. If the original and the derived chromosome are present in the same individual (inversion heterozygote), a configuration resembling that shown in the upper right corner of figure 1 will be formed in the salivary gland cells.

A chromosome once changed by an inversion may undergo a further change due to another inversion. The location of the second inversion with respect to the first may vary. Three possibilities present themselves. (1) The second inversion may occur in the part of the chromosome not affected by the first. A chromosome ABCDEFGHI may be changed first to AEDCBFGHI and then to AEDCBFHGI. An individual heterozygous for ABCDEFGHI and AEDCBFHGI will have in its salivary glands a chromosome forming a double loop shown in figure 1, second line from above. Such inversions may be termed independent. (2) An inversion may take place wholly inside of the region affected by another inversion, or it may include that region. The chromosome ABCDEF changes first to AEDCBF and then to AECDBF, or else the change is ABCDEF→ ABDCEF→AECDBF. Such inversions may be called included ones. An individual having chromosomes ABCDEF and AECDBF is expected to

have in its salivary gland cells a double loop configuration shown in figure 1, second line from below. (3) The second inversion may have one end inside and the other outside of the region included in the first inversion. For example, the chromosome ABCDEFGHI may change consecutively to AFEDCBGHI and to AFEHGBCDI. Such inversions may be described as overlapping ones. The chromosome configuration expected to appear in the salivary gland cells of an individual heterozygous for overlapping inversions is like that shown in figure 1, lower right corner.

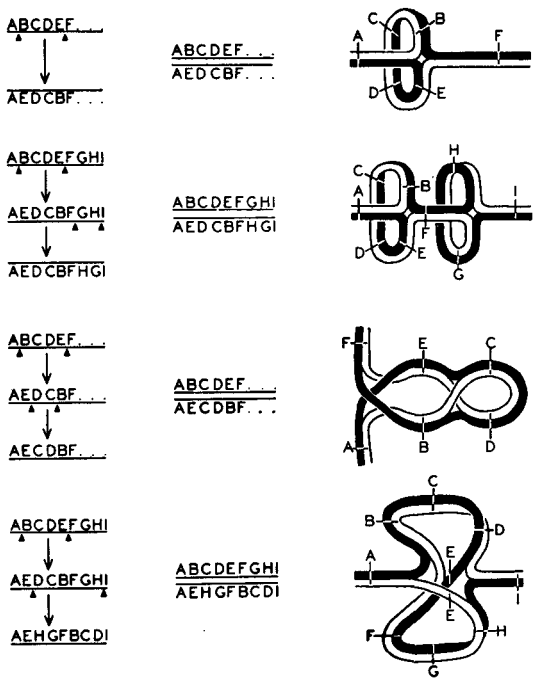


FIGURE 1.—A schematic representation of the pairing of chromosomes differing in a single or a double inversion. Above—a single inversion; second from above—two independent inversions; third from above—two included inversions; below—two overlapping inversions. Further explanation in text.

Overlapping inversions deserve an especially careful consideration. Suppose that three gene arrangements, ABCDEFGHI, AFEDCBGHI, and AFEHGBCDI, are encountered in a species. The first of these arrangements might have originated from the second through a single inversion; or else, the first might have given rise to the second, likewise through a single inversion. Similar relations exist between the second and the third of these arrangements: each of them might have originated from or given rise to the other by means of a single inversion step. But the supposition that the first has originated from the third, or vice versa, leads to great difficulties, which can be obviated only by supposing that the change has

been accomplished in two steps, the second arrangement being the intermediate stage. Indeed, in order that the chromosome ABCDEFGHI may become transformed at once into AFEHGBCDI, or vice versa, the chromosome must become broken simultaneously in four places: between A and B, D and E, F and G, and H and I. The occurrence of such a multiple breakage is in itself not very difficult to visualize, since such complex breakages have in fact been observed in some X-ray experiments with Drosophila. Whether they occur also without X-ray treatments is for the time being an open question. Much more important is the fact that the fragments of a chromosome broken in four places may reunite in a variety of ways: AGHEFBCDI, AEFBCDHGI, AEFHGBCDI, AFEHGBCDI, and others, all of which are theoretically equally likely to occur.

If the three arrangements, ABCDEFGHI, AFEDCBGHI, and AFEHGBCDI, are all observed to occur in nature, the probability of the direct origin of the first from the third, or vice versa, becomes almost nil. Indeed, this would involve the assumption that due to a mere coincidence the chromosome has been broken at exactly the same two places on at least two separate occasions. Since a chromosome of a moderate length may undergo breakage presumably at hundreds or even at thousands of points, such coincidences must be extremely rare. Even if the supposition is made that certain points in the chromosome are more likely to break than others, the coincidence of two breaks at exactly the same loci remains improbable, for at least some chromosomes are known to have undergone breakage at many points. It follows, then, that the phylogenetic relationships of the three gene arrangements represented above must be $1 \rightarrow 2 \rightarrow 3$, or $3 \rightarrow 2 \rightarrow 1$, or $1 \leftarrow 2 \rightarrow 3$, but not $1 \rightleftarrows 3$. In other words, although we can not determine directly which of the three arrangements is the ancestral and which are the derived ones, if any one is selected as the original then the course of the evolution is thereby fixed.

If only the first and the third arrangements are actually observed to occur in nature, then the second may be postulated theoretically as a form that either has lived in the past but has become extinct, or else is still living but has not been discovered. For if the change from the first to the third arrangement has taken place in two steps, each involving a single inversion, there could have been but a single intermediate, namely the second arrangement. Among the seventeen gene arrangements that are known in the third chromosome of *pseudoobscura*, at least one arrangement has been so predicted, and subsequently discovered to occur on Santa Cruz Island, off the coast of California, and in some other places.

The geometrical properties of the overlapping inversions that render them amenable to a phylogenetic analysis of the sort just outlined are not

found in the independent and the included ones. If only two sequences, ABCDEFGHI and AEDCBFHGI, are encountered, their genetic relationships are ambiguous. Supposing that inversions occur one at a time, the relationships ABCDEFGHI⇄AEDCBFGHI⇄AEDCBFHGI and ABCDEFGHI⇄ABCDEFHGI⇄AEDCBFHGI are equally probable. In other words, the intermediate steps in the phylogenetic series can not be reconstructed with a sufficient degree of assurance, while such a reconstruction is practicable for overlapping inversions. If one of the two theoretically possible intermediates (that is, AEDCBFGHI or ABCDEFHGI) is actually encountered in nature, it is of course likely that it, rather than its alternative, is the actual connecting link between the two end members of the series. But even in this most favorable case the method remains less precise than it is for overlapping inversions; it is possible, for example, that the arrangement ABCDEFGHI has given rise independently to AEDCBFGHI and ABCDEFHGI, and that a crossing-over between the latter has produced AEDCBFHGI.

In practice, it becomes important to establish whether two or more inversions observed in the same chromosome do or do not belong to the class of the overlapping ones. Fortunately, the salivary gland chromosome method is sufficiently accurate to permit a decision to be made in every adequately studied case. Even if the degree of the overlapping is small (that is, if one of the breaks in one of the inversions lies very close to a breakage point in the other), a careful investigation shows where in the "new" chromosome every section of the "old" one is located.

GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF RACE A

A composite map of the standard gene arrangement in the third chromosome of race A of *pseudoobscura* is represented in the upper part of Plate 1. It should be reiterated that the choice of this arrangement as a standard is arbitrary, and the results of the investigation would not be altered in the least if a different choice were made. TAN (1935) and DOBZHANSKY and TAN (1936) have already published maps of the same standard arrangement, but it is believed that the map here given is more accurate. We do not claim however that even this map represents all the faintest discs that may be found in this chromosome.

To facilitate description, the chromosomes of *pseudoobscura* have been subdivided into one hundred arbitrary sections; the third chromosome contains sections from 63 to 81 inclusive (DOBZHANSKY and TAN 1936). Each section is further subdivided into sub-sections denoted by letters A, B, C, etc. The limits of the sections and sub-sections are indicated by lines in Plate 1. In the standard arrangements the sections run in the natural order, from the proximal to the distal end, thus: 63 ABCDE, 64

ABC, 65 ABCD . . . 81 ABCD. Gene arrangements other than the standard one can be described most conveniently in terms of these numbered sections and sub-sections.

When the strain Arrowhead-5, derived from a single female collected near the village of Arrowhead, British Columbia, is crossed to the standard, the third chromosomes in the hybrid form a pairing configuration like that represented in Plate 1. From this configuration one can easily deduce that the Arrowhead strain differs from the standard in having a single inversion in the third chromosome. The origin of this inversion can be represented as being due to a breakage of the standard third chromosome in the sections 70B and 76B, and a rotation of the middle fragment by 180°. The arrangement of sections in the third chromosome of the Standard and the Arrowhead strains are as follows:

Standard 63,64 . . . 69,70A:70BCD,71 . . . 75,76AB:76C,77 . . . 81
Arrowhead 63,64 . . . 69,70A:76BA,75 . . . 71,70DCB:76C,77 . . . 81

The signs : indicate the loci of the breakages. Configurations similar to that observed in the Arrowhead/Standard hybrids have been found also in hybrids between the standard and many other strains. It follows that the gene arrangement first detected in the Arrowhead-5 strain is encountered rather commonly elsewhere; this will be referred to as the "Arrowhead" arrangement. In strains that are homozygous for the third chromosome of the Arrowhead type no loop-like configuration similar to that shown in Plate 1 is, of course, formed. The examination of the disc pattern in the third chromosome shows however that the seriation of the sections is like that just indicated for the Arrowhead rather than that for the Standard chromosome.

In crosses between certain strains collected on the slopes of Pikes Peak Colorado, and the standard strain, the third chromosome in the salivary gland cells has been observed to form a configuration like that shown in Plate 1 under the label "Pikes Peak/Standard." Although this configuration is identical in type with that in Arrowhead/Standard hybrids, a more careful inspection shows that different sections of the chromosome are involved in the formation of the loops in the two cases. It follows that the gene arrangement found in the Pikes Peak strains is different both from that found in the Arrowhead and in the standard strains. A comparison of the standard and the Pikes Peak arrangements may be represented thus:

Standard 63,64,65AB:65CD,66 . . . 75ABC:76 . . . 81
Pikes Peak 63,64,65AB:75CBA . . . 66,65DC:76ABC . . . 81

It is worth while to compare the Arrowhead and Pikes Peak arrangements with each other. Both can be thought of as being derived from, or giving rise to, the standard arrangement by a single inversion. The two

inversions are however of the overlapping type: the distal break in Pikes Peak lies further from the free end of the chromosome than the distal break in Arrowhead, while the proximal break in Pikes Peak is much closer to the base of the chromosome than the proximal break in Arrowhead (Plate 1). The origin of the Pikes Peak arrangement directly from Arrowhead, or vice versa, is therefore very improbable. The relationships of the three arrangements may be represented as Arrowhead⇄Standard⇄ Pikes Peak, but not as Arrowhead⇄Pikes Peak. In hybrids carrying one Arrowhead and one Pikes Peak chromosome a configuration resembling the double loop shown in figure 1, lower right corner, may be expected. One of these loops must be formed by the sections from 65C to 70A inclusive (corresponding to FGH in figure 1), and the other loop by sections from 70B 75C (corresponding to BCD in fig. 1), while the base (sections 63 to 65B) and the free end (sections 76B to 81) must pair normally (corresponding to I and A in fig. 1). Sections 76A and 76B are too short to be expected to pair frequently with their homologues (corresponding to E in fig. 1). The cross Arrowhead by Pikes Peak has actually been made, and the expected chromosome configuration has been observed in the hybrids.

Crosses between standard and certain strains from Santa Cruz Island California, show in the third chromosome a double-loop configuration depicted in Plate 1. An analysis of this configuration leads to the conclusion that the arrangement of sections in the third chromosome of the Santa Cruz strains is as follows:

63 . . . 67,68ABC:79BCD:76A,75 . . . 69,68D:79A,78,77,76CB:80,81

It is interesting to determine in what relation the Santa Cruz sequence stands to the previously described ones. An attempt to derive the Santa Cruz sequence from either Arrowhead or Pikes Peak shows immediately that at least three inversion steps are necessary, one of which transforms the Arrowhead or Pikes Peak sequences respectively into the standard one. Only two inversion steps are needed to derive Santa Cruz from the standard arrangement. These two steps can be represented as follows:

Standard 63 . . . 67,68ABCD,69 . . . 75,76A:76BC,77,78,79ABCD:80,81

Hypothetical 63 . . . 67,68ABC:D,69 . . . 75,76A,79DCB:A,78,77,76CB, 80,81

Santa Cruz 63 . . . 67,68ABC,79BCD,76A,75 . . . 69,68D,79A,78,77, 76CB,80,81

The hypothetical link between the standard and Santa Cruz has as yet not been discovered in *pseudoobscura*. It will be shown below that the essential characteristics of the hypothetical sequence are encountered in the X^2 chromosome of a related species, *miranda*. The relationships of the sequences described thus far are therefore as follows:

Arrowhead←→Standard←→Hypothetical←→Santa Cruz

↕

Pikes Peak

Crosses between the standard strain and certain strains derived from flies collected at Cuernavaca, Morelos, Mexico, showed in the third chromosome a configuration reproduced in Plate 1. This configuration, though at first sight very complex, can be resolved to indicate the following arrangement of sections in the chromosome of the Cuernavaca strain:

63,64AB,69DE,70 . . . 75,76A,79DCB,68CBA,67 . . . 64C,69CBA,68D, 79A,78,77,76CB,80,81

The following series of steps was worked out theoretically to account for the origin of the Cuernavaca sequence (IV) from the standard one (I):

(I) 63,64ABC,65 . . . 67,68ABCD,69ABCDE,70 . . . 75,76A:BC,77,78, 79ABCD:80,81

(II) 63,64ABC,65 . . . 67,68ABC:D,69ABCDE,70 . . . 75,76A,79DCB: A78,77,76CB,80,81

(III) 63,64AB:C,65 . . . 67,68,ABC79BCD,76A,75 . . . 70,69ED:CBA, 68D,79A,78,77,76CB,80,81

(IV) 63,64AB,69DE,70 . . . 75,76A,79DCB,68CBA,67 . . . 65,64C,69CBA, 68D,79A,78,77,76,CB,80,81

It may be noted that six breakages have to be assumed in the chromosome to derive the Cuernavaca sequence from the standard one; it would require eight breakages to derive Cuernavaca from either Arrowhead or Pikes Peak. The arrangements (II) and (III) were provisionally designated as "hypothetical 1" and "hypothetical 2" respectively. Shortly thereafter, due to accession of material from Santa Cruz Island, the Santa Cruz arrangement was discovered, and proved to be identical with the theoretically postulated "hypothetical 2" (=III). To test this identification, a simple experiment was made, namely a Cuernavaca strain was crossed to a Santa Cruz strain. If the Santa Cruz and the "hypothetical 2" arrangements are identical, a single inversion is expected in the hybrid, the sections from 64C to 69ED inclusive being in the inversion loop. The observations have borne out this prediction. Hence, no more than a single hypothetical arrangement need be postulated to account for the descent of the Cuernavaca arrangement from the standard one, or vice versa. The line of descent may be represented as follows:

Standard←→"hypothetical"←→Santa Cruz←→Cuernavaca

Certain strains from the Chiricahua Mountains, Arizona, produce when crossed to the standard a pairing configuration in the third chromosome shown in Plate 1. An analysis of this configuration leads to the conclusion that a gene arrangement not identical with those described previously is

found in the Chiricahua strains. This new arrangement, denoted Chiricahua I, turns out to have the following sequence of sections:

63 . . . 67,68ABC,79BCD,76A,75 . . . 71,78ABC,79A,68D,69,70,77,76CB, 80,81

A comparison of the Chiricahua I arrangement with the standard one shows that a triple inversion is present; six breaks in the chromosome have to be assumed to derive one from the other. The origin of the Chiricahua I becomes clearer if it is compared with the Santa Cruz arrangement, for they are identical, except in the sub-terminal portion of the chromosome. One can be derived from the other by a single inversion, as follows:

Santa Cruz 63 . . . 67,68ABC,79BCD,76A . . . 71:70,69,68D,79A,78CBA :77,76CB,80,81

Chiricahua I 63 . . . 67,68ABC,79BCD,76A . . . 71:78ABC,79A,68D,69, 70:77,76CB,80,81

The cross Chiricahua I× Santa Cruz gives the expected configuration, namely the single inversion loop shown in Plate 2. Chiricahua I can not be derived from Cuernavaca, or vice versa, by a single inversion, since they are related as overlapping inversions. They can be derived from each other only through the intermediate step of the Santa Cruz arrangement, as follows:

Chiricahua I←→Santa Cruz←→Cuernavaca

Three separate strains from Mammoth Lake, Sierra Nevada, California, produced when crossed to the standard a configuration not encountered in any other crosses. Unfortunately, these three strains were lost before this new arrangement, Mammoth, was studied in detail and before appropriate drawings could be made. The most probable (though not conclusively established) sequence of sections in the Mammoth chromosome appears to be as follows:

63 . . . 67,68ABC,79BCD,76A:76BC,77,78,79A,68D,69 . . . 75:80,81

If this interpretation is correct, the Mammoth arrangement can be derived from Santa Cruz by a single inversion. No test of this interpretation by crossing the two to each other has been made however.

Still another, and very interesting, arrangement has been detected in a strain coming from the tree line region of Pikes Peak, Colorado (elevation more than 11,000 feet). The heterozygote Tree Line/Standard shows a configuration in the third chromosome represented in Plate 1. The sequence of sections in the Tree Line chromosome, compared to that in Santa Cruz, is as follows:

Santa Cruz 63 . . . 67,68ABC,79BCD,76A,75,74C:74BA,73 . . . 69:68D, 79A,78,77,76CB,80,81

Tree Line 63 . . . 67,68ABC,79BCD,76A,75,74C:69 . . . 73,74AB:68D, 79A,78,77,76CB,80,81

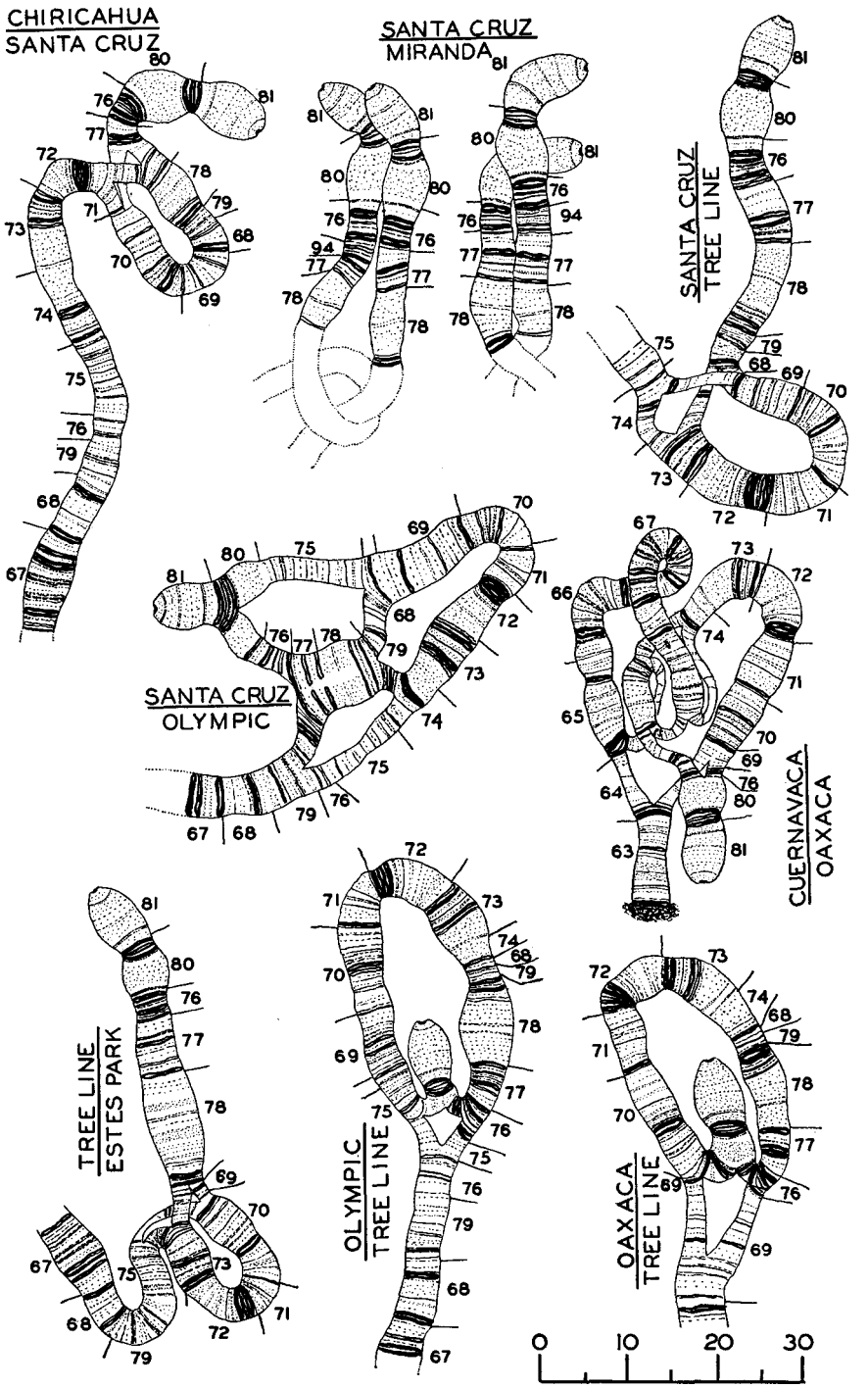


Plate 2.—Configurations observed in the third chromosome in various inversion heterozygotes. The scale below represents 30 micra.

The Tree Line arrangement may, consequently, be derived from Santa Cruz, or vice versa, by a single inversion. The derivation of Tree Line from any other arrangement described above demands more inversion steps, namely, two (Cuernavaca, Chiricahua I, Mammoth), three (Standard), or four (Arrowhead, Pikes Peak). In every case an arrangement identical with Santa Cruz has to be postulated if the origin is to be accomplished with a minimum of inversion steps. The heterozygote Tree Line/Santa Cruz shows, as expected, a single fairly short inversion in the third chromosome (Plate 2).

In turn, the Tree Line arrangement is very simply related to three other chromosome arrangements, each of which can be derived from Tree Line, and from no other arrangement, through a single inversion step. These three new arrangements were first recovered from strains coming respectively from Estes Park, Colorado, from mountains near Brinnon, Olympic peninsula, Washington, and from Cerro San Jose, Oaxaca, Mexico. They were designated as the Estes Park, Olympic, and Oaxaca arrangements respectively. A comparison of the Tree Line arrangement with the three new ones is shown below:

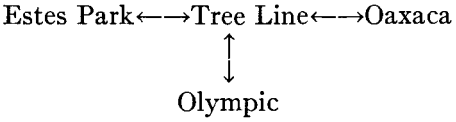
Tree Line 63 . . . 67,68ABC,79BCD,76A,75C:BA,74C,69ABC:DE,70 . . . 73,74AB,68D,79A:78,77,76C:B,80A:BC,81

Estes Park 63 . . . 67,68ABC,79BCD,76A,75CBA,74C,69ABC:79A,68D, 74BA,73 . . . 70,69ED:78,77,76,CB,80ABC,81

Olympic 63 . . . 67,68ABC,79BCD,76A,75C:80A,76BC,77,78,79A,68D, 74BA,73 . . . 70,69EDCBA,74C,75AB:80BC,81

Oaxaca 63 . . . 67,68ABC,79BCD,76A,75CBA,74C,69ABC:76C,77,78, 79A,68D,74BA,73 . . . 70,69ED:76B,80ABC,81

To test the above interpretation, the crosses Estes Park×Tree Line, Olympic×Tree Line, and Oaxaca×Tree Line were made. As expected, in each of the crosses the third chromosome showed a single inversion, but the sections included in the inversion loop were different in each case (Plate 2). All three inversions belong to the overlapping class, although the proximal breaks in Estes Park and Oaxaca are very close to each other, being located at approximately the middle of the length of section 69. The relationships of the four arrangements under consideration may be represented as follows:



The crosses Olympic×Santa Cruz (Plate 2), Olympic×Estes Park, and Oaxaca×Estes Park were also made. The third chromosomes in the hybrids showed double loop configurations with some unpaired sections, as

expected in overlapping inversions. The crosses Estes Park×Standard (Plate 1), Olympic×Standard (Plate 1), Oaxaca×Standard, Oaxaca×Cuernavaca (Plate 2), Oaxaca×Chiricahua I, Estes Park×Chiricahua I, and Oaxaca×Pikes Peak give very complex pairing configurations in which many chromosome sections usually fail to pair with their homologues. All these relationships are in accord with expectations, since Estes Park, Olympic, Oaxaca, and Santa Cruz can be derived from each other through two inversion steps, the Tree Line arrangement being in every case the connecting link which could be constructed theoretically if it were not actually observed to occur in nature. It takes however three steps to derive any of these arrangements, except Santa Cruz, from either Chiricahua I or Cuernacava, and four steps to derive them from the standard arrangement.

In addition to the eleven arrangements thus far described, one more, the twelfth, has been observed in race A. In a cross between a strain homozygous for the Arrowhead arrangement and Chiricahua-6, a larva was found that exhibited a configuration of an unusual type. The distal part of the third chromosome had a sequence of sections identical with that encountered in Arrowhead, but there was a short inversion in the middle part, involving approximately sections 69, part of 70, part of 76, and 75. Obviously, some of the flies in the Chiricahua-6 strain had a gene arrangement not hitherto encountered; this arrangement is labeled Chiricahua II. Attempts to recover the Chiricahua II sequence from the strain in which it was once observed proved unavailing, and this sequence has never been seen again. Chiricahua II is clearly derived from the Arrowhead arrangement through a single inversion, its relationship to other arrangements being as follows:

Standard←→Arrowhead←→Chiricahua II

GENE ARRANGEMENTS IN THE THIRD CHROMOSOME OF RACE B

The gene arrangement in the third chromosome of race B is apparently about as variable as it is in race A. Relatively few strains of race B have been examined however, and therefore the number of arrangements recorded in this race is smaller than in race A, namely six. TAN (1935) has shown that the standard arrangement is encountered in race B as well as in race A, but he has also found that race B usually has another gene sequence in the third chromosome, giving a single inversion in the third chromosome in interracial hybrids with Standard race A. This most frequent arrangement in race B is denoted as Klamath, according to the name of the locality (in northern California) from which one of the strains carrying it was collected.

The hybrids between race B strains possessing a third chromosome of

the Klamath type, and either race A or race B strains with the standard arrangement, show an inversion loop depicted in Plate 1. This inversion occupies approximately the same part of the chromosome as that found in Standard×Arrowhead heterozygotes (Plate 1), but a careful examination discloses that the two inversions are unquestionably not identical. The sequence of sections in the Klamath chromosome is as follows, the Arrowhead sequence being given for comparison:

Klamath 63 . . . 69, 70ABCD:77,76CBA,75 . . . 71:78,79,80,81
Arrowhead 63 . . . 69, 70A:76BA,75 . . . 71,70DCB:76C,77,78,79,80,81

In the Klamath B×Arrowhead A hybrids no inversion loop is present in the third chromosome, but two sections, one lying at about the middle and the other in the distal part of the chromosome, remain unpaired. These are sections 70BCD, 76C, and 77. Klamath may be derived from Standard, or vice versa, through a single inversion, but it takes two steps to pass from Arrowhead to Klamath. The relationships of the three sequences are therefore as follows:

Klamath←→Standard←→Arrowhead

Three strains of race B from Sequoia National Park, California, were crossed to a strain possessing the Klamath chromosome. The hybrids showed in the third chromosome a double inversion (fig. 2). The gene arrangement encountered in these three strains is referred to as Sequoia I; it is characterized by the following sequence of sections:

63 . . . 69,70:73,72,71:74,75 . . . 80,81

It may be noted at once that the Sequoia I arrangement may be derived through a single inversion from the standard, but it takes two steps to obtain Sequoia I from Klamath, or vice versa. Nevertheless, we are not dealing here with overlapping inversions, since the proximal breakages in Sequoia I and Klamath seem to lie at the same point, corresponding to the dividing line between sections 70 and 71. The Sequoia I inversion may be described as being included in the Klamath one, with one end apparently coinciding. Hence, the relationships between Sequoia I and Klamath may be pictured in the following two ways. First: 63 . . . 70:77 . . . 74:73 . . . 71:78 . . . 81 (Klamath)←→63 . . . 70:71 . . . 73:74 . . . 77:78 . . . 81 (Standard) ←→ 63 . . . 70:73 . . . 71:74 . . . 77:78 . . . 81 (Sequoia I), or, second, 63 . . . 70:77 . . . 74:73 . . . 71:78 . . . 81 (Klamath) ←→63 . . . 70:77 . . . 74:71 . . . 73:78 . . . 81 (hypothetical)←→63 . . . 70:73 . . . 71:74 . . . 77:78 . . . 81 (Sequoia I). The second of these two manners of representation demands a hypothetical connecting link, while the first does not. Since the standard arrangement is encountered in race B populations inhabiting the region of the Sequoia National Park, by far the most probable course of evolution is as follows:

Sequoia I←→Standard←→Klamath,

but it should nevertheless be remembered that the degree of probability with which this relationship is established is lower than that involved in other conclusions presented above.

Three other gene arrangements have been detected in strains of race B coming from, respectively, Cowichan Lake, British Columbia, the neighborhood of Wawona, California, and from Sequoia National Park, California. These arrangements are denoted as Cowichan, Wawona, and

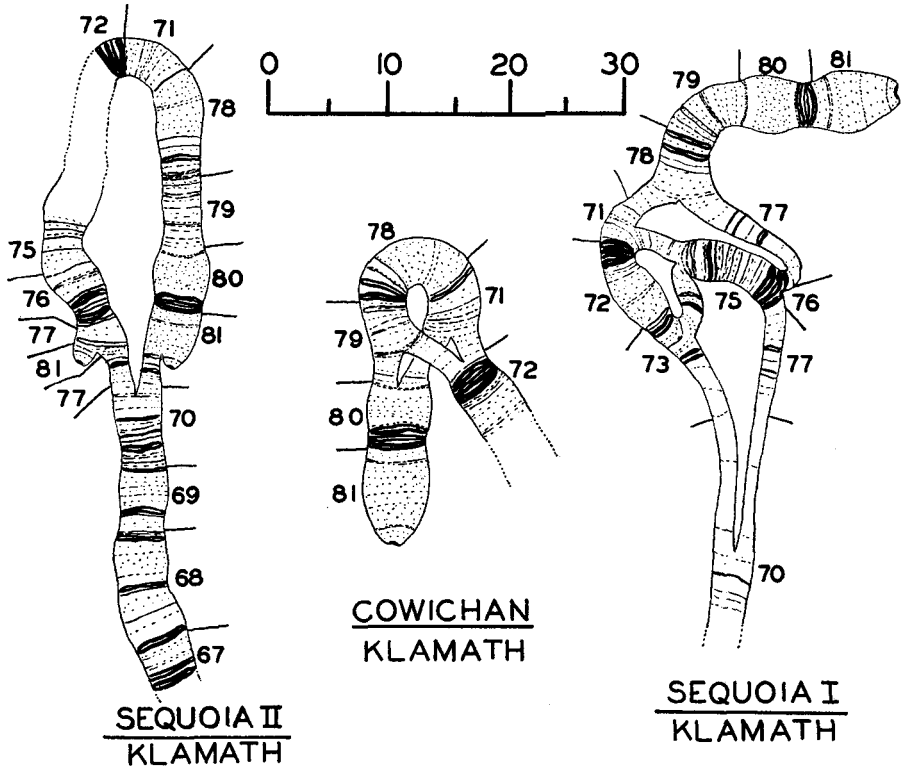


FIGURE 2.—Configurations in the third chromosome in hybrids between various strains of race B.

Sequoia II. The pairing configurations encountered in the hybrids Sequoia II×Klamath and Cowichan×Klamath are shown in fig. 2, and that in the hybrid Wawona×Standard in Plate 1. The distribution of sections in the three new arrangements is as follows (the data for Klamath being given for comparison):

Klamath 63 . . . 70, 77B:A, 76:75 . . . 71C:BA, 78A:BC, 79ABC:D, 80, 81AB:CD

Cowichan 63 . . . 70, 77BA, 76, 75 . . . 71C:79CBA, 78CBA, 71AB:79D, 80, 81ABCD

Wawona 63 . . . 70, 77BA, 76:78A, 71ABC . . . 75:78BC, 79ABCD, 80, 81ABCD

Sequoia II 63 . . . 70, 77B:81BA, 80, 79DCBA, 78CBA, 71ABC . . . 75, 76, 77A:81CD

Each of the three new arrangements can be derived from Klamath by a single inversion, while it takes two inversions to derive them from the Standard or from each other. The Cowichan and Wawona inversions are overlapping, and therefore the relationship

Cowichan←→Klamath←→Wawona

may be taken as established. The inversion encountered in Sequoia II is much longer than that found either in Cowichan or in Wawona, and includes the regions involved in the other two. The relationships between these arrangements are therefore similar to those discussed above in connection with the series Klamath-Standard-Sequoia I. In other words, since the Klamath arrangement is known to be present in nature, the most probable inference is that Sequoia II has descended directly from Klamath, or has given rise to the latter, independently from the origin of Wawona and Cowichan. If however, the Klamath arrangmeent were unknown, one could formulate two equally probable hypotheses, first, that the ancestral arrangement was like Klamath, or, second, that the ancestral arrangement was one combining the properties of Sequoia II and Wawona, or of Sequoia II and Cowichan. Such hypothetical arrangements might have given rise to the existing ones through single inversions.

On the other hand, the relationships between Sequoia II, Klamath, and Standard are quite clear. The inversions differentiating these arrangements are all overlapping, and therefore Sequoia II can not arise directly from Standard, or vice versa. The same is true for Wawona and Cowichan, Hence, the relationships are as follows:

Sequoia II←→Klamath←→Standard

Wawona←→Klamath←→Standard

Cowichan←→Klamath←→Standard

Taken as a whole, the six gene arrangements recorded in race B are not related to any of the arrangements known in race A, except through the Standard one. Since, however, the Standard is the only arrangement encountered in race A as well as in race B, this connecting link is sufficient to account for the origin of all arrangements in both races from a common source.

THE EFFECTS OF INVERSIONS IN THE THIRD CHROMOSOME ON CROSSING OVER

Standard sequence. The map of the third chromosome given by STURTEVANT and TAN (1937) is based on the results obtained from females homozygous for the standard sequence. The following loci are included: orange

(0), Blade (7.7), abrupt (9.7), Emarginate (13.3), Jagged (23.3), Scute and polychaete (28.3), purple (49.9), curved (65±), crossveinless (68.0). So far as tested, values similar to these are obtained when one of the "Standard" chromosomes is derived from race B, though the usual complications (differential viability and effects of inversions in other chromosomes) are evident here as in all crossing over experiments with A×B hybrids.

Arrowhead sequence. In females of the constitution Standard/Arrowhead the orange-Scute interval gives about 8 percent crossing over (192 out of 2511, =7.6 percent in one series), but the results appear to be variable. Orange, Blade, and Emarginate have all given crossing over with Scute; Scute and the inversion have never given crossing over. One doubtful case suggests a crossover between the inversion and purple, and a more certain one indicates that crossveinless lies to the right of the inversion. The total frequency of Scute-crossveinless crossing over is certainly very low, not more than 0.1 percent.

Several mutant genes have been found in Arrowhead chromosomes: a Jagged allelomorph, plexus, narrow, and rugose. We have not succeeded in getting crossing over between any of these and the inversion in Standard /Arrowhead. In homozygous Arrowhead the data indicate the following map: orange (0), Emarginate (14.1), plexus (22.1), Jagged (23.1), narrow (25.1), rugose (29.7). Taking these data, together with the results of TAN (1937), it may be concluded that Jagged and crossveinless are not in the inverted section of Arrowhead, Scute and rugose are in it, and purple and narrow are doubtful.

Pikes Peak. Standard/Pikes Peak has given crossing over between purple and crossveinless, a Pikes Peak crossveinless chromosome being easily obtained. No crossovers have been observed elsewhere. An experiment involving orange, Blade, Scute, and purple was carried out on the assumption that so long an inversion should give recoverable double crossovers, but none was obtained.

Klamath. In Standard-B/Klamath the results (orange Scute purple tested) are much like those from Standard-A/Arrowhead, plus the usual A×B complications. Orange-Scute crossovers are present in nearly every culture, Scute-purple ones have never been found.

Other sequences. No systematic study has been carried out for the remaining sequences, but a few data have been obtained in experiments using orange Scute purple (standard) against some of them. In such experiments Scute-purple crossovers have never been found; orange-Scute ones occur rarely (approximately 1 percent) when the opposing chromosome is Chiricahua, Estes Park, or Oaxaca. They have not been found in the cases of Santa Cruz, Cuernavaca, or Tree Line, but the numbers of flies seen are too low to be significant. Comparison of the cytological maps

(TAN, in press) with the limits of the inversions here given indicates that Pikes Peak and Cuernavaca would be expected not to give single crossovers between orange and Scute; Santa Cruz and Tree Line may be expected to produce them as frequently as do Chiricahua, Estes Park, and Oaxaca—a conclusion not negatived by the small amount of data available.

Combinations not involving standard. A few experiments have been carried out with Arrowhead/Pikes Peak and Arrowhead/Klamath, since these combinations might be expected to give single crossovers in the common inverted region (STURTEVANT and BEADLE 1936). None was found, and the probability is high that, if they occur, the crossover chromosomes are inviable. Such a result is not unexpected, since inversions as different as these in the X chromosome of *melanogaster* often give no viable crossovers. In the case of Arrowhead/Klamath, where the inversions are more alike, the present data are not conclusive, owing to the scarcity of usable mutant genes. The experiment is also complicated by the fact that the Klamath chromosome used was (of necessity) originally derived from race B, and the flies carrying it were not fully viable and fertile.

Disturbances in chromosome pairing. In connection with the data on the reduction of the frequency of crossing over in inversion heterozygotes, it has been found that the pairing of the chromosomes differing in gene arrangement is frequently disturbed in the salivary gland cells. As pointed out above, most of the drawings in Plates 1 and 2 and in figure 2 are made from selected figures showing as complete a pairing as can be found. In general, failures of pairing are rather common. Where only a single inversion is present (Standard/Arrowhead, Standard/Pikes Peak, Standard/Klamath, and others) the pairing is usually, though by no means always, nearly complete, only short sections immediately adjacent to the breakage points failing to pair. But where the homologous third chromosomes differ by double, triple, or multiple inversions some fairly long sections nearly always fail to pair with their homologues. In the hybrids Oaxaca/Standard, Estes Park/Standard, and many similar ones, the chromosomes usually pair only in the basal regions and in a few places in the parts affected by the inversions. Instances of cells where the third chromosomes have failed to pair almost entirely are not uncommon. No drawing of the Oaxaca/Standard configuration is given in the present article because we have not succeeded in finding a cell in which the third chromosomes of this hybrid show more than a negligible amount of pairing in the inverted region. Although small failures of pairing are sometimes encountered in structurally homozygous individuals as well, there can be no doubt that in inversion heterozygotes such failures are both more frequent and more extensive.

GENE ARRANGEMENT IN CHROMOSOMES OTHER THAN THE THIRD

The third chromosome is by far the most variable one with respect to the gene arrangement. Nevertheless, the gene arrangement has been found to vary to some extent also in all other chromosomes, except the small fifth, which, however, has not been carefully examined in most preparations. TAN (1935) found that the left limb of the X chromosome in race A differs from that in race B in having an inverted section. As far as we know, this difference is constant, in the sense that all strains of race A differ from all race B strains in having this inversion.

Three different gene arrangements are known in the right limb of the X chromosome. Race A differs from race B in having an inversion in this limb (TAN 1935), but the so-called "sex-ratio" strains in race B have the same arrangement as the normal representatives of race A (STURTEVANT and DOBZHANSKY 1936b). "Sex-ratio" strains in race A differ from normal A by an inversion, which is however not identical with that differentiating normal race B. Cytologically, the three sequences are related as overlapping inversions:

Sex-ratio A←→Normal A, or Sex-ratio B←→Normal B

Six different gene arrangements have been recorded in the second chromosome, three in race A and three in race B. This chromosome carries sections from 43 to 62 inclusive, which in most race A strains are arranged in the natural order; this is the "Standard" arrangement for the second chromosome. Most strains of race B have sections inverted from the distal part of 52 to 56 inclusive (TAN 1935, DOBZHANSKY and TAN 1936). Denoting the proximal part of a section as p, and the distal part as d, the two sequences may be represented as follows:

Standard 43 . . . 51p:51d, 52 . . . 56:57 . . . 62
Race B 43 . . . 51p:56 . . . 52, 51d:57 . . . 62

Three strains of race A proved to carry a gene arrangement deviating from the Standard. These strains are Zuni-4, Magdalena-2, and Pinos Altos-9, all from New Mexico. The new arrangement, denoted as Zuni, is therefore restricted to a relatively narrow geographical region, outside of which it is not encountered. Even in New Mexico most strains have the Standard arrangement, and the three exceptional strains just enumerated were all inversion heterozygotes, carrying one Standard and one Zuni second chromosome. The Zuni arrangement differs from the Standard in having the segment extending approximately from section 47 to 53 inverted. The limits of this inversion overlap those distinguishing race B from the Standard, and consequently the relationships of the three arrangements may be represented as follows:

Zuni←→Standard←→race B

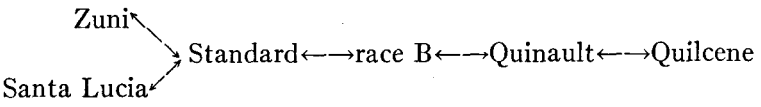
TAN (1935) has observed a long inversion in the second chromosome in

the hybrids between the strain Santa Lucia-7 (California) and the strain carrying the mutants Bare and Smoky. The latter is known to possess the Standard arrangement. From the published figure of this inversion it appears that the Santa Lucia arrangement is unquestionably different from both Zuni and Race B arrangements. Whether or not the Santa Lucia inversion overlaps Zuni and Race B is not clear, but in any case it takes one inversion-step to derive the Santa Lucia arrangement from the standard one, while it takes two steps to derive it from either Zuni or race B.

Two strains of race B have been found to deviate from the normal structure of the second chromosome in that race. Some of the larvae in the crosses cinnabar (race B) × Quinault-23 (Olympic Peninsula, Washington) had a fairly long inversion in the distal part of the second chromosome. Some of the hybrids in the cross cinnabar × Quilcene-4 (Olympic Peninsula) had a double inversion in the same part of the chromosome. The sequence of sections in these arrangements is as follows:

Race B 43 . . . 51p, 56d:56p, 55 . . . 52, 51d, 57:58 . . . 61, 62p:62d
Quinault 43 . . . 51p, 56d:62p, 61 . . . 58:57, 51d, 52 . . . 55, 56p:62d
Quilcene 43 . . . 51p, 56d, 62p, 61 . . . 58:56p, 55 . . . 52, 51d, 57:62d

The distal breakages in the Quilcene and Quinault inversions both lie in section 62, but it appears that in the former the exact point of the break is somewhat closer to the free end of the chromosome than in the latter. If this is the case, the two inversions are overlapping, but the material studied is not extensive enough to make this conclusion certain. In any event, the most probable relationship of the three arrangements is that Quinault is derived from the race B one, and Quilcene is derived from Quinault. The relationships of all the arrangements recorded in the second chromosome may be represented by the following scheme:



The fourth chromosome proves to be the least variable one (neglecting the small fifth). It has identical gene arrangements in race A and race B, as attested by the complete pairing of the homologues in the interracial hybrids. The only variation observed in this chromosome has been found in some larvae from the cross of the strain Cuernavaca-5 (Mexico) to a laboratory strain carrying the mutant gene plexus. The fourth chromosome in some of the hybrids showed a large inversion extending from almost the free end to the sub-basal region of the chromosome. Since the plexus strain has been repeatedly used for cytological examinations of various hybrids, and showed a normal fourth chromosome, the inversion came presumably from the Cuernavaca-5 strain.

COMPARISON OF DROSOPHILA PSEUDOOBSCURA WITH D. MIRANDA

DOBZHANSKY and TAN (1936) have compared the disc patterns in the salivary gland chromosomes of *D. pseudoobscura* with those in a closely related species, *D. miranda*. They found that the gene arrangements in the two species are profoundly different, at least one hundred breakages being needed to derive the chromosome structure encountered in one of the species from that in the other. Yet, one may inquire to which of the gene arrangements known in *pseudoobscura* the gene arrangement of *miranda* is most closely related. Fortunately, the overlapping inversion method permits an unequivocal answer to this question.

As pointed out by DOBZHANSKY and TAN (1936), it takes one less inversion step to derive the gene arrangement in the left limb of the X chromosome in *miranda* from that in race A than from that in race B of *pseudoobscura*. Furthermore, the interracial inversion overlaps the limits of the inversion distinguishing race A and *miranda*. Hence, the relationships are as follows:

Race B←→Race A←→*miranda*

A comparison of the right limbs of the X chromosome and of the second chromosomes leads to conclusions fully consistent with the above: in either case the *miranda* arrangement is related to that in race A rather than to that in race B of *pseudoobscura* (inversions are overlapping). As to the third chromosome, the data of DOBZHANSKY and TAN were inconclusive. One may note here that the homologue of the third chromosome of *pseudoobscura* is present in *miranda* in duplicate in the females but only once in the males, and is termed the X^2 chromosome (DOBZHANSKY 1935). The proximal part of the X^2 contains sections 65, 66, 68, 70, and 71, and is therefore similar to the proximal part of the third chromosome of *pseudoobscura* (standard arrangement). Section 71 in *miranda* is however followed distally by a long region the homology of which was left undecided in the article of DOBZHANSKY and TAN (loc. cit.), since this region has never been observed to be paired with anything in the *miranda*×*pseudoobscura* hybrids. However, this region contains parts much resembling in disc patterns the sections 72 and 73 of *pseudoobscura*, and arranged in a similar manner (cf. drawings of DOBZHANSKY and TAN, 1936). Finally, the free end of the X^2 has sections 80 and 81, a condition encountered also in *pseudoobscura*. Proximally from section 80 the X^2 carries a series of prominent discs, the origin of which has not been determined, except that a few of them were found to be homologous to a part of section 94 in the fourth chromosome of *pseudoobscura*.

When the Santa Cruz arrangement in the third chromosome of *pseudoobscura* became known, we were struck by the resemblance of the disc pat-

terns in the distal parts of that chromosome and of the X^2 chromosome of *miranda*. A cross of *pseudoobscura* Santa Cruz×*miranda* was therefore made, and the hybrid larvae were studied cytologically. Several cells have been found in which the distal portions of the X^2 and of the third chromosome were paired. Two drawings in Plate 2 show the configurations observed. An analysis of these configurations leads to the conclusion that the distal portions of these chromosomes are built as follows:

Third chromosome of Santa Cruz . . . 78, 77, 76CB, 80, 81

X^2 chromosome of *miranda* . . . 78, 77, 94 (part), 76CB, 80, 81

In other words, except for the insertion of a part of section 94 in *miranda*, the two chromosomes are similar in this region. Assuming, further, that the identification of sections 72 and 73 in the X^2 chromosome of *miranda* is correct, we are forced to conclude that the X^2 chromosome has a gene arrangement most similar to the "hypothetical" one, postulated as a connecting link between standard and Santa Cruz in *pseudoobscura* (cf. page 36). It does not follow of course that the "hypothetical" arrangement in *pseudoobscura* is identical with that observed in *miranda*. In fact, this is certainly not the case, since many differences between the two are present. Nevertheless, it may be taken for established that it takes less alteration to derive the *miranda* sequence from the "hypothetical" one in *pseudoobscura* than from any other.

Thus, all the information now available agrees in pointing to a closer similarity between race A of *pseudoobscura* and *miranda* than between race B and *miranda*.

PHYLOGENY OF THE GENE ARRANGEMENTS IN THE THIRD CHROMOSOME

The various separate phylogenetic schemes presented above have all been combined in one diagram in figure 3. In the earlier sections the schemes were presented with double-headed arrows, since there is no inherent method of determining the directions in which inversion phylogenies should be read. The overlapping inversion method would not prevent us from supposing that any one of the gene arrangements shown in figure 3 is the ancestral one; but once an arrangement is selected as ancestral, the course of the evolution is determined. In figure 3 most of the arrows are single-headed; it is the purpose of the present section to show how we have arrived at this result.

It is simplest to suppose that the common ancestor of *pseudoobscura* and *miranda* had the "hypothetical" sequence, since *miranda* is related to no other existing *pseudoobscura* sequence except through this one. Owing to its exceptional sex chromosome mechanism, *miranda* cannot be considered ancestral to *pseudoobscura*, but must be derived either from *pseudoobscura* itself, or from a common ancestor that was (at least as regards its X and

its third chromosomes) more like *pseudoobscura*. Further, it may reasonably be supposed that the separation took place long ago, in order to account for the great difference between *miranda* and *pseudoobscura*. The "hypothetical" sequence is thus to be considered a very ancient one.

Next to the difference between the species *pseudoobscura* and *miranda*, the most striking and important distinction in the group is that between races A and B, which have practically reached a species status, except for

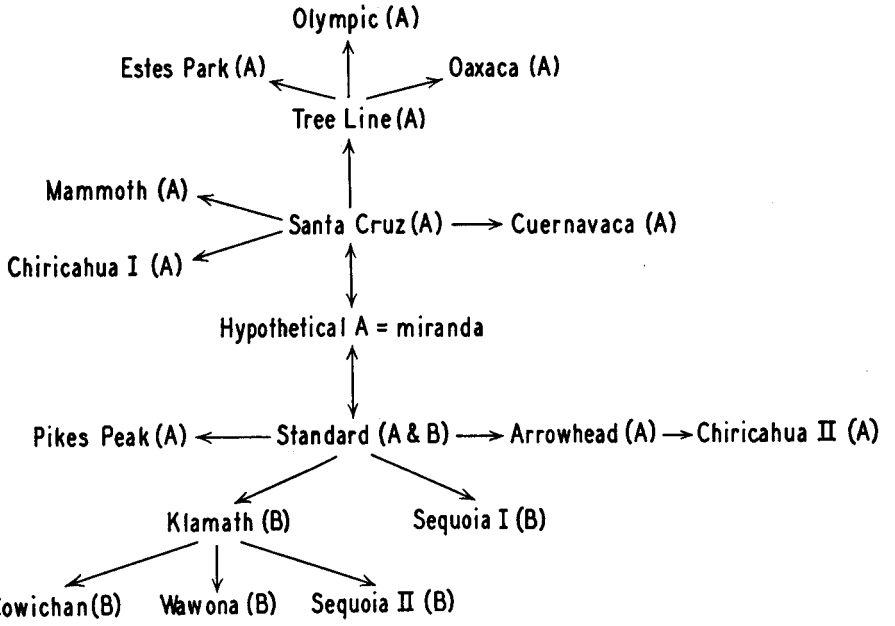


FIGURE 3.—Phylogeny of the gene arrangements in the third chromosome of *Drosophila pseudoobscura*. Any two arrangements connected by an arrow in the diagram differ by a single inversion. Further explanation in text.

the fact that they remain indistinguishable morphologically. The difference between these "races" may likewise be supposed to be of long standing. It is clear that the common ancestor of A and B had the Standard sequence, since this is the only one common to the two, and is a necessary connecting link between all other race A sequences on one hand and all other race B sequences on the other hand.

The great antiquity of the Standard and "hypothetical" sequences may thus be taken for established. There is one other arrangement that may be supposed to be relatively old, namely, Santa Cruz. Only Standard and Santa Cruz form centers to which a whole series of other arrangements are directly related (fig. 3), and only these two are derived by single steps from the "hypothetical" arrangement.

The most probable view seems to be that the original form was either

Standard or "hypothetical," with Santa Cruz still to be considered as a possibility. The argument cannot be taken as excluding other arrangements, though all those restricted to race B are highly improbable. It is, of course, entirely possible that the common ancestor of race A and race B, as well as that of *D. pseudoobscura* and *D. miranda*, was itself variable in gene arrangement, just as its living derivatives are.

GEOGRAPHICAL DISTRIBUTION OF THIRD CHROMOSOME GENE ARRANGEMENTS

A list of localities and strains in which the various third-chromosome gene arrangements have been recorded is given below; figures 4 and 5 present the same data in map form.

RACE A

Standard. British Columbia: Lytton-1; Merritt-2; Lake Okanagan-4, 5, 8; Lake Shuswap (Mara)-3; Kaslo-3, 4.

Washington: La Grande-2; Chelan-2, 8, 10, 12; Metaline Falls-1; The Dalles-2.

Montana: Bitterroot Mts.-6.

Oregon: Newbery Crater-3.

Northern California and Sierra Nevada: Dunsmuir-3, 9; Lassen-1, 19; Oakland-1; Pacific Grove-2; Santa Lucia-35; Wawona-1, 3, 6, 7, 8, 9; Mammoth Lake-22; Sequoia Park-3, 13, 15; Kern-1.

Southern California: Santa Cruz Island (table 1); Arroyo Seco (Pasadena)-1, 2, 23; Azuza, San Gabriel Canyon (table 1); Dollar Lake-2; Barton Flats-1, 7, 11; Banner (table 1); Julian (table 1); Lake Henshaw -2, 3, 4, 5.

Lower California: Guadelupe (table 1); Santo Tomas (table 1).

Nevada: Charleston-1, 5, 6, 7, 9, 10; Las Vegas-3.

Utah: Cedar City-A.

Idaho: Idaho Falls-2, 3, A (possibly introduced by man).

Nebraska: Scottsbluff-3.

Arizona: Santa Rita Mts.-6.

New Mexico: Carizozo-5, 7.

Texas: Georgetown-1.

Arrowhead. British Columbia: Yale-7; Pavilion-5; Kamloops-1; Merritt-2; Okanagan-2, 6, 8; Shuswap-3; Arrowhead-1, 5; Kaslo-4.

Washington: La Grande-2; Olympic-2; Chehalis-4; Chelan-7; Metaline Falls-2, 3.

Idaho: Lake Coeur d'Alene-2, 7, 8; Boise-1, 2; Idaho Falls-1, 2.

Montana: Bitterroot-3, 4, 6, 7.

Wyoming: Big Horn Mts.-5, 6.

South Dakota: Black Hills-3, 5.

Nebraska: Scottsbluff-4, 3.

Oregon: Sisters-6; Newbery Crater-3; Crater Lake-3.

Northern California and Sierra Nevada: Dunsmuir-10; Lassen-16; Wawona-1, 7, 8, 9; Mammoth-2, 4, 8, 22; Lake Tahoe-2; Sequoia-11, 15; Greenhorn Mts.-11.

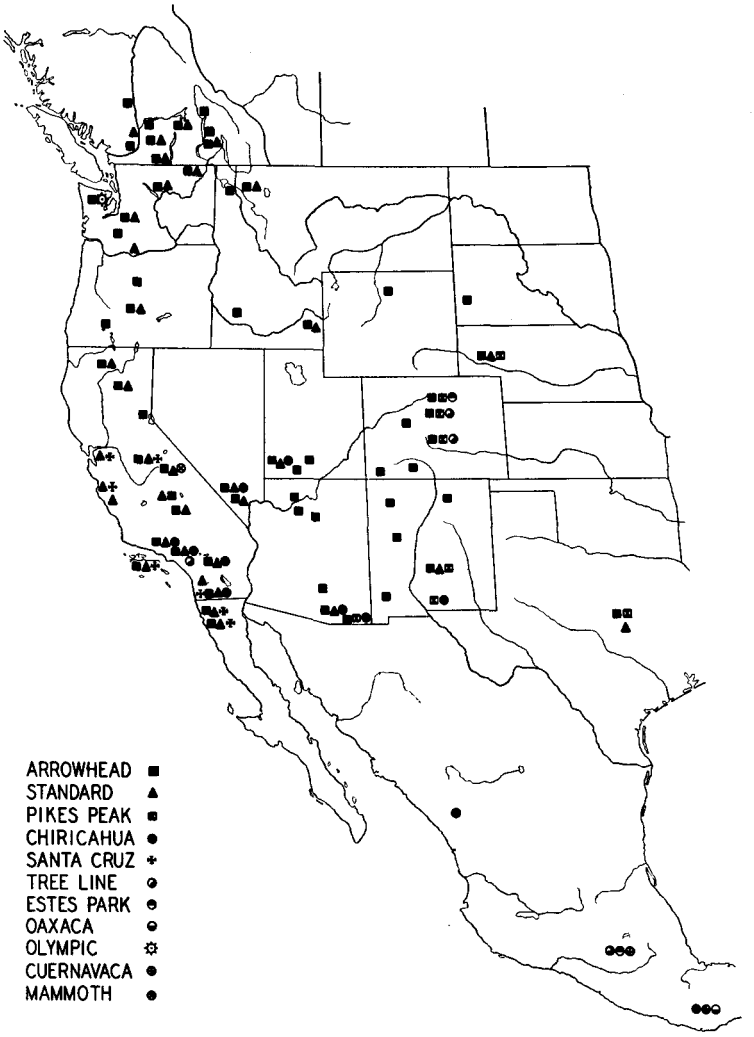


FIGURE 4.—The geographical distribution of the gene arrangements recorded in race A.

Southern California: Santa Cruz (table 1); Arroyo Seco-22, 23; San Gabriel-(table 1); Big Bear-1, 2; Dollar Lake-2, 3; Barton Flats-2, 4, 5, 6, 8, 10, 13, 14, 15; Hidden Springs-1; Banner-(table 1); Julian (table 1); Providence Mts. (table 1).

Lower California: Guadelupe; Santo Tomas (table 1).

Nevada: Charleston-2, 4, 5, 6, 8; Las Vegas-1, 2, 3.

Utah: Cedar City-1, 3, 4, 5, 6, 7, 9, 10, 11, B, C, D; Zion-4, 5; Bryce-4, 5, 6, 7, 8, 9, 10.

Colorado: Aspen-1; Estes Park-2, 3; University Camp-1, 4, 5, 6; Pikes Peak-1, 2, 3, 4, 5, 6, 7; Tree Line on Pikes Peak-2, 3; San Juan Mts.-1, 2, 3, 4, 5, 6, 7, 8, 9; Mesa Verde-1, 3, 4, 5, 6, 7, 8.

Arizona: Grand Canyon North Rim-1, 2, 3; South Rim-1; Flagstaff-1, 2, 5, 7, 8; Gila near Yuma-1, 2; Santa Catalina Mts.-2, 3, 4, 5, 6, 9, 12; Santa Rita Mts.-1, 2, 3, 4, 5, 7; Chiricahua Mts.-2, 4, 6, 7, 8, 10, 11.

New Mexico: Zuni Mts.-2, 3, 4, 5, 6, 7, 8, 9; Taos-1, 3, 4, 6, 7, 8, 9, 10, 11; Magdalena-1, 2, 4, 7, 8, 9; Pinos Altos-1, 2, 3, 4, 7, 8, 9; Carizozo-2, 4, 5, 6, 7, 8, 9, 10.

Texas: Florence-1.

Chiricahua II. Arizona: Chiricahua-6.

Pikes Peak. Nebraska: Scottsbluff-3, 5, 9.

Colorado: Estes Park-2, 3, 5; University Camp-3, 4, 6; Pikes Peak-1, 2, 4, 5, 6, 7.

New Mexico: Carizozo-9; Carlsbad-1, 2.

Texas: Florence-1, 2.

Arizona: Chiricahua-6.

Santa Cruz. California: Wawona-4, 6; Oakland-1; Pacific Grove-1; Santa Cruz (table 1); Banner (table 1); Julian (table 1).

Lower California: Guadelupe; Santo Tomas (table 1).

Chiricahua I. California: Arroyo Seco-21; San Gabriel (table 1); Big Bear-1, 2; Dollar Lake-1; Barton Flats-1, 2, 9, 10; Banner (table 1); Julian (table 1); Providence Mts. (table 1).

Lower California: Santo Tomas (table 1).

Nevada: Charleston-2, 4.

Utah: Cedar City-4, E.

Arizona: Santa Rita-1; Chiricahua-3, 5, 4, 9, 11, 12.

New Mexico: Carlsbad-1.

Mexico, Durango: Otinapa-3; Oaxaca: Cerro San Jose-4, 5.

Cuernavaca. Mexico, Morelos: Cuernavaca-2, 5, 6, 8; Oaxaca: Cerro San Jose-4, 5.

Mammoth. California: Mammoth Lake-2, 8, 19.

Tree Line. California: San Gabriel (table 1); Dollar Lake-2.

Colorado: University Camp-1; Tree Line on Pikes Peak-1.

Mexico, Morelos: Cuernavaca-2, 5.

Estes Park. Colorado: Estes Park-1, 2.

Mexico, Morelos: Cuernavaca-2, 6.

Oaxaca. Mexico, Oaxaca: Cerro San Jose-4.

Olympic. Washington: Olympic-2.

RACE B

Klamath. British Columbia: Campbell River-3, 4; Quesnel-5; 150-mile House-5; Pavilion-6; Yale-3; Merritt-4.

Washington: Cape Flattery (La Push)-7; Olympic-5; Quinault-15, 23; Quilcene-4; Seattle-4, 6; The Dalles-7.

Oregon: Reedsport-2; Sisters-9; Crater Lake-2.

California: Klamath-5; Shelter Cove-5; Lassen-2, 8; Mammoth-3, 12; Sequoia-14.

Standard. California: Shelter Cove-5; Santa Lucia-11; Nojogui (Santa Barbara)-8; Dunsmuir-8; Lassen-2, 8; Mammoth-1, 3, 4, 7, 9, 10, 11, 12, 13, 15, 17, 20; Sequoia-5, 8, 14, 16.

Sequoia I. California: Sequoia-5, 8, 17.

Wawona. California: Wawona-4.

Sequoia II. California: Sequoia-16.

Cowichan. British Columbia: Cowichan Lake-6.

The Standard arrangement is commonest on the Pacific Coast, but occurs sporadically to the limits of the range of the species in the United States. It is nowhere found alone. The Santa Cruz sequence is known from a crescent-shaped area extending from the central Sierra Nevada to the Monterey peninsula, extreme southern California, and Lower California. *D. miranda* occurs in the Puget Sound region (Washington and British Columbia). These three gene sequences seem the best indices of the geographical origin of the group, and they agree in pointing to the Pacific Coast region. With this as a guiding hypothesis we may take up the various families of arrangements in order.

Race B is still confined to the Pacific Coast. Within this area, the standard sequence has been found only in California, and seems to become progressively more frequent to the south. Klamath is the common sequence in race B, occurring practically throughout the range. The other three sequences in race B are known from a single locality each; it should be observed that Sequoia I, the only one of them derived directly from the standard, occurs within the region where standard is frequent. One may surmise that race B arose somewhere in the southern part of its present range, and later spread northward; a conclusion that is not inconsistent with the fact that it is now found further north than race A, and at greater elevations when the two occur in the same region. These relations do, however, suggest caution in accepting the suggested conclusions.

The race A sequences fall into two groups—the Standard and Santa Cruz with their respective derivatives. The Arrowhead arrangement is the commonest one throughout the range of race A in the United States and British Columbia, but has not been found in Mexico (except in northern Lower California). That it is nevertheless of relatively recent origin is sug-

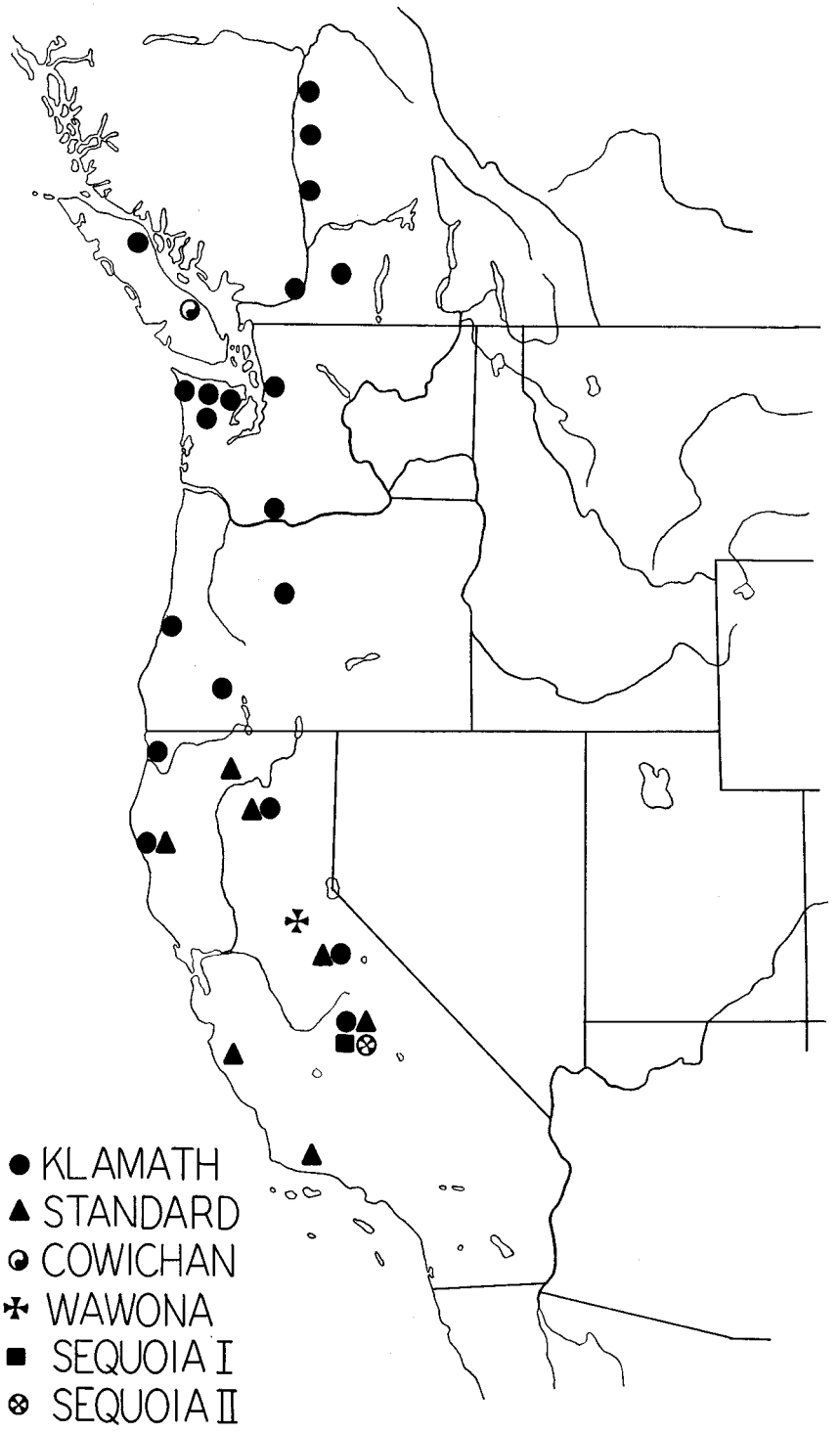


FIGURE 5.—The geographical distribution of the gene arrangements recorded in race B.

gested by the fact that it has given rise to only one new sequence, and that one has been found only in a single stock. Arrowhead is the only sequence that appears to occur over any considerable area unmixed with any others. This area—northern Arizona, western and northern New Mexico, southern Utah, and a portion of southern Colorado—is completely surrounded by regions in which other sequences are frequent. Its historical significance is not clear. The Pikes Peak sequence occurs in the eastern Rocky Mountains with outliers in central Texas and southern Arizona. It presumably arose in this area, where its parent, the Standard, still occurs sporadically.

Sequences derived from Santa Cruz are in general southern in distribution. Tree Line is unusual in two respects, both of which suggest that it is a very ancient sequence. It has given rise to three new sequences (Estes Park, Oaxaca and Olympic), and it has a strikingly discontinuous distribution. It is known from southern California (where it almost overlaps the distribution of Santa Cruz), from the Rocky Mountains of Colorado, and from southern Mexico. Of the three sequences derived from it, Estes Park likewise has a sharply discontinuous range—Colorado and southern Mexico. Olympic is known only from the Olympic peninsula, Washington and Oaxaca only from southern Mexico. The significance of these facts is uncertain; more data from the regions of northern Mexico, Texas, Montana and Idaho are needed. Tentatively, in view of the general southern trend of Santa Cruz derivatives, one may surmise that the occurrence of Tree Line and related arrangements in Colorado and Washington is the result of northward migrations.

Of the other three Santa Cruz derivatives, Mammoth is known only from the eastern slope of the Sierra Nevada, just across the divide from the area where Santa Cruz itself has been recorded. Chiricahua is a widely distributed form, rather frequent in southern California (where it overlaps the distribution of Santa Cruz), and extending from Nevada and southern Utah to southern Mexico. Cuernavaca is thus far known only from southern Mexico. Its occurrence there, together with the probable southern origin of Tree Line, suggests that Santa Cruz is likely to be found in the still unexplored regions of northern and western Mexico.

The inference that the Pacific Coast region is the original home of the species *Drosophila pseudoobscura* finds support in a study of the distribution of other species of this genus. The closest known relatives, aside from *D. miranda*, are evidently *D. obscura*, *tristis*, and *subobscura*—all of which are European. No species from eastern North America or from any other part of the world has yet been found that is structurally so much like *pseudoobscura* as are these. Until collecting is done in northern Asia (a region still almost completely unknown as far as its Drosophilid fauna is concerned), and until more genetic and cytological data concerning the

Palaearctic forms are available, it does not seem profitable to speculate concerning the time or the direction of the migrations between Europe and North America. The next most closely related forms seem to be the members of the *D. affinis* group, confined to North America. We have discussed these elsewhere (STURTEVANT and DOBZHANSKY 1937), and came to the conclusion that *D. athabasca* (ranging from Alaska south to Oregon and Colorado) and *D. azteca* (Mexico) formerly occupied a continuous range, and were separated by an eastward extension of the range of the ecologically similar *D. pseudoobscura*—a conclusion which again stands in agreement with the supposition that the latter originated on the Pacific Coast.

To summarize, the presumed history of the group may be sketched as follows, with the proviso that what we have here is merely a working hypothesis, every point being in need of further study. The original American member of the group lived somewhere on the Pacific Coast of the United States, and had the standard or the hypothetical gene arrangement in its third chromosome. Race A of *D. pseudoobscura*, among the known forms, is most like this ancestral type. From it *D. miranda* arose, probably in the northern part of the area. The hypothetical sequence gave rise also to Santa Cruz, this event happening further to the south. Standard gave rise to race B, probably somewhere in California. Race B has remained on the Pacific Coast, gradually spreading northward. The Standard sequence in race A has spread much further east than the other old sequences, but has remained more frequent in its original home near the Pacific. In the Rocky Mountains it has given rise to the Pikes Peak arrangement. One may surmise that the wide range of the Arrowhead sequence is due to its being by chance predominant in the population that played the most important part in the eastward migration; it arose probably in or near the original coastal area.

The Santa Cruz sequence arose very early, probably in California; its greatest development has been in the south. Its derivatives are the only types so far known to occur in Mexico; perhaps the great differentiation occurred there, and was followed by a northward movement (which would then be postglacial in time) of Tree Line and Chiricahua, bringing them into the Rocky Mountains, among the standard derivatives that moved in from the west. One member of this group, the Olympic sequence, has penetrated as far as the Puget Sound region.[1]

[1] In the Puget Sound region are to be found three race A sequences, of which Olympic is only remotely related to the other two; two race B sequences; three different gene sequences in the second chromosome of race B and one in race A; an endemic type of Y chromosome in race B; *Drosophila miranda; D. athabasca.* No other area of similar size is known in which diversity approaching this occurs.

THE RELATIVE FREQUENCIES OF DIFFERENT GENE ARRANGEMENTS WITHIN A REGION

Most of the data available are based on samples that are too small to give adequate determinations of the frequencies of different sequences in a given region; they must be considered as qualitative rather than quantitative. Quantitative data promise to be interesting, and are now being collected. So far the most satisfactory results are all from southern California and adjacent Lower California; they are shown in table 1.

TABLE 1

Frequencies of different gene arrangements in wild populations from some localities. The figures indicate the percentage frequencies among the total third chromosomes (n) tested from a given locality.

LOCALITY	STAND-ARD	ARROW-HEAD	CHIRICA-HUA	SANTA CRUZ	TREE LINE	N
1. Santa Cruz Island	54.7	16.7	—	28.6	—	42
2. Santo Tomas and Guadelupe	59.4	28.1	3.1	9.4	—	32
3. East of Julian	45.2	25.8	25.8	3.2	—	31
4. West of Julian	47.0	35.3	11.8	5.9	—	17
5. Banner	57.1	26.2	14.3	2.4	—	42
Total 3, 4, and 5	**51.1**	**27.8**	**17.8**	**3.3**	—	**90**
6. San Gabriel Canyon 1936	14.8	27.8	40.7	—	16.7	54
ditto, 1937	57.5	27.7	10.6	—	4.3	47
7. Providence Mts.	8.0	82.0	10.0	—	—	300

The two collections from Julian were made on two successive days, in October, 1936, at points about four miles apart; that from Banner was made in the following April at a point about seven miles east of the more eastern Julian locality, and at several thousand feet less elevation. These collections appear to have had substantially the same frequencies of the four gene sequencies concerned; they are little if any different from the Lower California populations (Santo Tomas, Guadelupe), which were collected in April 1936 at points somewhat over fifty miles from Julian but connected by an area that is presumably inhabited throughout by large numbers of *pseudoobscura*. The other four populations shown in table 1 all appear to be significantly different from these and from each other. In the case of the two from San Gabriel Canyon this is of particular interest, since these represent collections from a single small locality, made in November 1936 and in April 1937. Evidently the proportions of the gene sequences concerned changed markedly during the winter, though the same four occur in each collection. It should be noted that this locality is much disturbed by man, being close to permanent dwellings and also a popular week-end resort. It is possible that such great fluctuations are not

usual under natural conditions; only further studies can decide this point. In general, the material on which the present investigation is based has been collected in localities as far as possible removed from human habitation.

DISCUSSION

As shown above, the overlapping inversions may be used as a tool for the study of historical problems. In this connection it is necessary to consider the question whether the same inversion may arise repeatedly. It is evidently of importance to decide how safe it is to assume that two chromosomes having a given gene arrangement are descended from a common ancestor having the same arrangement.

One of us (STURTEVANT 1931) had concluded that such recurrences are frequent; we are now of the contrary opinion. The earlier argument was based on the wide geographical range of specific gene sequences in *D. melanogaster;* we should now interpret the facts as indicating a high degree of constancy in gene sequence. GERSHENSON (1930) and GRÜNEBERG (1936) have reported cases of "reinversion," where a sequence has given rise to the ancestral sequence from which it was derived. We are not completely convinced that other possible explanations (such as crossing over or contamination) have been excluded in these cases. In general, the chance of the occurrence of a new inversion, both of whose ends shall coincide with those of a previous one, seems very remote. A few instances are known in which one of the ends in two inversions seems so to coincide, but we are not acquainted with any examples in which such a coincidence has been fully demonstrated by a detailed comparison in salivary gland chromosomes.

From our data it appears that the end-points of the inversions in the third chromosome of *D. pseudoobscura* are not distributed at random through the chromosome. Thus, several breakages are known in the short sections 76 and 77, and only two in the interval including sections from 63 to 67 inclusive which constitutes more than one third of the total length of the chromosome. But even in the sections in which the breakages are numerous there are no unequivocal cases of exact correspondence—clearly the usual thing is that the inversion-points, even when they lie in the same region, are not identical.

We can only conclude that, while recurrences of the same inversion must be recognized as possible, they are too improbable to be postulated in any given case.[2] We have used a number of strains that were homozygous for

[2] One reservation may be made here, however. In many cases we have identified sequences only by the configurations seen in heterozygotes. There exists a remote possibility that in some instances we may be dealing with "mimic" sequences, differing by a few discs from those with which they are identified. In every instance where a sequence has a markedly discontinuous dis-

sequence, and have kept them for several years. It is clear that these, like similar strains of other species, have not undergone changes in sequence. The uniformity with which the Arrowhead sequence is found in the southern Rocky Mountain region may be taken as a further indication of the rarity with which new inversions occur.

If new inversions arise rarely, it becomes of interest to inquire how some of them come to be established in wild populations inhabiting large territories. We have no answer to give to this question, other than to say that inversions are in this respect not different from gene mutations and do not require any different theory—except in one respect. It has been questioned whether gene mutations that are wholly neutral in relation to natural selection can be supposed to exist. Inversions would seem to come closer to such a neutral condition than any other genetic character, if one neglects the possibility of position effects that they may produce. In general, heterozygosis for inversions decreases the amount of crossing over, and this may be of selective value in connection with heterosis effects. The magnitude of this effect of inversions cannot be evaluated satisfactorily at present, though experiments under way in this laboratory should enable us to reach definite conclusions.

On the other hand, heterozygosis for some inversions may have a slight unfavorable effect. Long inversions, such as Pikes Peak/Standard, should give an appreciable frequency of crossing over, and there should be some mortality among the offspring as a result (STURTEVANT and BEADLE 1936). When two sequences differ by two inversions whose ends are not too different, crossovers should occur giving inviable or poorly viable zygotes (STURTEVANT and BEADLE, loc. cit.). Perhaps the most likely combination for this effect is Pikes Peak/Arrowhead. It would seem that the Pikes Peak sequence should be at a selective disadvantage whenever it occurs in the same population either with Standard or with Arrowhead. And yet just these sequences are the ones with which Pikes Peak is most often associated; it is probable that it does not exist in any considerable area that is free of the Arrowhead sequence. One is led to wonder how effective are slight increases or decreases in mortality in determining the fate of chromosome structures or genes in wild populations of this species. The observation that autosomal lethals are present in approximately one-sixth of the third chromosomes of wild strains gives further point to this question.

Drosophila pseudoobscura is, as species go, relatively constant in external morphology. We have had under observation some hundreds of wild

tribution, representatives from the different regions have been tested directly, by studying the salivary gland chromosomes of homozygotes. A fairly large number of such direct tests has been made also for sequences showing continuous distribution. No "mimics" have been found. The reservation made here is not intended to cast doubt on the validity of the distribution maps shown in figures 4 and 5.

strains, from localities scattered from British Columbia to southern Mexico and from the Pacific to Texas. None of these have been distinct enough so that we should feel certain of distinguishing unlabeled cultures from the external appearance of the flies. Yet this same material has been found to show striking heterogeneities in a variety of characters. The "strength" (testis size in B ♀ × A ♂ hybrids) has been shown to be extremely variable (DOBZHANSKY and BOCHE 1933; also unpublished data); seven different types of Y chromosomes occur (DOBZHANSKY 1935b, 1937); the present account shows the existence of a wide diversity in gene arrangements. In addition, unpublished results show that there are frequent and marked differences in the modifiers affecting bristle number, in the genes affecting the number of male offspring produced by A-B hybrid females, and in the ease with which the various strains cross with *Drosophila miranda*.

A comparison of the variability of the gene arrangement in the third chromosome with that of the structure of the Y chromosome leads to results that are suggestive. Each type of Y chromosome has a geographical distribution that roughly corresponds to that of one or more of the third chromosome sequences, as follows:

Y chromosome	Third chromosome	
Type I	Oaxaca, Cuernavaca, Chiricahua	Race A
Type IV	Standard	Race A
Type V	Arrowhead	Race A
Type VI	Pikes Peak, Chiricahua	Race A
Type VII	Estes Park, Tree Line	Race A
Type I	Klamath	Race B
Type II	Cowichan	Race B
Type III	Standard, Sequoia	Race B

These agreements are rather rough, but they may be made into a consistent scheme if it be assumed that the original Y chromosome was Type I (which is, morphologically, the largest of all, and looks as though all the others could be derived from it by losses of parts). This Y was, on such a basis, originally associated with the Standard, "hypothetical," or Santa Cruz sequences, probably with all three. In race A it has persisted only in part of the region that is occupied by Santa Cruz derivatives; in race B it has persisted throughout the range, but has become less common to the south.

Type IV of Y chromosome may be supposed to have arisen from Type I by loss of most of the short arm, Type V from Type IV by loss of a portion of the long arm. Types II, III, and VII perhaps came directly from Type I; Type VI, because of its structure, seems more likely to have come from I than from IV or V, and may therefore be tentatively associated with Chiricahua rather than with Pikes Peak.

All these suggestions are to be considered as speculative; they are presented here as an example of the way in which such historical problems may be approached with this material.

SUMMARY

1. Strains of *Drosophila pseudoobscura* coming from the same or from different geographical localities are frequently dissimilar with respect to the gene arrangement in their chromosomes. The variations in the gene arrangement are due to inversion of chromosome sections, no translocations or other chromosome aberrations having been found.

2. The third chromosome is the most variable one, seventeen different gene arrangements having been recorded; the second chromosome with six follows next; next are the right limb of the X chromosome with three, and the left limb of the X and the fourth chromosomes with two arrangements each. The fifth chromosome has not been sufficiently studied.

3. Each gene arrangement occurs in a definite geographical area. Some arrangements have been recorded so far only in a single locality, while others are distributed fairly widely (figs. 4 and 5). In many localities the populations are mixed, four or more gene arrangements being present. However, none of the seventeen arrangements known in the third chromosome is present in the entire distribution region of the species.

4. A chromosome once changed by an inversion may undergo another change due to a second inversion. The second inversion may be independent, included, or overlapping the first (fig. 1). Overlapping inversions are especially interesting, since they allow conclusions to be drawn regarding the historical sequence of changes in the chromosome involved. With three gene arrangements, A, B, and C related as overlapping inversions, the phylogenetic series is A⟷B⟷C, but not A⟷C (cf. pages 31–34).

5. The gene arrangements recorded in the chromosomes of *D. pseudoobscura* are as a rule related as overlapping inversions. This permits the drawing of phylogenetic charts representing the historical sequence of changes in the chromosomes in question (fig. 3 for the third, p. 48 for the second chromosomes).

6. The overlapping inversion method itself does not permit the determination of the original (ancestral) gene arrangement in a given chromosome. Among the gene arrangements recorded in the third chromosome there exist however three arrangements (Standard, "hypothetical," and Santa Cruz, see fig. 3) which on other grounds are likely to be ancestral ones.

7. The overlapping inversion method is applicable for comparison of distinct species as well as strains of the same species. It is shown that

Drosophila miranda is more closely related to race A than to race B of *D. pseudoobscura*.

LITERATURE CITED

DOBZHANSKY, TH., 1935a *Drosophila miranda*, a new species. Genetics **20**: 377–391.

1935b. The Y chromosome of *Drosophila pseudoobscura*. Genetics **20**: 366–376.

1937 Further data on the variation of the Y chromosome in *Drosophila pseudoobscura*. Genetics **22**: 340–346.

DOBZHANSKY, TH., and BOCHE, R. D., 1933 Intersterile races of *Drosophila pseudoobscura* Frol. Biol. Zbl. **53**: 314–330.

DOBZHANSKY, TH., and TAN, C. C., 1936 Studies on hybrid sterility III. A comparison of the gene arrangement in two species, *Drosophila pseudoobscura* and *Drosophila miranda*. Z.i.A.V. **72**: 88–114.

DUBININ, N. P., SOKOLOV, N. N., and TINIAKOV, G. G., 1936 Occurrence and distribution of chromosomal aberrations in nature. Nature **138**: 1035–1036.

GERSHENSON, S.M., 1930 Phenomenon of reinversion in the sex-chromosome of *Drosophila melanogaster*. Reports IV Congress Zool., Kiev (Russian) p. 7.

GRÜNEBERG, H., 1936 A case of complete reversion of a chromosomal rearrangement in *Drosophila melanogaster*. Nature **138**: 508.

STURTEVANT, A. H., 1917 Genetic factors affecting the strength of linkage in Drosophila. Proc. Nat. Acad. Sci. **3**: 555–558.

1931 Known and probable inverted sections of the autosomes of *Drosophila melanogaster*. Pub. Carnegie Instn. **421**: 1–27.

STURTEVANT, A. H., and BEADLE, G. W., 1936 The relation of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

STURTEVANT, A. H., and DOBZHANSKY, TH., 1936a Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc. Nat. Acad. Sci. **22**: 448–450.

1936b Geographical distribution and cytology of "sex-ratio" in *Drosophila pseudoobscura* and related species. Genetics **21**: 473–490.

1937 Observations on the species related to *Drosophila affinis*, with descriptions of seven new forms. Amer. Nat. **70**: 574–584.

STURTEVANT, A. H., and TAN, C. C., 1937 The comparative genetics of *Drosophila pseudoobscura* and *D. melanogaster*. J. Genet. **34**: 415–432..

TAN, C. C., 1935 Salivary gland chromosomes in *Drosophila pseudoobscura*. Genetics **20**: 392–402.