GENETIC AND CYTOLOGICAL STUDIES ON THE AFFINIS GROUP OF DROSOPHILA

Thesis by

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In Partial Fulfillment

of the

Requirements for the Degree of

Doctor of Philosophy

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

1942

TABLE OF CONTENTS

THE CORRELATION OF THE SALIVARY GLAND CHROMOSOMES WITH THE LINKAGE GROUPS OF DROSOPHILA AFFINIS

	page
Introduction	1
Methods	3
Procedure	4
Results	5
Salivary Gland Chromosome Analysis	9
The Correlation of the Linkage Groups with the Salivary Gland Chromosome	12
Discussion	14

STRUCTURE AND VARIATION OF THE CHROMOSOMES OF DROSOPHILA ATHABASCA

Introduction	17
Materials	19
Methods	20
Technique	22
Structure of the Chromosome	23
Sequences in Element C	23
Sequences in Element E	27
Sequences in Elements A and D	28
Sequences in Element B	29
The Phylogeny of the Sequences in Element C	29
Some Consequences of the Geographical Distribution of the Various Sequences	33

A Theory of the Origin of Certain Inversions 34

SOME GENETIC NOTES ON AN ANOMALOUS SEX RATIO CONDITION IN DROSOPHILA AFFINIS

Introduction 43

"Sex Ratio" in Affinis 44

Discovery of the "Male Sex Ratio" Phenomenon 45

The Genetic Composition of MSR Males 46

The Characteristics of the Progeny of MSR Males 54

The Nature of the Action of the MSR Complex 55

Geographical Distribution of MSR and the Gene a 57

Discussion 57

THE HOMOLOGIES OF THE CHROMOSOME ELEMENTS IN THE GENUS DROSOPHILA

Introduction 60

Consideration of the Various Species 61

Homologous Genes in the Various Species 71

Lengths of the Elements in the Salivary Gland Nuclei 71

Sequence of Corresponding Genes in Different Species 72

Discussion 75

A Note on the Relations Between the Species Groups of Sophophora 78

Summary 82

SUMMARY 83

ACKNOWLEDGMENTS 84

BIBLIOGRAPHY 85

THE CORRELATION OF THE SALIVARY GLAND CHROMOSOMES WITH THE LINKAGE GROUPS OF DROSOPHILA AFFINIS

Introduction

The genetic evidence available at present indicates that certain groups of genes tend to remain linked in the various members of the genus Drosophila (Donald, 1936; Sturtevant and Tan, 1937; Sturtevant, 1938a, 1940; Sturtevant and Novitski, 1941). These elements are comparable to the arms of the chromosomes of melanogaster and have accordingly been lettered A, B, C, D, E, and F for X, IIL, IIR, IIIL, IIIR, and IV, respectively. In view of the extensive cytological studies on the various members of the affinis group (Dobzhansky and Socolov, 1939; Miller, 1939; Novitski, unpublished; Sturtevant, unpublished), it has seemed pertinent to extend the homologies with the elements to the salivary gland chromosomes of this group. The high degree of similarity of the salivary gland chromosome arms of the various affinis group species examined renders it possible to make such a correlation within one species, in this case affinis, with the reasonable certainty that the results obtained will apply to other members of the group (athabasca, algonquin, and azteca).

Sturtevant (1939) has homologized a number of mutant genes in affinis with those of melanogaster and pseudoobscura,

applying to the linkage groups of affinis the same designations as those of their homologs in pseudoobscura. In terms of the above mentioned elements, consequently, the affinis linkage groups, XL, XR, II, III, IV, and V correspond to A, D, E, C, B, and F, respectively. Miller (1939), in a study of the salivary gland chromosomes of algonquin, has arbitrarily lettered the units found therein: a two-armed X-chromosome (XL and XS), a single armed autosome (A), two two-armed autosomes (B and C), and a microchromosome (D). In order to avoid confusion, the symbols to be used until the complete correlation has been established will be those of Sturtevant for the linkage groups and those of Miller for the salivary gland chromosomes.

The metaphase chromosomes of males of the affinis group include a V-shaped X-chromosome, a J-shaped Y-chromosome, two pairs of J-shaped autosomes, a pair of rods and a pair of dots (Metz, 1916; Sturtevant and Dobzhansky, 1936; Dobzhansky and Socolov, 1939; Miller, 1939). It seems clear that there is a correspondence between the number of large chromosome arms in the metaphase plate and the euchromatic arms in the salivary gland nucleus (Miller, 1939, for algonquin; Sturtevant, unpublished, for affinis; Novitski, unpublished, for athabasca; Novitski, in Sturtevant, 1940, for azteca.

Methods

The ability of X-irradiation to induce chromosomal aberrations has been repeatedly demonstrated (review in Dobzhansky, 1936). The most common type of aberration produced is the translocation, which results when two chromosomes exchange segments. When such a reciprocal translocation is present in the heterozygous state in an individual, the only completely balanced gametes that are produced contain either both normal or both translocated chromosomes; other combinations of chromosomes carry both duplications and deficiencies for some sections of the chromosomes and generally exert a lethal effect. The differential survival value for the different combinations results in an apparent linkage of the genes on the chromosomes involved in the translocation.

A somewhat similar situation exists with respect to translocations involving the Y-chromosome, but since it is relatively inert, deficiencies and duplications for sections of it are less deleterious than correspondingly large fragments of the autosomes and consequently unbalanced complements may be much more frequent. The detection of such a translocation depends upon the linkage of certain genes, not to each other, but to the Y-chromosome or, in other words, upon the presence of the translocation in the male sex only.

Since, in the salivary gland nuclei, the homologous chromosomes derived from each parent tend to pair and fuse,

any translocation present in the heterozygous state is represented by irregular configurations, in which a part of one chromosome strand tends to pair with its homolog, now located in another chromosome. It is thus possible to identify the salivary gland chromosomes involved in a translocation discovered by genetic tests.

Procedure

The stocks of affinis used in these experiments include the wild-type strains Woods Hole 9.42 and Victoria, and strains of the mutants rugose (rug, linkage group II), cinnabar (cn, linkage group III), and jaunty net (jt net, linkage group IV). In order to detect translocations involving more than one autosome, it was necessary to synthesize strains carrying combinations of mutants of different autosomal linkage groups. The combinations chosen were rug cn and cn jt net.

Wild-type males were X-rayed at the Huntington Memorial Hospital with a dosage of 4000 roentgen units. These males were then mated to females of the constitution rug cn in one series of cultures and cn jt net in another series. F_1 male progeny, heterozygous for the mutant markers and also for any aberrations that might have arisen during the irradiation of the sperm cells of the treated males, were backcrossed individually to females homozygous for the marker

genes. If no translocation is present, the progeny of such a cross should show a random segregation of the marker genes on the different chromosomes, both with respect to each other and with respect to sex. On the other hand, if there is present in such F_1 males a translocation involving the two autosomes marked, or either of the marked autosomes and the Y-chromosome, aberrant linkages should result, for the reason discussed above.

Results

Of 56 matings to rug cn females of males heterozygous for rug, cn, and the corresponding chromosomes from the irradiated males, 12 cultures were sterile and 7 of the remaining ones gave clear evidence of the presence of translocations (table 1). Each translocation found has been labeled with arabic numerals according to the linkage groups determined to be involved, and has been assigned a letter distinguishing it from other translocations involving the same linkage groups. Cultures 9, 11, 18 and 31 showed a complete linkage of the mutants of groups II and III; they were consequently labeled as containing T (translocation) 2-3B, T2-3C, T2-3A and T2-3D, respectively. In the progeny of culture 29, all the females were cn and all the males were not. Evidently the chromosome carrying the wild-type allele of cn had undergone a translocation with the Y-chromosome.

Table 1.

Analysis of aberrant linkages obtained in backcrosses of F_1 (rug cn ♀♀ x X-rayed ♂♂) ♂♂ to rug cn ♀♀. (Culture 33 showed normal segregation and is presented here for comparison.)

Culture number	Progeny								Genetic analysis
	+		rug cn		rug		cn		
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
1-33	10	17	15	20	9	20	12	10	Normal
1- 9	2	4	2	10	--	--	--	--	T2-3B
1-11	33	37	19	28	--	--	--	--	T2-3C
1-16	37	--	22	38	33	42	30	--	TY-2A
1-18	19	9	5	11	--	--	--	--	T2-3A
1-29	12	--	--	3	11	--	--	2	TY-3B
1-31	5	10	4	16	--	--	--	--	T2-3D
1-38	31	--	15	22	21	27	28	--	TY-2B

It was labeled TY-3B. In the progeny of cultures 16 and 38, all the females were rug; the males were both rug and non-rug. One may assume that the chromosome corresponding to linkage group II underwent a translocation with the Y-chromosome and that the rug males in the progeny of these cultures resulted from eggs fertilized by sperm carrying a rug chromosome and a duplication for the part of the chromosome carried by the Y-centromere, the duplication not containing the wild-type allele of rug. The complementary class, expected to consist of wild-type females, did not appear and it may be surmised that the deficiency for which that class would be heterozygous exerts a lethal effect. These cultures have been labeled TY-2A and TY-2B.

A series of cultures comparable to those described above had jt net and cn as the marker genes. Of 54 such matings, 18 were sterile and 12 of the remaining ones showed evidences of induced translocations (table 2). The same type of analysis suggested above for the rug cn series can also be applied here. Departures from apparently complete linkage occur in some of the cultures, caused, presumably, by the viability of certain classes carrying a duplication and a deficiency. In some cases individuals of these exceptional classes were morphologically abnormal; they have been noted in table 2 by an asterisk.

Since in each of the series one linkage group was

Table 2

Analysis of aberrant linkages obtained in backcrosses of F_1 (cn jt net ♀♀ x X-rayed ♂♂) ♂♂ to cn jt net ♀♀. (Culture 32 showed normal segregation and is presented here for comparison. Those classes marked with an asterisk were morphologically abnormal.)

Culture number	Progeny +		cn jt net		cn		jt net		Genetic Analysis
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
2-32	7	17	10	15	12	15	12	8	Normal
2- 4	32	--	1*	14	11	--	1	22	TY-4A
2-14	27	--	--	28	18	--	--	9	TY-4D
2-18	18	17	27	23	--	--	--	--	T3-4C
2-20	35	--	1*	27	11	--	2*	31	TY-4B
2-24	31	--	--	17	13	--	7	30	TY-4G
2-30	1	20	19	23	4	--	--	--	TY-3-4B
2-37	18	1	6	20	--	26	24	--	TY-3A
2-38	11	--	2	9	--	--	5*	--	TY-3-4A
2-44	19	--	6	15	16	--	12*	12	TY-4F
2-48	14	--	--	16	8	--	--	26	TY-4C
2-51	11	--	--	11	12	--	--	12	TY-4E
2-54	12	7	15	17	--	--	--	--	T3-4A

left unmarked, the genetic analysis used here may not always have given a true picture of the number of chromosomes involved in an aberration; in some of the cases the chromosome of the unmarked linkage group may have participated in the translocation.

Stocks were made of the translocations detected, except of those that were obviously complex, such as translocations Y-3-4A and Y-3-4B. Translocations involving the Y-chromosome were maintained without selection, since, in such cases, all fertile males must carry the translocation. Those translocations involving only autosomes were kept by selection. Wild-type males, heterozygous for the marker genes and for their wild-type alleles on the translocated chromosomes, were mated in each generation to females homozygous for the markers. The absence of crossing over in the male sex of Drosophila insured the invariable association of the wild-type alleles of the marker genes with the translocated chromosomes.

Salivary Gland Chromosome Analysis

As has already been mentioned, the salivary gland chromosome complement of all the affinis group species examined consists of seven euchromatic strands, besides the microchromosome. These strands are generally seen to be united at the basal heterochromatic region. The frequent attachment of certain of the strands to each other in cases

where the chromocenter has been broken suggests the basic attachment of those pairs of strands as arms of the same chromosome. One such pair appears to be haploid in the salivary gland nuclei of males and is interpreted to represent the two arms of the X-chromosome. Miller (1939) was able to make this preliminary identification of the two arms of the B chromosome more secure by analyzing both in the salivary gland nuclei and giant ganglion cell metaphases an inversion across the spindle attachment found in wild strains of algonquin. Of the three remaining long arms, two represent the long and short arms of the C chromosome and the third, the single-armed A chromosome.

No attempt has been made to correlate the two arms of the X-chromosome with the right and left ends of the genetic map of affinis. The greater length of X long (the longer limb of the X-chromosome) of the salivary gland chromosomes as compared with X short parallels the greater genetic length of XR (X right) as compared with XL (X left). Furthermore, there are clear indications of homology at the terminal and basal regions of X short of affinis with those regions of X short of pseudoobscura, in which species the correlation of the longer limb of the salivary gland X-chromosome with the right limb of the genetic map and of X short with X left has already been made (Tan, 1935). The correlation between the microchromosome and the small linkage group

V is obvious; nothing additional has been attempted with it.

It has been pointed out that heterozygous translocations may be obvious in the salivary gland nuclei as abnormal configurations which reveal the structure of one chromosome as composed of sections of different chromosomes. When one of the breakpoints occurs in heterochromatin (such as the Y-chromosome which is visible in the salivary gland nuclei of males only as a small heterochromatic mass), the translocation configuration describes a loop, in which a point on the euchromatic strand involved in the translocation seems to be associated with the chromocenter. Translocations with breaks in the heterochromatic regions of both chromosomes involved do not yield any obvious aberrant configurations in the salivary gland nuclei.

Of the translocations obtained, several, such as TY-3-4A and TY-3-4B, were discarded because of their obvious complexity. TY-4A and TY-4B did not yield any obvious aberration in the pairing of the salivary gland chromosomes, probably as a result of the location of the breakpoints of the translocation in heterochromatic regions. T2-3E and T3-4A turned out to be complex aberrations involving more than two autosomes and therefor worthless, since no conclusions could be drawn as to the chromosomes corresponding to the marked linkage groups.

Table 3 lists those translocations in the cases of which it was possible to determine clearly which chromosomes were involved. The particular salivary gland chromosome arms involved in the translocations are given in column 2, without reference to the exact locations of the breakpoints on those arms.

The Correlation of the Linkage Groups with the Salivary Gland Chromosomes

There are two ways in which the correlation of the linkage groups with the salivary gland chromosomes may be deduced - either by determining possible identities or by eliminating impossible ones. The assumption may be made that the chromosomes determined cytologically to be involved in a translocation correspond to the linkage groups known also to be involved. Column 3 of table 3 shows such a series of conclusions for the translocations examined. By taking into account involvements of linkage groups in different translocations, it may be determined which of the salivary gland chromosomes corresponds to each of the linkage groups. For the various aberrations analyzed, there is one and only one set of equalities which agrees completely with the observed possibilities. When A = 3, B = 2, and C = 4, all the genetic and cytological observations are consistent.

An objection to the analysis given above lies in the possibility of misinterpreting an aberration in which the

Table 3.

Salivary gland chromosome analysis of certain translocations, with a list of the linkage groups known to be and known not to be involved.

Translocation	Salivary gland chromosomes involved	Possibilities	Linkage groups not involved	Impossibilities
TY-2A	Y, B long	II = B	III	III = B
TY-2B	Y, B long	II = B	III	III = B
TY-3A	Y, A, B short	III = A or B	IV	IV = A or B
TY-3B	Y, A, C long	III = A or C	II	II = A or C
TY-4D	Y, B long, C long	IV = B or C	III	III = B or C
TY-4E	Y, B long, C short	IV = B or C	III	III = B or C
T2-3C	A, B long	II or III = A or B		
T2-3D	A, B short	II or III = A or B		
T3-4C	A, C long	III or IV = A or C		

chromosome corresponding to the linkage group known to be involved has been broken in the heterochromatic region and thus may not be obviously affected in the salivary gland nuclei, while another chromosome, not tested genetically, has undergone a cytologically clear translocation. To avoid this possibility, the linkage groups known not to be involved in a given translocation can be eliminated as corresponding to the salivary gland chromosomes involved in that translocation. (These have been listed in columns 4 and 5 of table 3.) Summing the results from the individual translocations, it turns out that linkage group II cannot be represented by salivary gland chromosomes A or C, III cannot be represented by B or C, nor can IV be represented by A or B. The only alternatives in each case are those suggested above by the simple comparison of involvements.

Discussion

It has been shown that translocations which would transfer genic material from one linkage group to another are established as specific differences in the genus Drosophila only very rarely (see discussion in Sturtevant and Novitski, 1941). For species as closely related as those in the affinis group, the probability of such a translocation difference is extremely small. For this reason, in addition to the morphological similarity of the salivary gland chromosomes of the

members of the group examined, the results obtained above by using affinis may be projected to the other members of the group. The complete correlation of the salivary gland chromosome arms of affinis with the linkage groups of affinis and with the elements is presented in Sturtevant and Novitski, 1941.

The data presented here are not in complete agreement with those of Miller (Thesis, 1940) who attacked the same problem using algonquin. By studying the segregation of certain mutants marking the different linkage groups, with respect to inversions marking the various salivary gland chromosomes, he was able to differentiate between cases where both the inversions and markers were carried on the same chromosome and cases where they were not. By this technique, the linkage group containing "cinnabar," proved by feeding tests to be true cinnabar, appeared to be represented in the salivary gland chromosomes by chromosome C, whereas the present work shows that it is represented by chromosome A. There are several possible pitfalls inherent in the method to which he was limited; nevertheless, it must be admitted that this discrepancy may be a true one, resulting from an interspecific translocation difference, although all other evidence, both theoretical and experimental, speaks against it. A similar inversion - mutant gene analysis had been carried out in athabasca with a mutant later determined by

Miller to be true cinnabar with a result comparable to that obtained by studying translocations in affinis, that the cn linkage group corresponds to salivary gland chromosome A.

The results of Dobzhansky and Socolov (1939) with respect to the existence of translocation differences between azteca and pseudoobscura, contrary to the theory of stable elements, has been evaluated elsewhere (Sturtevant and Novitski, 1941).

STRUCTURE AND VARIATION OF THE CHROMOSOMES OF DROSOPHILA ATHABASCA

Introduction

Drosophila athabasca Sturt. and Dobz. (1936) is one of the six species comprising the affinis group. It is closely related to Drosophila affinis Sturt. (1916) with which it, as well as the remaining species narragansett, seminole, azteca and algonquin, had been previously confused. The various members of this group have presumably diverged from their common ancestor relatively recently and for this reason should prove valuable for a population analysis.

The known geographical distribution of athabasca is shown in figure 1. It has been found in northwestern United States, southwestern Canada, southern Alaska and in the eastern part of the continent, from Quebec to North Carolina. Complete data on its distribution in the middle section of the continent is lacking; it has been found, however, in Ohio and in Michigan. It seems quite likely that the range of athabasca is actually continuous across the continent.

The other species in this group have a more limited range. Affinis is found in eastern United States, extending in the Southeast as far west as Texas. The range of

<u>Figure 1</u>. The known distribution of athabasca. Open circles denote localities at which this species has been captured; closed ones indicate the localities from which strains have been established and analyzed. The insert is a view of southern Alaska.

algonquin extends from northeastern United States through the Middle West to Texas. The known distribution of narragansett is the narrow strip of territory running from Massachusetts to Ohio, and that of seminole is a small area in southern Alabama. Azteca, the only exclusively western species, has been found in California and Arizona and is common in Mexico and Central America.

All three species hybrids known in the affinis group involve athabasca. It has been shown to produce viable offspring when mated with azteca (Sturtevant and Dobzhansky, 1936), with affinis, and with algonquin (Miller, Thesis, 1940). These facts, in combination with the data on the geographical distribution of all the members of this group, suggest that athabasca is the link by which some of the others, particularly the eastern as opposed to the western species, are related.

The eastern form of athabasca had originally been separated from the western form as a subspecies, mahican, on the basis of a somewhat paler coloration (Sturtevant and Dobzhansky, 1936). During the course of the investigations to be described, no evidence has been found that any distinction need be made between the two forms and the subspecific designation has been disregarded.

Materials

Strains of athabasca, derived from single fertilized females captured at various localities, have been established in the laboratory. Those strains analyzed are represented in figure 1 by closed circles at the locality from which they were taken. Table 4 lists localities of the strains analyzed, with the designation applied to those strains.

Table 4

Locality	Number of Strains	Designations
Gravina Island, Alaska	1	Alaska
Quinault, Washington	1	Washington
Sundance, Wyoming	1	Wyoming-1
Grey's River, Wyoming	2	Wyoming-2, -3
Perpetua, Oregon	2	Oregon-1, -2
Hope, British Columbia	4	British Columbia -1, -2, -3, -4
Montreal, Quebec	1	Quebec
Gray, Maine	1	Maine
Franconia Notch, New Hampshire	2	New Hampshire
Amherst, Massachusetts	1	Massachusetts-1
Woods Hole, Massachusetts	1	Massachusetts-2
Chautauqua, New York	1	New York
Clingman's Dome, North Carolina	1	North Carolina

Methods

The analysis of the various strains of athabasca has been accomplished by a study of the salivary gland chromosomes of the larva. The detail obvious in preparations of those chromosomes makes it possible to distinguish small differences in the structure of the two strands which form the complete chromosome.. Since each of the two strands comes from each of the parents of an individual larva, differences in the structure of the two reveal themselves as abnormal pairing tendencies. The most common type of difference in chromosomes derived from wild strains is the inversion, in which a section of one chromosome has become inverted with respect to the same section of the original. Thus, if a chromosome is arbitrarily lettered along its length 12345678, section 23456 may become inverted to produce a chromosome of the constitution 16543278. A larva which is heterozygous for both sequences will show, in its salivary gland chromosomes, a loop, formed by the two different strands as they attempt to synapse completely (see figure 3B).

Two successive inversions which overlap give a final sequence of sections which enables one to predict the constitution of the first inversion, although it may never have been seen (Sturtevant and Dobzhansky, 1936). A chromosome of the constitution 154762389 differs from the standard

sequence 123456789 by two such overlapping inversions; the simplest and most probable explanation of the changes that must have occurred to produce the final doubly inverted sequence is that an intermediate step, 154326789, differing from "standard" by a single inversion, was itself modified by an inversion of section 3267. This type of analysis can be extended to include a large number of different sequences of the same chromosome; the phylogenetic relationship of those sequences can then by graphically represented on a chart.

In practice, inversions other than overlapping ones can yield more or less comparable phylogenetic information. Two included or two independent inversions whose breakpoints are such that the probability that crossing over will either combine or separate them is negligible can be adapted to a phylogenetic series. In these cases it is not possible to say which of the inversions must have occurred first but it seems clear that one of them probably did and that this ambiguous hypothetical sequence represents an intermediate step in the production of the double inversion.

Furthermore, two independent inversions which theoretically might undergo crossing over to produce various combinations of sequences may be separated geographically so that these combinations are never found. In this case the phylogeny of such inverted sequences is reasonably

clear: the "standard" sequence is interposed between the inverted sequences. The argument of geographical isolation applies equally well to the hypothesis that ohe inversion may have arisen on a chromosome already inverted at a different region with crossing over subsequently separating the two.

Since, in a study of wild populations, the "standard" sequence must be chosen arbitrarily, there is no reason to assume a priori that the direction of change is from the "standard" to the relatively inverted sequences and not vice versa. For this reason, the direction of change is generally represented by a double arrow (⟷).

Technique

The salivary gland chromosomes have been stained, for the most part, with aceto-carmine; aceto-orcein has been used during the latter part of the work. Favorable preparations of the salivary gland chromosomes of athabasca are fairly rare; for this reason, some strains have not been analyzed completely and, on the other hand, data on certain chromosomes as, for instance, element B, has been particularly difficult to obtain. In no case has it been possible to derive the essential characteristics of a given configuration from a single nucleus. All the drawings presented here are, consequently, composites taken from a

number of different camera lucida drawings of a given configuration in various cells.

Structure of the Chromosomes

The gross structure of the salivary gland chromosomes and of the chromosomes at metaphase in the giant ganglion cells has already been discussed. Reference to the various chromosomes will be made in terms of elements, rather than the arbitrarily chosen letters used by others working with this group.

The drawings of the salivary gland chromosomes presented here are complete only insofar as they illustrate the gross structure of naturally occurring sequences. Chromosomes which show no variation have been illustrated by drawings of the basal and terminal regions for purposes of identification. The various elements shall be considered in the order in which they yielded phylogenetically useful information.

Sequences in Element C

The sequence chosen as standard, for purposes of reference, was one found to be homozygous in the North Carolina strain and occuring also in the strains New Hampshire-1, -2, Maine, Massachusetts-1, -2 and New York. This sequence, which was arbitrarily lettered A is depicted in figure 2.

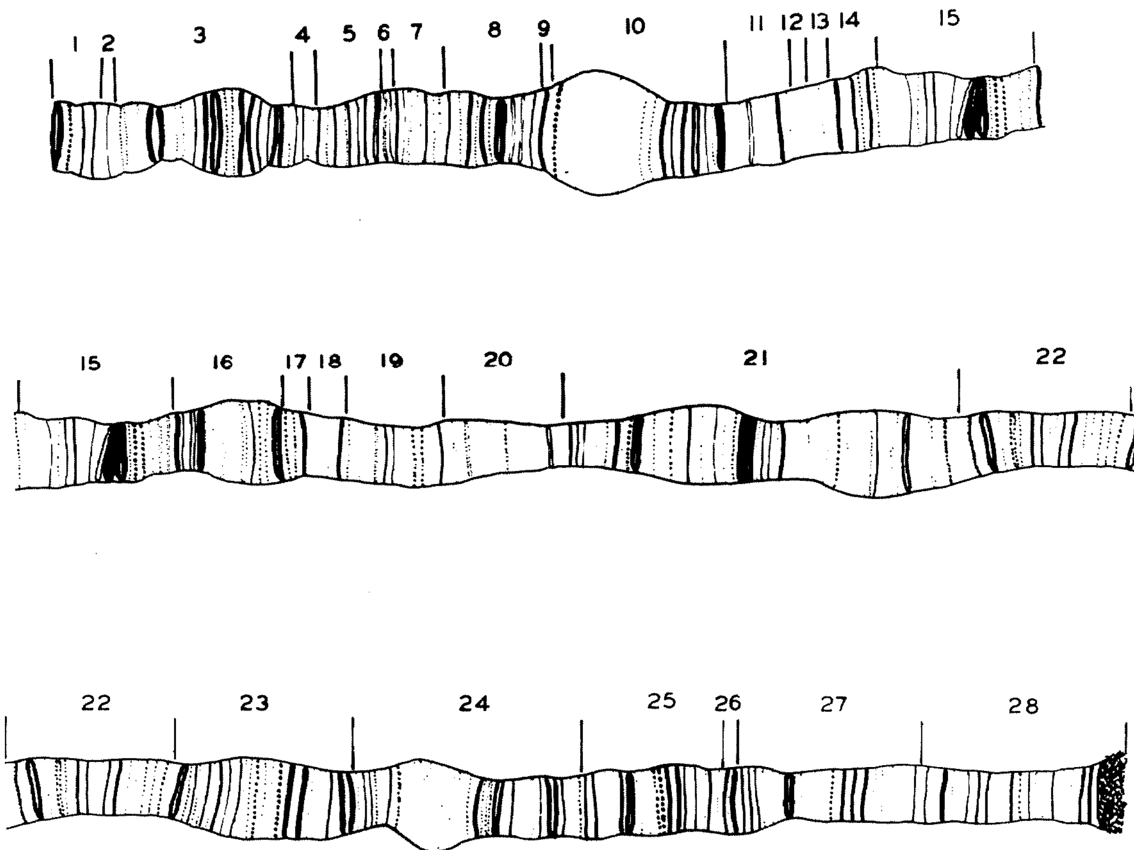


Figure 2. The salivary gland chromosome representative of element C. The divisions have as their endpoints the regions at which inversion breakpoints are known to occur.

The sections into which it has been divided have, as their endpoints, the regions at which breakages of the chromosome, by inversion, are known to occur. This system of numbering facilitates the description of relatively inverted sequences in terms of sections.

A double inversion with respect to A has been found in Maine, both Massachusetts and both New Hampshire strains. It has the sequence:

1.....7.11.....22.10 9 8.24..... (C)

This is either an included or an overlapping inversion (fig. 3) with two of the breakpoints of the two individual inversions occurring in close proximity. No sequence has been found which might represent an intermediate step (B).

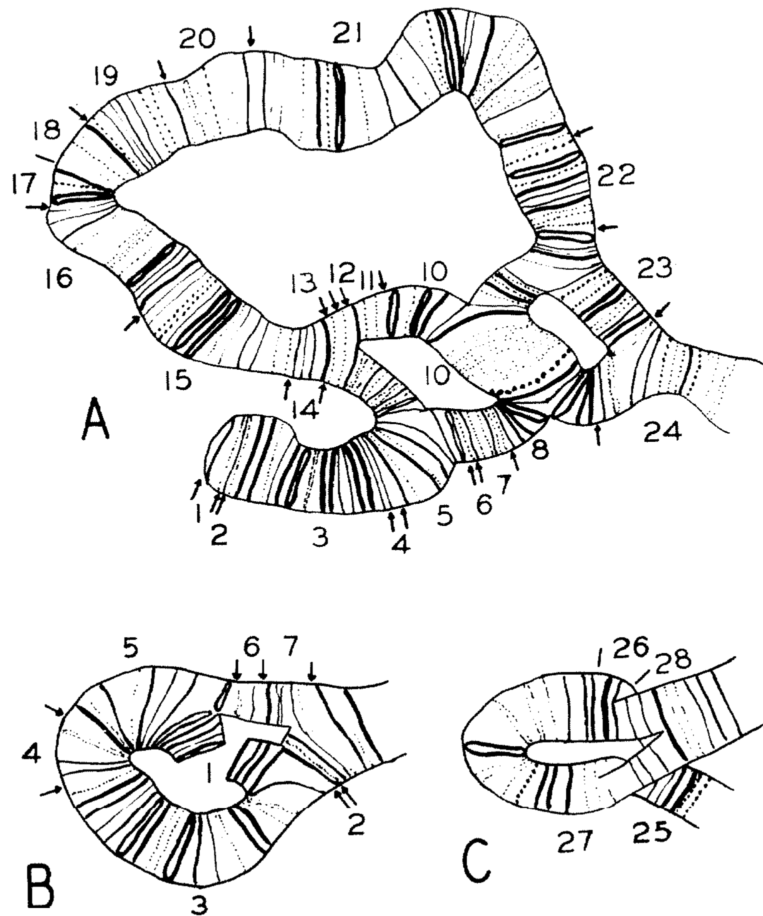
The strain of New Hampshire-1 yielded two simple inversions with respect to A (fig. 3, B and C). The order of sections on the sequences were:

1.6 5 4 3 2.7 8..... (D)

and:

.....25.27 26.28..... (E)

Both were found together in combination with sequence C; since they do not overlap C and are sufficiently distant from it that crossing over may have combined them with C after their origin on another sequence, the exact phylogeny is not clear. The sequence actually observed was DCE; the dotted lines on the phylogenetic chart (fig. 10) represent the possible origins of this final sequence.



<u>Figure</u> <u>3.</u> Inversions in element C. A. A/C inversion. B and C. Distal and proximal inversions, respectively, of the BCD/C combination.

In New Hampshire-2, a sequence of at least six break points with respect to A has been found (fig. 4A). The sequence of sections is:

1 2.16.....6.4 5.20 3.17 18 19.21..... (I)

In order to derive this from A, which is the most closely related of the sequences, it is necessary to postulate four inversion steps. Two pairs of the breakpoints must occur in close proximity and have not been differentiated. The three hypothetical sequences between A and I are represented by the letters F, G, and H (fig. 10).

A small inversion found in the Massachusetts-2 strain (fig. 4B) differs from A by the following sequence:

.....14.9 10 11 12.8..... (J)

The phylogenetic relationship is obvious, since it is a direct derivative of A.

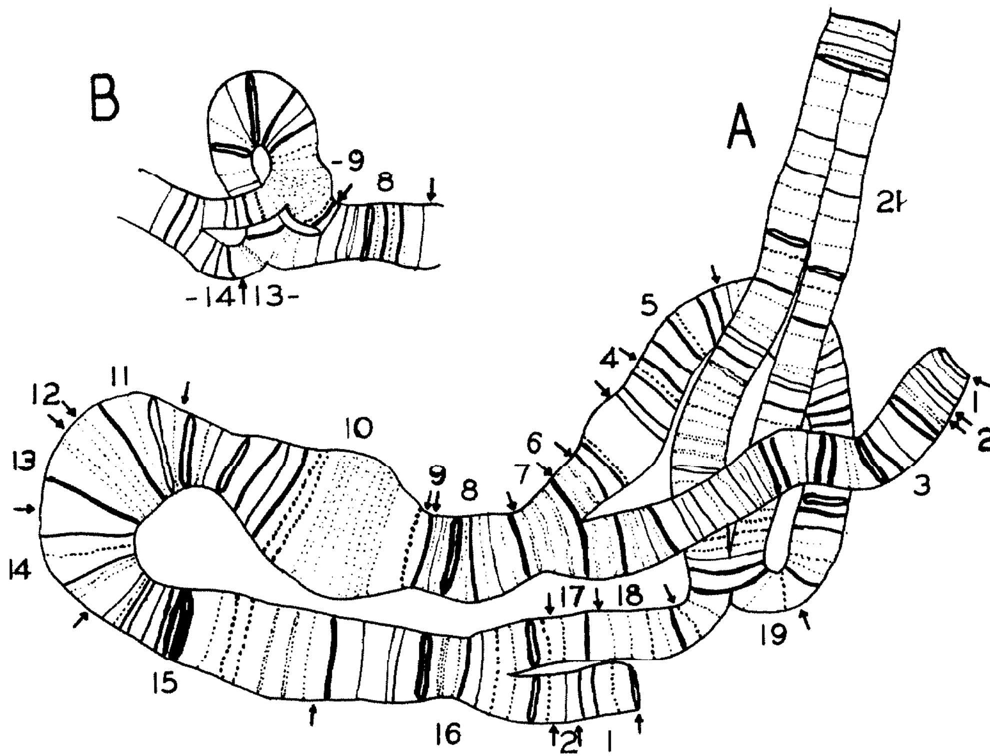
In the Quebec strain a double inversion with respect to A is found in the proximal end of element C (fig. 5A). The sequence of sections of this relatively inverted chromosome are:

.....16 17.24 23 22.18 19 20.25..... (L)

The intermediate step K to this double inversion has not been seen. All western forms show this sequence, or derivations of it, in the basal part of element C.

The western forms differ from the eastern ones by an additional distal inversion (fig. 5B). It has the sequence:

1 2 3 4.12.....5.13..... (M)



<u>Figure</u> <u>4</u>. Inversions in element C. A. A/I multiple inversion. B. A/J inversion.

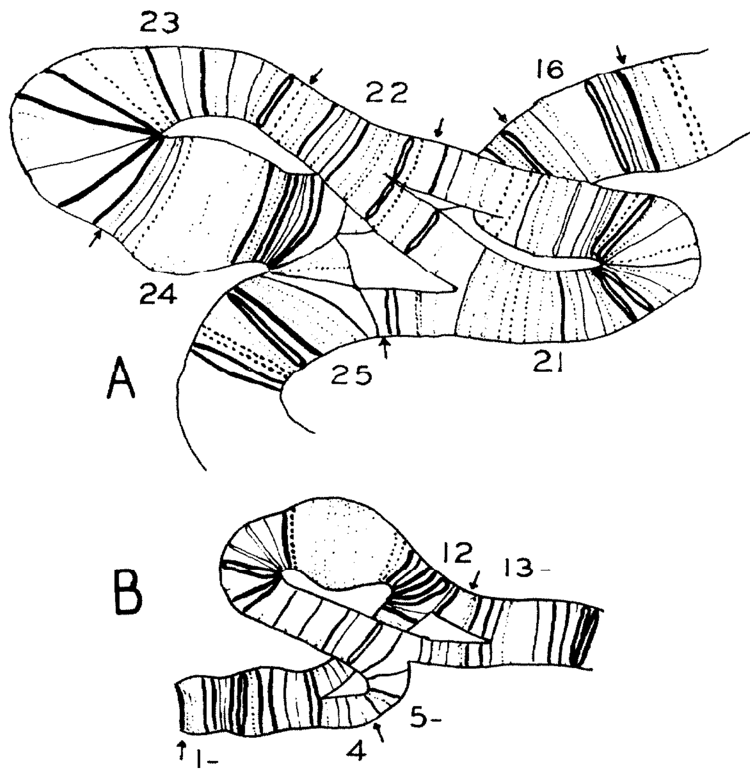


Figure 5. Inversions in element C. A. A/L inversion. B. A/M inversion.

The LM combination of sequences has been found in Alaska, Wyoming-1, British Columbia-1, -2, -4, Washington and Oregon-1. This combination represents the closest relationship of the western sequences to A. All other sequences found in the western range of athabasca are derivations of LM.

A double inversion with respect to LM (fig. 6A) has been found in Washington, Wyoming-2, -3, and British Columbia-3. The order of sections on it is:

1 2 3.9 8 7 6 5 13 14 12.4.10 11.15..... (O)

The intermediate step necessary to derive this overlapping inversion sequence from sequence LM is:

1 2 3.4 12 14 13 5 6 7 8 9.10 11 15..... (N)

No sequence that might represent this intermediate has been observed. In order to prove that the standard A sequence was not, in fact, this hypothetical sequence, despite the obvious displacement of some bands in hypothetical as compared to standard, larvae were obtained which were heterozygous for A and O. Instead of the single expected if A were the hypothetical, the salivary gland chromosomes showed a triple inversion.

A simple inverted sequence with respect to LM (fig. 5C) found in British Columbia-1, -2, -3 and -4, Oregon-1 and -2 strains has the order:

.....27.22 21 18 19 20 25 26.23..... (P)

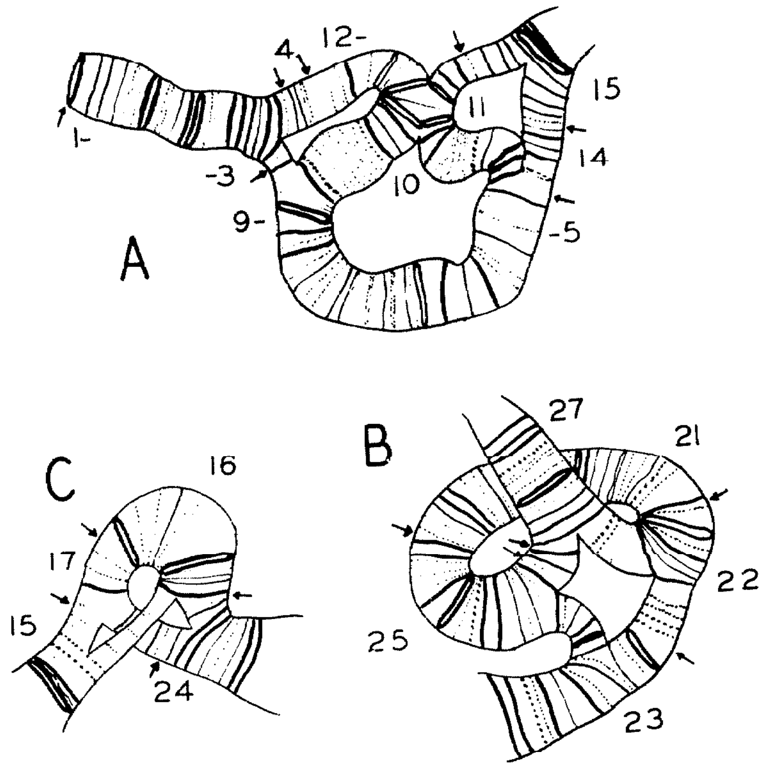


Figure 6. Inversions in element C. A. LM/O inversion. B. LM/Q inversion. C. LM/P inversion.

It differs from the sequence LM by a single inversion; the phylogeny of this can be represented as LM ⟶ P.

Another simple inversion with respect to LM (fig. 5C) has the order:

.....5.17 16.24..... (Q)

It has been found only in the Oregon-1 and -2 strains.

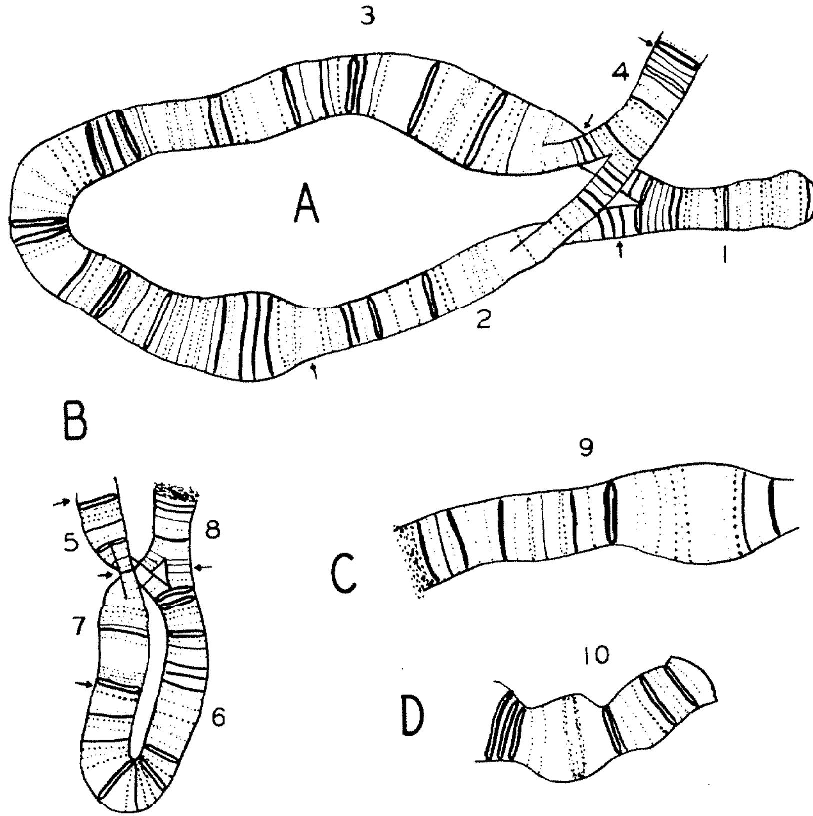
Combinations of the above sequences can often occur in the same chromosome, when the nearest breakpoints of the two independant inversions are sufficiently separated. The sequence PO has been found in British Columbia-3, -4, and Oregon-1 and -2 strains.

Sequences in Element E

Element E is composed of two arms, a long and a short one. The longer arm has been numbered from 1 to 8; no inversions have been found in the shorter arm and it has consequently been numbered 9 at the basal region (fig. 7C) and 10 at the distal end (fig. 7D).

The sequence 1 2 3 4..... (A) has been found in the following strains: North Carolina, New Hampshire-1, Maine and Quebec. The relatively inverted sequence 1.3 2.4..(B) (fig. 7A) has been found in New Hampshire-1, -2, New York, Maine, Quebec in the eastern range. All the western strains studied are homozygous for this sequence.

The sequence 5 6 7 8..... (C) occurs in the western



<u>Figure 7</u>. Inversions in element E. A. The A/B inversion at the distal end. B. The A/B inversion at the proximal end. C. and D. Base and tip, respectively, of the short arm.

strains Alaska, Wyoming-1, -2, -3, Washington, British Columbia-1, -2, -3, -4, Oregon-1 and -2 and in the eastern strains North Carolina, New Hampshire-1, Maine, Massachusetts-1, -2 and Quebec.

The relatively inverted sequence .....5.7 6.8..... (fig. 7B) occurs in the western strains Washington, Wyoming-3, British Columbia-1, -3, -4 and Oregon-2; among the eastern strains it is found in New Hampshire-1, Maine, Quebec and Massachusetts-2.

Sequences in Elements A and D

Elements A and D are combined in athabasca, as in pseudoobscura and other closely related forms, to form the X-chromosome. Element A has been numbered from 1 to 5 proceeding proximally, element D has been numbered from 6 to 13 proceeding distally.

Only two arrangements have been found in element A. Together they form a subterminal inversion (fig. 8A). The sequence 1.3 2.4 (B) is limited to the Alaska, Wyoming-1, -3, Oregon-1 and -2 strains. All other strains studied and also Wyoming-1 have the sequence 1 2 3 4 (A).

Element D, the longer limb, has as its most common sequence 6 7 8 9 10 11 12 13 (C). It has been found in all strains except New Hampshire-1, New York and Massachusetts-2. These three strains, along with New Hampshire-2, showed a

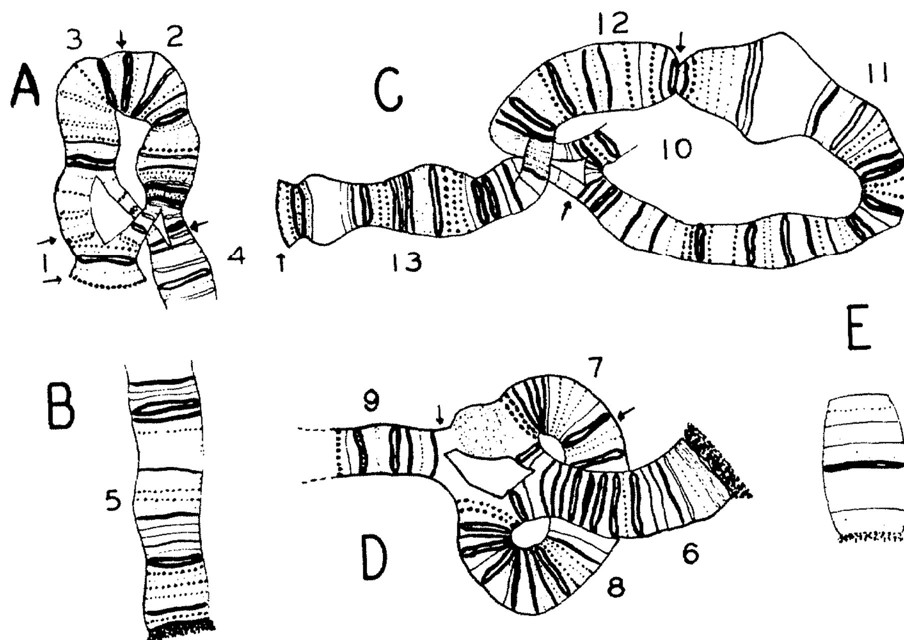


Figure 8. Inversions in elements A and D (A to D) and the microchromosome (E). A. The A/B inversion in element A. B. Basal region of element A. C. The A/B inversion at the distal end of element D. D. A/B inversion at the proximal end of element D.

sequence differing from A by three independent inversions, the basal and distal of which are shown in figure 8C and 8D. The sequence of these chromosomes is 6.3 7.9.....10.12 11.13 (B).

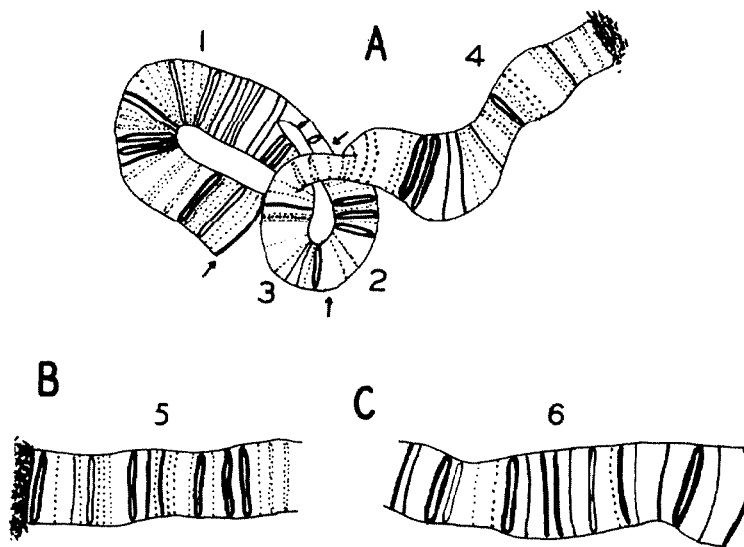
Sequences in Element B

Element B has two unequal arms; the longer arm has as its most prevalent sequence one that is found in both eastern and western strains. The tips and base of this sequence are illustrated in figures 9 B and C. Inversion configurations in this arm have been seen occasionally, but because of the rarity with which satisfactory preparations involving this arm are obtained, no attempt has been made to analyze these occasional variations.

Two sequences occur in the shorter arm (fig. 9A). The sequence 1 3 2 4 (A) occurs in all the strains examined except two; the relatively inverted sequences 1.2 3.4 (B) has been found in the Massachusetts-1 and -2 strains.

The Phylogeny of the Sequences in Element C

As has been pointed out previously, the actual order in which the various sequences have originated is not immediately obvious from their relationships as sequences. It is pertinent to attempt to establish such an order so that the geographical distribution of the various sequences may be



<u>Figure</u> <u>9</u>. Element B. A. The A/B inversion in the short limb. B. and C. The basal and proximal ends, respectively, of the longer limb.

interpreted in terms of the history of the species itself.

In the case of element C of pseudoobscura (Dobzhansky and Sturtevant, 1938). An attempt has been made to homologize one of its sequences, Santa Cruz, with a sequence found in miranda, a closely related species with which pseudoobscura hybridizes. The argument for this procedure is this: assuming that the miranda and pseudoobscura sequences show close parallelism in the salivary gland chromosomes of their hybrids, both must represent very primitive arrangements and, consequently, either that particular pseudoobscura (and miranda) sequence or its immediate derivatives are probably the source of all the other sequences. The salivary gland chromosome representative of element C in pseudoobscura had been divided arbitrarily into 19 more or less equal sections. Approximately 3 distal sections of Santa Cruz show some resemblance to a sequence of miranda except for a small intercalation of several dark bands in the middle of this homologous segment of miranda. This intercalation would necessarily imply at least a double overlapping inversion difference between this small segment and the corresponding Santa Cruz sections. Since there is some variation in sequence in miranda itself, another sequence of miranda may show greater resemblance to a different sequence of pseudoobscura than the case given above. The assumption that miranda has evolved with the most primitive sequence of element C present

in the common ancestor rather than with a derivative or even with several sequences is open to question. The type of inference suggested by Sturtevant and Dobzhansky as a means of determining direction in phylogeny is basically sound but, for the reasons given above, is impracticable in pseudoobscura and other closely related species, as those of the affinis group.

The probable base of the phylogenetic tree of sequences (fig. 10) has been arrived at simply on the basis of the geographical distribution. The eastern and western forms of athabasca have probably been isolated from each other for a long period in their history, although not long enough nor completely enough for their divergence into separate species. Any sequence that they might have in common would seem to be the primitive sequence or a close relative of it. Actually they have none in common, but each of the two forms has as its most prevalent sequence two that are very closely related (_A_ and _LM_). (The letters which represent known sequences are underlined; letters representing hypothetical sequences are not.) It seems likely that one of these two, or one of the intermediates between them (K, _L_, or M) represent the original type. All the arrows between these, consequently, are doubly pointed. Further derivatives of these are designated by arrows pointing away from this group.

Starting with the "standard" _A_ sequence, _J_ differs

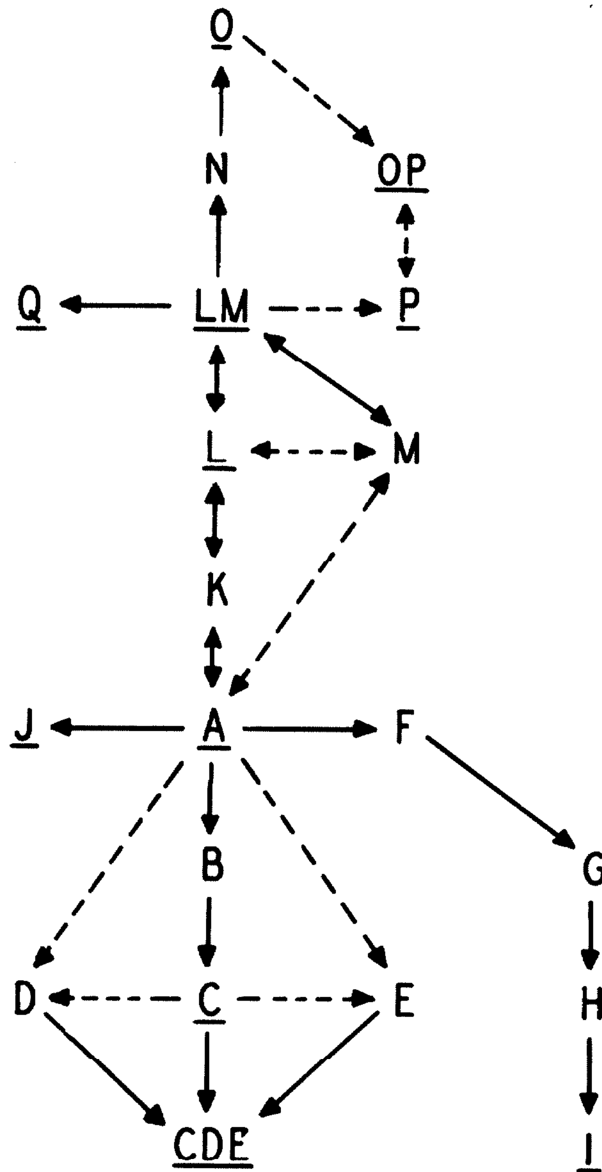


Figure 10. The phylogenetic tree of sequences in element C. Underlined sequences are known ones, the others are hypothetical. Dotted lines indicate ambiguous paths in the phylogeny.

from it by a single inverted section. The relationship is *A* → *J*. (Frequent references to figure 10 may be helpful during the following discussion.) Sequence *I* differs from *A* by four consecutive inversions; three hypothetical intermediates must come between *A* and *I* (*A* → F → G → H → *I*). Between *A* and the doubly inverted sequence *C* is the hypothetical B (*A* → B → *C*). The sequence *CDE* differs from *C* by two independent inversions, D and E. Since crossing over might have combined them with C after their origin on another sequence, as *A*, the exact phylogeny is ambiguous. Either may have originated from *A* or *C* (*A* --→ D ←-- *C* and *A* --→ E ←-- *C*) but all three, *C*, D, and E have been combined to produce *CDE* (D → *CDE* ← E).
↑
C

Sequence *L* differs from *A* by two inversions, the hypothetical intermediate K has not been seen (*A* ↔ K ↔ *L*). Sequence M does not occur alone but occurs only in combination with *L* as *LM*. Since crossing over may have combined M with *L* after its origin on another sequence such as *A*, the origin of *M* is ambiguous and can be represented thus: *L* ←-→ M ←-→ *A*. *L* and M, however, together produce the widely distributed *LM* (*L* ↔ *LM* ↔ M). The derivation of the singly inverted sequence *Q* can be simply represented as *LM* → *Q*. *O*, a double inversion with respect to *LM*, has a hypothetical intermediate N (*LM* → N → *O*). The origin of

P is ambiguous: It occurs both with and without sequence O and since there is ample opportunity for crossing over either to combine or to separate them, P may have arisen on the LM sequence or it may have arisen on the O sequence to give PO, later separating from O. The various paths that these sequences may have taken are illustrated in figure 10.

Some Consequences of the Geographical Distribution of the Various Sequences

Since both the eastern and western strains of athabasca have their individual set of element C sequences, it is obvious that all the derivative sequences are fairly recent and have originated after the migration of the species across the continent, regardless of the actual direction in which that might have taken place. This conclusion eliminates the possibility that many of the sequences found have been carried over from the ancestral type, a possibility which might have explained the unusually high variability in element C only of both pseudoobscura and athabasca.

A relatively limited sample of strains from the eastern and western distributions has been analyzed; it is consequently difficult to draw conclusions from the variations within each distribution. It may be suggested that sequence O, found throughout the entire western range, is older than sequence Q, found only in Oregon. It is evident that sequences

intervening between the central ambiguous group and the further derivatives are more ancient than those derivatives but are more recent than those of the central group, as in the case of A, C and CDE (fig. 10). Figure 11 shows the geographical distribution of the various sequences found in element C.

All the other elements have at least one sequence common to both the eastern and western ranges (BC and BD in element E, A in element A, C in element D, A in element B). These sequences are probably older than most of those found in element C. In only one case are there two different sequences of the same element found in both eastern and western ranges. These are sequences BC and BD of element E. All other inversions are limited to one of the two ranges and, in some cases, to a limited part of that range. These derived sequences are obviously more recent than the "standard" sequences.

A Theory of the Origin of Certain Inversions

Element C of athabasca shows a disproportionately high variability as compared with the other chromosomes. This is the same element which has the greatest variability in pseudoobscura (Dobzhansky and Sturtevant, 1938). In azteca, elements C and E show a relatively high variability compared to the others (Dobzhansky and Socolov, 1936) whereas

Figure 11. The distribution of the various sequences of element C.

in algonquin, all the elements have a uniformly low variability (Miller, 1940). These numerical relationships are presented in table 5.

The reason why certain elements are more variable than others has not been satisfactorily explained. One possible answer is that the more variable elements differ from the others in possessing higher inherent "breakability." Helfer (1940), in a study of the aberrations produced by x-irradiation of pseudoobscura, found no differences in "breakability" of the various elements. The basic assumption of that investigation, that naturally occurring inversions are produced by stimuli as catastrophic as x-irradiation, has little in its favor.

For a clue to the mechanism by which inversions arise in a natural population, it is pertinent to examine in detail the nature of the inverted sequences found. Figure 2 shows the breakpoints of the known inversions in element C of athabasca. If that salivary gland chromosome is divided into four equal parts, 10 breakpoints occur in the most distal quarter, 10 in the second quarter, 3 in the third quarter and 4 in the basal quarter. The distal clustering of breakpoints is obvious.

For the purpose of this analysis, element C of pseudoobscura serves better than that element of athabasca because of the extent of the phylogenetic tree and the absence of

Table 5.

A comparison of the numbers of different sequences found in the various elements of athabasca and closely related species.

Species	Elements				
	A	B	C	D	E
athabasca	2	4	10	2	2
algonquin	3	3	4	1	4
azteca	2	1	6	3	6
pseudoobscura, Race A	1	2	12	2	3
pseudoobscura, Race B	1	1	6	2	3

large numbers of hypotheticals. A similar distal clustering is found here. Of 38 breakpoints, 19 are found in the distal quarter, 10 in the second, 7 in the third and 2 in the basal quarter. Since these breakpoints have been related to "standard" sequence and inversions differing from standard by more than one step originate on sequences which have sections out of order, the position of the breakpoints may be recalculated with respect to their relative position at the time of the formation of the inversion. For those cases for which the necessary data are available for this transformation, 14 breakpoints fall in the distal quarter, 11 in the second quarter, 5 in the third quarter and 2 in the basal quarter. Applying the Chi-square method of determining "goodness-of-fit," the probability of obtaining as good a fit or worse from an equal number of random breaks is .01. If a correction is made in this value to include the very significant order in which these values occur (from the lowest value proximally to the highest distally), the probability of getting as good a fit or worse is 4×10^{-4}.

Elements C of pseudoobscura and athabasca show the same distal clustering; since each must differ from the other by many inversions, with a consequent relative scrambling of sections, the distal clustering is not the result of a higher inherent "breakability" of specific regions but is rather a general property of sections which happen to fall in the distal region.

In the pseudoobscura phylogenetic series of sequences in element C, there are two branches with three derivatives each, involving no hypotheticals [A. Santa Cruz → Tree Line → Olympic (1), Oaxaca (2), and Estes Park (3); B. Standard → Klamath → Sequoia II (1), Wawona (2), and Cowichan (3)]. For the sake of convenience at this point, the set of three derivatives in each series shall be called secondary sequences; the sequences from which they were derived shall be called primary ones.

If the salivary gland chromosome is represented by a line (fig. 12A and B) with the basal heterochromatin at the extreme left, the relative positions at which breakpoints on the Santa Cruz (A) and Standard (B) sequences have produced Tree Line (A) and Klamoth (B) sequences, respectively. The pairs of connected arrows below the lines representing the chromosomes indicate the points at which the already inverted sequences must have been broken to produce the secondary sequences. The numbers after each secondary sequence so indicated correspond to the numbers given to the sequences above. The distribution does not seem to be random with respect to the inversion of the original: Treating each section, that included within the original inversion and those on each side as units with an anticipated number of breakpoints in proportion to their length, the probability of getting such a distribution, or a worse one, on the

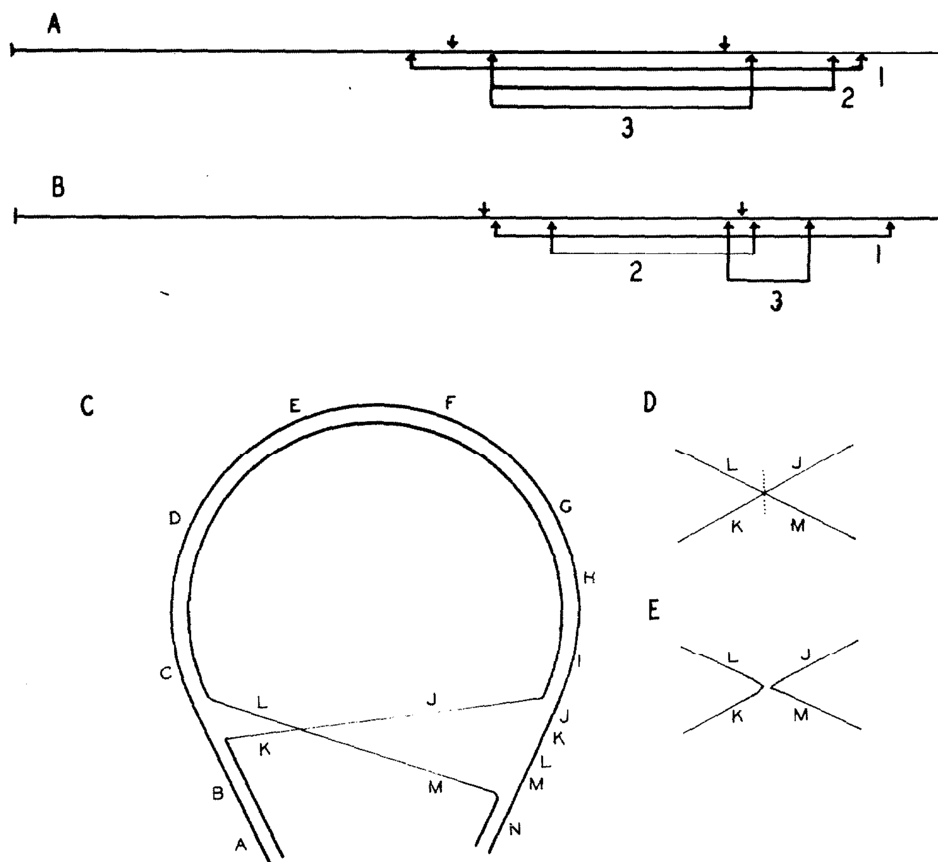


Figure 12. A theory of the mechanism of the origin of certain inversions. For complete explanation see text.

basis of chance alone is .05- for the two sets taken together. This type of analysis overlooks the actual position of the breaks, a refinement not practicable with the low numbers involved.

The relation of the position of the breakpoints which produced the secondary sequences to those breakpoints which produced the primary ones can be stated as follows: There is a tendency for the proximal breakpoints of both types of sequences to lie near each other and the same applies to the distal breakpoints but in each case there is a tendency toward a distal displacement, which is more pronounced for the distal breakpoints than for the proximal ones.

The only conceivable situation in which the breakpoints of an inversion are differentiated from other sections is the sort that is found at the pachytene stage of the first meiotic division of an individual heterozygous for an inversion. The tendency for complete synapsis of the two different sequences results in an asynapsis of the regions at which the inverted segment begins and ends.

Figure 12C is a simplified (two-strand, which condition will be assumed throughout this discussion for the sake of simplicity) schematic representation of such pairing in a heterozygote for sequences ABCDEFGHIJKLMN and AB.KJIHGFEDC.LMN. This configuration leads to a juxtaposition of two unsynapsed sections that would ordinarily be widely separated; these

sections are those containing the breakpoints of the inversion (fig. 12D). Breaks in this region of the two single strands, followed by a refusion of the pairs of broken ends on the same sides (fig. 12E) would lead to the production of another inversion on either sequence. If it occurred on the uninverted sequence, a "mimic" inversion would be produced, if it occurred on the already inverted sequence, a derivative of this inversion would be formed. In the example given in figure 12, the new sequence formed is ABK.LCDEFGHIJ.MN, an inversion that overlaps with a distal shift the first inversion. The predominance of distal over proximal shifts in the inverted sequences found in nature agrees with genetic and cytological observations that synapsis tends to be more complete proximally than distally, i.e., that the single strands available for breakage and reunion are more apt to represent distal sections than proximal ones.

The question of the cause of breakage of the single strands has two possible explanations. The first is that breaks originating in the same manner as those that produce the primary inversions are here more likely to produce an inversion than they would ordinarily because of the proximity of the strands. Secondly, the tendency of the inverted sequences to pair may impose a strain upon the unsynapsed sections of the chromosomes. This strain may be relieved by a sort of unequal crossing over at a point of contact of the

two strands (fig. 12 C and D) or both strands may be broken to relieve the tension, with a subsequent uniting of broken ends on the same side.

It seems likely that the breakage is not of the type that ordinarily produces inversions for then it would be expected that all species which show variability in sequence (and all those studied do) would tend equally to have high variability. The difference between pseudoobscura, athabasca, azteca and other species as melanogaster, virilis, etc. can be more readily explained on the basis of a secondary sequence producing "breakability" which is a function of the structure of the chromosomes of those species.

With the above relations of sequences in mind, it is now pertinent to redefine "primary" and "secondary" sequences. Primary sequences are those which have originated in the same way that natural aberrations generally do. They will generally be found near the base of the phylogeny and so represent rather primitive forms (as, for example, Pikes Peak in pseudoobscura or sequences A or K of athabasca). Secondary sequences are those which seem to have originated in the manner described above, bearing a definite relationship of its breakpoints with respect to the breakpoints of its immediate predecessor, generally with a distal shift of those breakpoints, the shift being more pronounced for the distal breakpoints. They tend to occur near the branches of the

phylogeny and so represent relatively recent derivatives with a relatively limited range (as the sequences in pseudoobscura discussed above, also sequences C and O of athabasca, and sequence A-2 of algonquin). Obviously the distinction between primary and secondary sequences in many cases is difficult to make, but the distinction in other cases is clear.

This theory offers an explanation of the high variability of elements C in pseudoobscura and athabasca and of elements C and E in azteca. When a certain element is favored by the chance distribution of inversions, by a slightly greater tendency to produce primary inversions or by the origin in it of primary inversions having a high tendency to produce secondary inversions, the frequency with which it produces secondary inversions increases geometrically resulting in a much higher variability of that element.

SOME GENETIC NOTES ON AN ANOMALOUS SEX RATIO CONDITION IN DROSOPHILA AFFINIS

Introduction

Natural populations of several species of Drosophila have been found to contain a gene which causes males carrying it to produce all or almost all female offspring. This "sex-ratio" gene has been found in a member of the affinis group (Sturtevant in Morgan, Bridges, and Sturtevant, 1925), in obscura (Gershenson, 1928) in athabasca, azteca, affinis and pseudoobscura, races A and B, (Sturtevant and Dobzhansky, 1936) and in melanica (Sturtevant in Sturtevant and Novitski, 1941).

It has been shown by Gershenson that females of obscura heterozygous or homozygous for this X-chromosome gene behave quite normally and that males carrying it seem to produce nearly all X sperm, instead of the normal 50 per cent.

Sturtevant and Dobzhansky have allocated the factor responsible to element D, (the right limb of the X-chromosome) of pseudoobscura, in both races. A cytological investigation revealed that the X-chromosome of "sex ratio" males undergoes an equational division at each meiotic division, the Y-chromosome usually degenerates so that each spermatid generally receives an X-chromosome, but no Y.

This section is devoted to a discussion of the genetic nature of a condition which results in the production by certain affinis males of only male offspring. This phenomenon has been named "male sex ratio", (abbreviated hereafter as MSR) to distinguish it from the "sex ratio" described above.

"Sex Ratio" in Affinis

Since, as will be shown later, MSR is associated genetically with "sex ratio," some of the genetic characteristics of the latter must be considered first.

Sturtevant (1940) has recorded for affinis two widely distributed sequences of the X-chromosome, "standard" and "inverted." In some cases the "inverted" sequence seemed to carry the "sex-ratio" gene. The ratio of the two sexes appeared to be extremely variable in different cultures in which the male parents had the same "sex ratio" X-chromosome.

During the course of these experiments, only one chromosome with the "inverted" sequence was used. At no time did it show any clear evidence of carrying the "sex ratio" gene. Instead, the "sex ratio" effect that has been investigated here is produced by an X-chromosome carrying a third sequence. This sequence reduces the crossing over between hairy (h) and scarlet (st), 62 units apart on the "inverted" sequence, and between white (w) and miniature (m), 35 units apart on the "standard" sequence, to practically

zero. An examination of the salivary gland chromosomes of heterozygotes for the "sex ratio" X-chromosome and "standard" show that the former sequence cannot be a simple crossover derivative of the other two.

The ratio of the sexes obtained from "sex ratio" males is quite variable, although the source of all the "sex ratio" X-chromosomes used here can be traced to one such X-chromosome found in a series of cultures manifesting the MSR effect. In one set of 8 cultures, an average of 170 ♀♀ to .6 ♂♂ per culture was obtained; in another set of 18, the average ratio per culture was 97 ♀♀ to 46 ♂♂. These data indicate the presence of one modifying factor or more on the other chromosomes, but an analysis of these has not been attempted. The danger of mistaking "sex ratio" cultures for normal ones and vice versa has been reduced by using only "standard" and "inverted" sequences marked with recessive mutants, their respective wild type alleles automatically marking the "sex ratio" sequence.

Discovery of the "Male Sex Ratio" Phenomenon

During the course of the translocation studies already described, 3 cultures, 1-17, 1-21 and 1-24 yielded only male or almost only male offspring. The nature of the matings which showed this effect may be described as cn rug ♀♀ X F_1 (cn rug ♀♀ X Woods Hole X-rayed ♂♂) ♂♂. Two

transfer cultures of 1-17 produced a total of 12 ♀♀ and 208 ♂♂, culture 1-21 produced 10 ♂♂ and culture 1-54 produced 25 ♂♂. This phenomenon was later observed by Professor A. H. Sturtevant in the F_2 from a pair mating of F_1 individuals from a cross of a pink-like ♀ by a cinnabar ♂. Only 1 ♀ but 64 ♂♂ constituted the entire progeny of this bottle.

The discovery of MSR in crosses involving irradiated chromosomes suggested that the causal factor was an induced mutation or a chromosomal aberration. The later appearance of this condition in cultures derived only from wild strains detracts from that possibility. The fact that all four crosses showing this effect involve stocks carrying the cinnabar mutant is strongly suggestive.

The Genetic Composition of MSR Males

The preliminary crosses designed to isolate the aberrant genetic system responsible for MSR were rather ineffective. Several pertinent points, however, were established. The appearance of MSR in cultures in which the female parents were of the compositions w, h st, asc st, h st/w, h st/+, w/+, dachs and jt net showed that the genetic composition of the male alone was the determining factor. Inbreeding experiments indicated that the genetic composition of MSR males was not simple, and that "sex ratio" was in some way associated with it. The rather high sterility of MSR males and the unisexual

inclination of the cultures from both MSR and "sex ratio" males made inbreeding difficult. Outcrossing individuals from the strains known to contain, to some degree, the MSR system to mutant strains for the purpose of marking the chromosomes generally led to the loss of the MSR effect, as tested by subsequent inbreeding. In only one case was the outcrossing followed by a recovery of the MSR effect; the strain extracted has been the basis of all the later work.

The single female appearing in the F_2 of the mating of a p ♀ x a cn ♂ was crossed with a w male. Tests by mating to w females of 16 of the male offspring from this cross showed clear evidences of the presence of a "sex ratio" X-chromosome (to be denoted as sr hereafter) in 5 of the males. Females of the constitution sr/w from the culture showing the most extreme "sex ratio" (101 ♀♀:1 ♂) were mated to w m males in order to obtain a stock carrying the sr X-chromosome in a balanced condition. After one generation of inbreeding (sr/w m ♀♀ x w ♂♂), males carrying sr were tested and were found, in a few cases, to produce only male offspring; the majority of the fertile cultures showed the typical "sex ratio" phenomenon. A large series of matings of the type w/sr ♀♀ x w m ♂♂ was set up, with a subsequent selection of those in which at least some of the sr males were in reality MSR. The strain finally derived, all fertile sr males of which showed the MSR effect, regardless of the genotype of its mate,

has been maintained for a period of ten months by selection of sr/w m ♀♀ and w m ♂♂ as the parents for each generation.

An obvious conclusion is that the sr X-chromosome is a necessary prerequisite for the MSR effect of a male. Never has a male with "standard" sequence (marked by w) produced only male offspring; certain cultures involving h st, in which all the sr males from a h st/sr female were MSR and all h st males were not, indicate that the "inverted" sequence is also not involved in MSR.

Several types of experiments indicate the nature of the difference between "sex ratio" males and MSR males. If a female of the constitution w m/sr is outcrossed to certain unrelated strains, as asc st or jt net, the F_1 sr males invariably show the typical "sex ratio" effect. MSR males can be found among the sr males of the F_2. When the male parent comes from the same inbred strain as the female parent, all the sr males show MSR. There is an intermediate possibility, that male parents derived from certain strains, produce, in the F_1 of matings to w m/sr females, both MSR and "sex ratio" males.

The above results can be explained by the assumption that an autosomal recessive gene (a) present in the homozygous state in sr males (sr a/a) converts it from a "sex ratio" male into a MSR male. Assuming that this factor exists, there is no evidence that it has any effect in the heterozygous state.

In order to demonstrate the existence of the a gene, an attempt was made to allocate it to a particular element. W m/sr females, of the inbred MSR strain and so with the hypothetical constitution w m/sr, a/a, were mated to males carrying jt net (element B), to males carrying cn (element C) and to males carrying rug (element E). Stocks of the constitution w jt net, w cn and w rug were derived from this mating to eliminate the unmarked X-chromosomes from the autosomal mutant stocks. The factor a might be present in these stocks (1) if selection for a specific mutant gene did not eliminate them by virtue of their location on different chromosomes or by virtue of an appreciable amount of crossing over between it and a specific gene on the same chromosome, and (2) if the a factor was present in the original autosomal mutant individuals mated to w m/sr, a/a females. This latter possibility was most serious for the cross involving cn, because of the evidence from earlier crosses which had shown the presence of the a factor in the cn stock. Tests of F_1 sr males, which should reveal whether the particular mutant individuals used carried the a factor, either in the homozygous or heterozygous state by the appearance of the MSR effect either in all or in half the males, gave negative results.

Males from the multiple mutant stocks were then mated to w m/sr, a/a females and F_1 sr males were tested for the

presence of the homozygous a factor in the way described above as a test for the presence of the a gene in the autosomal mutant stocks.

The results of these tests were quite striking: the w rug strain was homozygous for the a gene, the w cn was heterozygous for it, and the w jt net strain did not carry it at all. A strain of the composition w m/sr, a, rug was immediately obtained; a comparable strain homozygous for cn instead of rug was obtained within two generations. The attempt to obtain the comparable strain carrying jt net failed completely; the suggestion is that jt, net and a are on the same chromosome and that selection for jt net automatically eliminated a.

Corroboratory evidence that a is located in element B has been obtained in another way. Segregation of a Y-autosome translocation in a male heterozygous for it is such that the chromosome which has exchanged fragments with the Y-chromosome will generally be present in all males.

Consequently, repeated backcrossing of such males to females carrying marker genes on the chromosome corresponding to that involved in the translocation is ineffective; the males will always be heterozggous for those chromosomes involved.

With the series of Y translocations available in affinis, such a test is relatively simple. Males carrying

the translocations involving element B (TY-4A), involving elements B and E simultaneously (TY-4E), involving elements B and C simultaneously (TY-3B) and involving element E (TY-2A, TY-2B) were mated individually to females of the composition w m/sr, a. F_1 w m males, heterozygous for the translocation and for a, were backcrossed to w m/sr, a females. If the gene a is carried on the corresponding chromosome or on one of the corresponding chromosomes involved in the translocation, all the sr males from this cross must be heterozygous for a and so will behave as "sex ratio" males. If the gene a segregates independently of the translocated chromosomes, half the sr males of the backcross generation will be heterozygous for a and behave as "sex ratio" males, the other half will be homozygous for a and consequently may be expected either to be MSR or to show a characteristic sterility. Another possibility is that the translocated chromosomes of any given aberration may itself carry a rather than its wild-type allele, since MSR was first discovered in that set of cultures from which the translocations were derived. In this case such backcrossing should lead to sr males of which all would be expected to show the MSR effect instead of "sex ratio." These two expectations, males all of one kind or of two kinds have been represented in Table 5 by 1 and 2, respectively, for the translocations used, when a is located on each of the three long autosomes.

Table 5

The numbers of the kinds of males expected from back crosses to w m/sr, a females of males carrying certain translocations, considering individually the various possibilities for the location of a on the three long autosomal elements.

Translocation	Position of a		
	If On Element B	If On Element C	If On Element E
Y - 2A	2	2	1
Y - 2B	2	2	1
Y - 3B	1	1	2
Y - 4A	1	2	2
Y - 4E	1	2	1

From these crosses, males of two different behaviours were actually produced. In the order of the translocations as given in Table 5, the different types of male per series were 2, 2, 1, 1, 1 or exactly the distribution expected if <u>a</u> is located on element B as previous observations indicate.

The two distinct types of males were not "sex ratio" and MSR, however, but "sex ratio" and normal. All of the cultures (17) of the TY-3A series were clearly "sex ratio" the average number of females and males per culture being 77 and 4.5 respectively. The 50 cultures of the TY-4E series showed an average of 70 females and 4 males. The 49 cultures of TY-4A, on the other hand, gave an average of 95 females and 85 males. In the TY-2A series 9 cultures were "sex ratio;" 4 were normal; 3 were doubtful and 3 were sterile. In the TY-2B series, 22 were "sex ratio;" 18, normal and 13 sterile.

The conclusion to be drawn from the above results is that the presence of a translocation in a male of the composition sr <u>a</u> inhibits the male-producing mechanism so that normal offspring are produced. It follows that the Y-4A translocation must have the <u>a</u> gene on the translocated element B.

This unexpected effect of the Y-chromosome makes necessary a reexamination of the data for the possible role of the Y-chromosome as the modifier of the sr X-chromosome.

The hypothesis that a certain type of Y-chromosome present in a sr male, converts the action of the sr

X-chromosome from "sex ratio" into MSR can immediately be eliminated. Outcrosses of the inbred MSR strain was generally carried out by the use of females which should ordinarily not carry the Y-chromosome of the MSR strain. The appearance of MSR males in the F_2 rather than the F_1 of those matings in which females of the inbred strain were outcrossed is not the expectation if the Y-chromosome is in fact involved.

An alternative possibility is that the genetic constitution of MSR males includes either two Y's, or no Y. Preliminary cytological investigations on the spermatocytes of MSR males show that one, and only one, Y is present. (Several cases in which no Y was visible will be explained below.) The strongest argument, against the Y-chromosome as a determining factor, however, is the apparent association of the hypothetical gene a with the element B.

The Characteristics of the Progeny of MSR Males

During the course of this work, a total of 95 fertile cultures involving MSR males has yielded an average of 25.5 individuals per culture. Females appeared in 10 of the cultures with an average of 3.2 per culture that produced females. This gives a total sex ratio of 76.8 males per female.

The male offspring are morphologically normal. They carry the X-chromosome of the mother (w/h st ♀♀ x MSR ♂♂ ⟶ w ♂♂ and h st ♂♂). A cytological analysis of spermatogonial metaphases of three progeny of MSR males crossed to females from the same inbred line revealed that the F_1 males either may (one case) or may not (two cases) receive the Y-chromosome of the father. This observation is in agreement with the fact that both w and h st males produced from such crosses tend to be sterile more often than fertile (in one series, 22 fertile males carrying "standard" or "inverted" sequences, to 38 sterile ones). The ventral receptacles of the females from 4 such sterile cultures were examined and proved to be devoid of sperm.

The occasional female offspring of a MSR male have one X-chromosome from each parent (w ♀ x MSR ♂ ⟶ mostly w ♂, rare w/sr ♀). Whether or not they also may have the Y of the male parent has not been established. Such females are normal, with regard both to appearance and to fertility.

The Nature of the Action of the MSR Complex

When females mated to MSR males are dissected and the ventral receptacle is examined, copious quantities of sperm are generally (7 cases out of 8) found therein, although such females have usually produced no or very few offspring. The sperm, then, must have a lethal effect upon the zygote.

The recessive nature of the factor a indicates that this potential lethal effect originates during spermatogenesis rather than at the time of fertilization. This time of origin is also suggested by the fact that the MSR complex has as its basis the sr X-chromosome, which exerts its influence during spermatogenesis also.

The relationship between MSR and "sex ratio" is by no means clear. The translocation studies indicate that a fragmented Y-chromosome inhibits the action of MSR, leading to a normal sex ratio. The Y-chromosome, then, must be involved in some way, perhaps mechanically, in the MSR effect. The failure of Y translocations to suppress "sex ratio," as shown both by the experiments described above and by routine observations of translocation stocks, indicate the absence of any very obvious Y-chromosome effect.

If these Y-chromosome relations are true, MSR can be neither an incomplete nor an enhanced expression of "sex ratio." If the former were the case, then the Y translocations suppressing MSR should also suppress "sex ratio." Under the latter possibility, Y translocations, in preventing the MSR complex from exerting its effect, should convert MSR males into "sex ratio" males rather than into normal ones. The cytology of MSR males should throw light on this problem.

Geographical Distribution of MSR and the Gene *a*

A systematic analysis, for the sr X-chromosome and for the factor *a*, of strains from various localities has not been carried out. From the results of some of the experimental cultures, however, it can be deduced that these factors must have been present in certain strains.

The sr X-chromosome has been found in strains from Woods Hole, Massachusetts; Coffeyville, Kansas; Gatlinburg, Tennessee (Sturtevant, 1940); it has also been found the stock of pink-like (origin, Woods Hole, Massachusetts) and in the stock of cinnabar (origin, Austin, Texas). The factor *a* can be traced in various crosses to the stocks of pink-like, cinnabar, h st (origin ambiguous, including strains from both Gatlinburg, Tennessee and Woods Hole, Massachusetts),and also in wild type strains from Plano, Texas and from Woods Hole, Massachusetts.

These points of distribution suggest that both factors are present over the entire range of affinis.

Discussion

The behavior of populations of those species of Drosophila having factors altering the sex ratio is difficult to understand.

From the evidence obtained from laboratory cultures, "sex ratio" should automatically increase in frequency each

generation and it can be inferred that wild populations in which this type of chromosome is found should soon become almost completely homozygous for it (Gershenson, 1928). Such a population, the few males in which would produce all or almost all female offspring, would remain in constant danger of extinction. Sturtevant (unpublished) has suggested that the elimination of subpopulations by a deficiency of males may hold the total frequency of the sr X-chromosome at a low level.

Since half the autosomal genes in a population must come from males, those genes which, when present in the male sex, induce the production of more than the average number of male offspring automatically increase in frequency, for the increased number of male offspring will hold a greater than average number of chromosomes carrying those genes. Consequently, any genes which would tend to eliminate sr X-chromosomes in a population, or to suppress the action of such chromosomes would be selected for rather rapidly.

Actually the selection pressure for the factor a can hardly be very strong, for just those males which would serve as the agents of a frequency increase are largely sterilized by the lack of a Y-chromosome.

Without a pronounced positive selective pressure or even with a slight negative selective pressure for such a

gene, however, it may be possible for it to increase in frequency. The subpopulations of a distribution will vary on either side of the mean frequency of a given gene. Those subpopulations having a higher frequency of the gene, by chance, are more likely to survive than those with a lower frequency. Such selection, acting over a long period of time, can raise the frequency of a gene to an appreciable level.

In conclusion, it seems likely that one of the selective mechanisms described above, or possibly a somewhat similar unknown one, is responsible for the wide distribution of the *a* gene.

Reprinted from Genetics 26: 517-541, September 1941

THE HOMOLOGIES OF THE CHROMOSOME ELEMENTS IN THE GENUS DROSOPHILA

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Received April 13, 1941

INTRODUCTION

IT IS becoming increasingly clear that certain elements in the chromosome complexes of the Drosophilas maintain their identity from species to species, though undergoing various changes in their relations to each other and in their internal structure (DONALD 1936; STURTEVANT and TAN 1937; STURTEVANT 1938a, 1940). The terminology for these various elements, however, is far from uniform, since several conflicting systems of lettering and numbering are in use. The most satisfactory general solution of the problem of nomenclature seems to be that suggested by MULLER (1940). According to this system the recognizable elements are lettered, in the sequence familiar in *melanogaster*. The X of that species becomes A; IIL, B; IIR, C; IIIL, D; IIIR, E; IV, F. It is not expected that this system will replace those established by long usage, as in *melanogaster*, but it is strongly recommended that it be applied in those cases where the change might more easily be made. Furthermore, arbitrary systems of numbering or lettering used to facilitate studies on the genetics and cytology of unexplored species should be selected with full appreciation of the ambiguities that may arise when the homologies of the various elements become known.

In those cases where species are so closely related that their salivary gland chromosomes show obvious similarities, or that their hybrids can be obtained, the task of homologizing the elements is greatly simplified; for all other species it is necessary to resort to a comparison of their mutant genes with those of species whose elements have been determined. (The term "element" seems the most appropriate one for this unit since chromosome, arm, and limb have all been used with other connotations.) Since only a fraction of the mutants can be regarded as "good" parallels, comparisons must be made with caution; on the other hand, the tendency to minimize otherwise good homologies which do not appear to be consistent with the scheme must be watched carefully.

It is our purpose in the present paper to extend these homologies as far as now seems possible, and to examine critically evidence contrary to the working hypothesis that the elements have remained essentially intact. The species to be so examined include, among others, *affinis, algonquin, ananassae, azteca, busckii, funebris, hydei, miranda, montium, pseudoobscura, simulans, virilis*, and *willistoni*.

TABLE 1

Compositions of the chromosomes in terms of the elements.

SPECIES	A	B	C	D	E	F	AUTHORITY
melanogaster	X	IIL	IIR	IIIL	IIIR	IV	
affinis	XL	IV	III	XR	II	V	STURTEVANT 1940
algonquin	XS	C	A	XL	B	D	MILLER 1939
ananassae	X part IV part	IIIR	IIIL	IIR	IIL	IV	KIKKAWA 1938
azteca	XS	C	A	XL	B	D	DOBZHANSKY and SOCOLOV 1939
miranda	XL	IV	X2	XR	II	V	DOBZHANSKY and TAN 1936
pseudoobscura	XL	IV	III	XR	II	V	LANCEFIELD 1922
simulans	X	IIL	IIR	IIIL	IIIR	IV	STURTEVANT 1922
virilis	I	VI	V	III	II	IV	METZ, MOSES and MASON 1923
virilis	V	II	IV	I	III		HEITZ 1934
virilis	X	IV	V	III	II	VI	CHINO 1936
virilis	X	D	E	C	B	M	HUGHES 1939

THE AFFINIS GROUP

The metaphase chromosome configurations of females in this group (*affinis*, *algonquin*, *athabasca*, *azteca*) contain a large pair of V-shaped X chromosomes, two pairs of V-shaped autosomes, a pair of rods, and a pair of microchromosomes (METZ 1916). The original account (DOBZHANSKY in STURTEVANT and DOBZHANSKY 1936) of the salivary gland chromosomes in the members of the *affinis* group has been confirmed and extended by MILLER (1939). The observations of DOBZHANSKY and SOCOLOV (1939) on *azteca* failed to include the longer salivary gland chromosome arm of their "C" chromosome, a circumstance that invalidates their conclusion that "Some genes found in *D. azteca* in a single linkage group must belong to different ones in *D. pseudoobscura*; conversely, some genes linked in *D. pseudoobscura* may be expected to be independent in *D. azteca*."

The salivary gland chromosomes of the different members of the *affinis* group are sufficiently similar so that one familiar with any one of these species can recognize the homologous elements in any other. [Since there is considerable variation in gene sequence within each species, the salivary gland chromosomes of interspecific hybrids from strains taken at random tend to possess quite remotely related chromosomes. These need not be interpreted as interspecific differences, as they have been (BAUER and DOBZHANSKY 1937; DOBZHANSKY 1937), for it is possible that identical or simply related sequences exist within each species.] The homology of the *affinis* elements being known through a comparison of mutant types (STURTEVANT 1940), it was necessary only to correlate the genetic linkage groups with the salivary gland chromosomes of this species. XL of *pseudoobscura* agrees cytologically with the short arm of the X in *affinis* in having

an interstitial heterochromatic region near the base and identical banding at the tip. It follows that the longer arms of the X chromosomes of the two species, containing the same genetic material, are homologous. For the identification of the genetic autosomes with the arbitrarily lettered salivary chromosomes of *azteca* (DOBZHANSKY and SOCOLOV 1939) and of *algonquin* (MILLER 1939), a study was made of nine X-ray induced translocations in *affinis*, with the result shown in table 1. From this correlation it is evident that both elements B and E have median centromeres, yielding the V-shape obvious in two of the metaphase and salivary gland chromosomes.

D. ANANASSAE

The metaphase chromosome configuration of *ananassae* indicates that, while the two large autosomes are not unlike those of *melanogaster*, the centromere of the X chromosome has been shifted from a terminal to a median position and the dot chromosome has been transformed into a small V-shaped chromosome (METZ 1916). The nucleolus-forming region and the bobbed gene, ordinarily related to the sex chromosomes in other species, are associated with the small V-shaped fourth chromosome (KAUFMANN 1937; KIKKAWA 1938). This may have been achieved by a simple exchange of centromeres of the dot and X chromosomes, along with adjacent heterochromatin. If this be the case, the exchange must have occurred prior to the median shift in the centromere of the X; that this sequence is correct is borne out by the cytological observations of KIKKAWA (1936) on *montium*, a relative of *ananassae*, in which the V-shaped fourth chromosome is present, but the X chromosome retains its rod shape. There are other possible explanations, involving an exchange of the Y and fourth chromosome centromeres.

A number of mutants in *ananassae* furnish means for comparing its chromosomes with those of *melanogaster* and with the elements. A more complete discussion of these mutants and their linkage data can, in general, be obtained from the papers of MORIWAKI (1935, 1938) and KIKKAWA (1938). *Melanogaster* mutants with which those of *ananassae* are compared are followed by a symbol showing the limb in which they occur

The X Chromosome

Yellow, scute, white, Notch, cut, singed, miniature, dusky, forked, and Beadex seem good homologies with the same sex-linked mutants of *melanogaster*. The terminology for singed and forked may be reversed, as in *pseudoobscura*, but their value is not thereby impaired, since both occur in the same element. The close linkage of yellow with scute and of miniature with dusky (also found in *melanogaster*, *pseudoobscura*, and *virilis*) adds weight to already good comparisons. Vermilion, ordinarily a good parallel

between the X chromosomes of two species, lacks the confirmation of a feeding or transplantation test.

Chromosome II

Cardinal in the left limb of this chromosome corresponds to cardinal (IIIR) and can be differentiated from its mimics, scarlet and cinnabar, by virtue of its striking change in color with age. Plexate probably represents Delta (IIIR), agreeing both in phenotype and in mutability. Off is much like Bare of *pseudoobscura* (element E), although its dominance, its effect of clipping and removing the bristles, and the occasional viability of the homozygote are not conclusive. Puffed has no homolog in *melanogaster*. but agrees with Puffed in *virilis*, which has small, reddish, and "inflamed" eyes (MORIWAKI 1938). This mutant is located on the second chromosome of *virilis*, which will be shown to correspond to element E. For this reason, we believe that the locus of this gene in *ananassae*, which has not been determined because of its association with an inversion, will prove to be in the left arm.

The right limb of this chromosome has no mutants suitable for homologies, but it may be concluded that it represents the element associated with E in *melanogaster*—that is, D—since elements B and C are both accounted for below.

Chromosome III

Plum, in the left limb of chromosome III, is like Plum (IIR) in *melanogaster* in having a dominant mottled eye color, a recessive lethal effect, and an association with an aberration involving heterochromatin (KIKKAWA 1938). Furthermore, it produces a mottled white eye color in combination with vermilion. This indicates its allelomorphism to brown.

Gap compares with gap (IIR) in weakening the fourth longitudinal vein and in giving the posterior crossvein an oblique direction. This is not conclusive in itself, but serves to confirm the identity of the left limb of the third chromosome of *ananassae* with the right limb of the second chromosome of *melanogaster*.

Only one mutant, plexus, has been found on the right limb of this chromosome. Since we would expect true plexus to occur in the same limb as Plum, we feel that this is in fact net (IIL), one of the most common mutant types in other species.

Chromosome IV

The fourth linkage group contains a dominant mutant, similar to Shaven. This dominant form is not known in *melanogaster*, but is in *simulans* (unpublished data). Haplo-IV individuals correspond quite closely to those of *melanogaster*. The linkage of bobbed with these two has already been discussed.

From the above homologies, it would seem that the nomenclature for the autosomal linkage groups has been reversed with respect to that found in *melanogaster* and, in each case, the significance of left and right as applied to the chromosome arms has also been reversed (table 1).

D. BUSCKII

The metaphase chromosome configuration here is essentially the same as in *melanogaster*, except for the dot chromosomes which are absent as such and may be attached to the X chromosomes as satellites (METZ 1916; KRIVSHENKO 1939). KRIVSHENKO has obtained a considerable number of mutants in this species after treatment with X-radiation. Forked, miniature, Notch, scute, singed, vermilion, white and yellow, all on the X chromosome, may be homologous to the sex-linked mutants in *melanogaster*. Autosomal dominants resulting from X-ray treatment, the most abundant type in KRIVSHENKO's studies, are usually unsuitable for drawing comparisons, partly because of their non-specific effects and partly because of their frequent association with chromosomal aberrations which make linkage studies difficult. One exception in this case is Delta, which occurred seven times, once associated with a IIL–IIIL translocation and once with a IIL–IIR–IIIR translocation, in each case one of the break points occurring in the same region of IIL. This would point to the correspondence of IIL of *busckii* with element E.

D. FUNEBRIS

The mutants in *funebris* available for drawing comparisons are generally unsatisfactory, both because of their indefinite nature and of their unknown linkages, with the exception of a few sex-linked types. Notch corresponds closely to Notch (X) (STURTEVANT 1918). Forked may be either forked (X) or singed (X) (MORGAN, BRIDGES, and STURTEVANT 1925). The similarity of bobbed to bobbed (X, Y) has been pointed out by LUERS (1937). STUBBE and VOGT (1940), by transplantation of eye disks, have shown vermilion to be homologous to vermilion (X). They have also confirmed the identity of autosomal cinnabar with cinnabar (IIR) by the same technique. Radius incompletus bears some resemblances to radius incompletus (IIIL) (TIMOFÉEF-RESSOVSKY 1927) and cubitus incompletus to cubitus interruptus (IV); but any attempt to elaborate these and other possible autosomal homologies seems unprofitable in the absence of linkage data.

D. HYDEI

Notch, white, vermilion, and bobbed in the X chromosome of *hydei* (CLAUSEN 1923; SPENCER 1927) seem probable homologs of the same sex-linked mutants in *melanogaster*. For this species in particular and for many

others there remains unavailable a considerable body of unpublished information, which, as far as we are aware, is not in disharmony with the scheme of permanent elements but which, in a great many cases, furnishes additional evidence in its favor.

D. MIRANDA

The homologies of the salivary chromosomes of *miranda* with those of *pseudoobscura* have been deduced from studies of their hybrids (DOBZHANSKY and TAN 1936). Besides a large number of differences between homologous elements arising, presumably, from the effects of cumulative inversions, DOBZHANSKY and TAN conclude that at least five translocations (involving all the chromosomes) have become established between the two species. In view of MACKNIGHT's criticism (1939) on cytological grounds, WRIGHT's mathematical calculations (1940) for the incorporation of one such translocation (see below) and the absence of conclusive evidence to the contrary, we are forced to conclude that these cases very probably do not represent exceptions to the rule of the indivisibility of the elements.

D. MONTIUM

A number of mutants described by OSIMA (1940) provide means for drawing parallels between the linkage groups of montium and the elements. On the X chromosome, white, Notch, and vermilion compare favorably with the similar sex-linked mutants in other species.

Curled-b (left limb of II) simulates curled (IIIR) by curling the wings up and raising and crossing the posterior scutellar bristles. Confluent (left limb of II), obtained twice after X-ray treatment, would correspond to Delta (IIIR), one of the most frequent of mutants found after raying. Hairless, also on chromosome III, agrees with Hairless (IIIR) in its bristle and wing vein diminishing effect, in its dominance and in its lethality when homozygous. This mutant has not yet been allocated to a definite limb; it can be suggested here that it will prove to be on the left limb.

Curled (left limb of III) and jaunty (IIL) seem good parallels; plexus (right limb) would consequently suggest plexus (IIR) rather than net (IIL) since it falls on the anticipated element. On this basis, Plexate, located in the middle of chromosome III, would correspond to Plexate (IIR) and would be expected to occur on the right limb along with plexus. The centromere, therefore, might be expected to fall to the left of Plexate.

The metaphase chromosomes, reported by KIKKAWA and PENG (1938), suggest that this species may be like *ananassae*, but with a terminal instead of a median centromere in the X. The genetic data do not help in checking this supposition.

D. PSEUDOOBSCURA

The metaphase chromosomes of a *pseudoobscura* female consist of a pair of V-shaped X chromosomes, three pairs of autosomal rods, and a pair of dot chromosomes. LANCEFIELD (1922) suggested that a portion of the V-shaped X chromosome of *pseudoobscura* might exist in other species as autosomal material. CREW and LAMY (1935) pointed out, among other comparisons, that certain mutants in the right arm of the X chromosome could be homologized with similar mutants in IIIL of *melanogaster*, although this was not immediately obvious from this paper because of the authors' peculiar terminology. The argument that the elements have remained essentially intact in *pseudoobscura* as compared with *melanogaster* was applied by DONALD (1936) who studied more mutants and implied the necessary corrections to the work of CREW and LAMY. STURTEVANT and TAN (1937) confirmed and extended the main conclusions, leaving no doubt of the identity of the elements in the two species (see table 1). Two additions (STURTEVANT and TAN, unpublished) may be made to the information published in this latter paper: The order of the loci on the X chromosome in the neighborhood of 72 has been shown to be forked, dusky, and bobbed, not dusky, forked, and bobbed as surmised before; also the distance between compressed and sepia is 30 units rather than the indicated 7, thereby increasing all loci from sepia to the right end inclusive by about 25 units.

TAN (1935) correlated the salivary gland chromosomes with the genetic linkage groups in this species.

D. SIMULANS

Since *simulans* hybridizes with *melanogaster*, suspected homologies can be tested directly by mating the corresponding mutants. Thus ordinarily insecure comparisons become unquestionable. The genetic evidence (STURTEVANT 1921a, 1921b, 1929) indicates a close agreement between the limbs of the *simulans* chromosomes and those of *melanogaster* (table 1). The hybrid salivary gland chromosome analyses by PÄTAU (1935) and by KERKIS (1936) confirmed the homologies of the chromosomes and verified the existence of an inverted sequence of genes in the third chromosome, the first inversion to be found in Drosophila (STURTEVANT 1921c). The more detailed examination by HORTON (1939) revealed that as many as 23 additional intra-chromosomal differences, all very small, may exist between the two species.

Six unpublished corresponding mutant types of *simulans* may be noted here: scute (X, 0), ocelliless (X, 24), javelin (III, 0), radius incompletus (III, 58), recessive hairless (III, 61), and dominant Shaven (IV). The sex-

linked parallel vermilion (X, 31.8) was reported by STURTEVANT (1932), without its locus. All of these have been shown to be allelic to the *melanogaster* types whose names they bear and which they closely resemble.

DROSOPHILA VIRILIS

D. virilis remains the only species in the subgenus Drosophila (STURTEVANT 1939) with a sufficient array of mutants to make possible homologizing all the linkage groups with the elements. The six linkage groups agree with the metaphase configuration of five pairs of rods and one pair of microchromosomes. METZ, MOSES, and MASON (1923) and CHINO (1929, 1936) have drawn many comparisons of *virilis* mutants with those of *melanogaster*. *Melanogaster* mutants are followed by a notation of the chromosome limb in which they occur, all others being *virilis* types. We will attempt to evaluate these and, in some cases, make additional suggestions.

X Chromosome

Convincing parallels have been drawn in the cases of yellow, forked, singed, glazed (METZ, et al.), of crossveinless (WEINSTEIN 1920), of scute, white, Notch, miniature, dusky, rudimentary, Beadex, and bobbed (CHINO) with the identically-named mutants (except lozenge for glazed) in the X chromosome of *melanogaster*. HOWLAND, GLANCY, and SONNENBLICK (1937) have established the identity of vermilion of *virilis* with vermilion (X) by the transplantation of eye disks.

The comparisons of echinus with echinus (X), magenta with ruby (X), vesiculated with vesiculated (X), ragged with cut (X), decline with wavy (X), apricot with garnet (X), and small bristle with tiny (X) (CHINO) are open to doubt, but they do serve as corroborative evidence that the composition of the X chromosome of *virilis* and *melanogaster* is essentially the same.

Chromosome II

Confluent is very probably homologous to Delta (IIIR) (CHINO), although the weaker alleles described by METZ et al. show some discrepancies. Concave and crumpled (IIIR) constitute one of the most striking cases of homology (METZ et al.); the case is strengthened by the occurrence of similar types in element E of *affinis* and *pseudoobscura*. Our examination of varnished indicates that it is a good parallel to glass (IIIR).

It has been suggested that ebony corresponds to ebony (IIIR), brick to claret (IIIR), and broken to crossveinless-b (IIIR) or to crossveinless-c (IIIR) (CHINO). These are not diagnostic mutants and may be questioned, although they do fall in the proper limb.

Radius incompletus (IIIL) has been compared to both incomplete and

detached of virilis (CHINO). From the published figures, it would seem that incomplete is quite like radius incompletus (IIIL), but this homology loses its force since it is not in the expected element. A similar situation is found in the case of lanceolate (see below).

Thus chromosome II of *virilis* corresponds to IIIR of *melanogaster*, or element E.

Chromosome III

Short veins suggest veinlet (IIIL) (CHINO) but the comparisons of rose with rose (IIIL), spread with dihedral (IIIL), and rolled with rolled (IIIL) (CHINO) are less convincing.

The striking resemblance of hunch to ascute (IIIL) has been pointed out (METZ et al.) but was discarded as a possible homology because a similar mutant was sex-linked in *D. pseudoobscura*. Since it is now known that the limb of the X chromosome of *pseudoobscura* in which this mutant is located corresponds to IIIL of *melanogaster* (DONALD 1936), this can be considered a convincing parallel. Telescoped has no homolog in *melanogaster*, but agrees closely with deformed and its allele serrate in *willistoni* (LANCEFIELD and METZ 1922) and to compressed in *pseudoobscura* where it is located in element D (STURTEVANT and TAN 1937). Garnet, like Henna (IIIL), is a dominant dark eye color; the only other possible homology would be Plum (IIR), which may be eliminated because of its mottling effect and allelism to brown (see eosinoid below).

The evidence favors the correspondence of *virilis* chromosome III with *melanogaster* IIIL or element D. On this basis, it may be safely predicted that cinnabar, after appropriate tests, will prove to be scarlet (IIIL) and not cinnabar (IIR).

Chromosome IV

Dachsous and dachsous (IIL), Star and Star (IIL) and reduced and reduced (IIL) seem good comparisons (CHINO). Clipped probably represents one of the truncate alleles of dumpy (IIL) (CHINO).

However, CHINO's identification of rough-4a with roughish (IIL), black with black (IIL), Squat with Squat (IIL) and flipper with pupal (IIL) are less convincing. Since the suggested homologs lie in the proper arm, they do tend to confirm the correspondence of these limbs. His figure of veinlet indicates that it is not veinlet (IIIL), as he suggests (see short vein, above)

Plexus might correspond to either net (IIL) or plexus (IIR). We would favor the first alternative since it falls in the proper limb. Lanceolate has been compared to lanceolate (IIR). This is one case where a mutant suitable for homologizing does not have an equally good or better comparison in the anticipated element. The force of this discrepancy is diminished by DONALD's observation (1936) that a similar mutant is found in element D

in *pseudoobscura*, in addition to that found later in element C (STURTEVANT and TAN 1937). Also, KIKKAWA (1938) lists two such mutants, lance and lanceolate, one on the second, the other on the third chromosome of *ananassae*.

The bulk of the evidence favors the view that the third chromosome is element B.

Chromosome V

Eosinoid agrees with brown (IIR) in giving a white eye color in combination with scarlet or cinnabar (MORI 1937). Vestigial and vestigial (IIR) and straw and straw (IIR) seem excellent homologies (CHINO). Comparisons of ruffled with intwined (IIIL), fat with fat (IIL), dachsoid with four-jointed (IIL), mahogany with clot (IIL) and with sepia (IIIL), Beaded with Beaded (IIIR) and morula with morula (IIR) (CHINO) are questionable. Beaded may be compared with Jagged (C) of *pseudoobscura*.

Branched might be either net (IIL) or plexus (IIR); the latter seems more likely on the basis of the other homologs. For the same reason, we believe that scarlet corresponds to cinnabar (IIR) and not to scarlet (IIIR) (see cinnabar above).

Chromosome VI

The comparison of abdomen rotatum with abdomen rotatum (IV) (CHINO) is convincing; Gap, which shortens the fifth longitudinal vein, may be questioned as a homolog to cubitus interruptus. To these may be added the parallel of stubby with shaven (IV). Thus the dot chromosomes of both species are apparently the same.

As far as the above data can show, the elements have remained essentially intact. We are therefore reluctant to accept in its entirety CHINO's conclusion that "it seems to be justified to assume that in the evolutional course of Drosophila there occurred many inversions, mutual translocations, and attachments or fragmentations of all chromosomes."

FUJII (1936), by means of four translocations and an inversion, has correlated the salivary gland chromosomes with the linkage groups, as indicated in table 1.

SPENCER (1940) has described a native American subspecies, *virilis americana*, in which elements D and E are united to form a V; elements A and B are also fused, the additional B necessary to the diploid complement of the male being present as a single rod (HUGHES 1939; STALKER 1940; PATTERSON, STONE, and GRIFFEN 1940). In another subspecies, *virilis texana*, elements B and D have a single centromere (PATTERSON, STONE and GRIFFEN 1940). In each case the remaining elements are present as rods.

D. WILLISTONI

The X chromosome of this species is V-shaped, as in *pseudoobscura*, while the autosomes are comprised of one V and one rod (LANCEFIELD and METZ 1921). From the metaphase chromosome length relationships, it is clear that one of the elements that is autosomal in most species is part of the X in *willistoni*. The homologies of the sex-linked mutants here are consequently of considerable interest.

Yellow has been compared to yellow (X), scute to scute (X), vermilion to vermilion (X), stubby to forked (X), triple to bifid (X), forked to singed (X) (LANCEFIELD and METZ 1922; FERRY, LANCEFIELD, and METZ 1923). In addition, square agrees with rudimentary (X); unpublished data show that a newly arisen vermilion (locus in X unknown) lacks the v^+ substance, as determined by the feeding technique. Deformed and its allele serrate correspond quite closely to telescope of *virilis* (element D) and to compressed of *pseudoobscura* (element D) (STURTEVANT and TAN 1937); stump compares favorably with radius incompletus (IIL), and short and veinlet (IIIL) seem good parallels.

The evidence thus indicates that elements A and D have fused to form the X in *willistoni*, as in *pseudoobscura*. This conclusion is strengthened by the distribution of the mutants homologized: all those paralleling element A mutants are found on one half of the X (34.5 to 84) while all those paralleling element D mutants are on the other half (0 to 34). The genetic locus of the centromere on this basis would be between 34 and 34.5. Low interference values for this region, as deduced from the crossing over data of LANCEFIELD and METZ (1922) support this hypothesis.

The autosomal mutants do not yield a satisfactory account of the composition of the V and rod-shaped autosomes. Balloon (chromosome II) has been homologized with balloon (IIR) (FERRY, LANCEFIELD, and METZ 1923). Apterous (chromosome II) agrees well with apterous (IIR); both remove the wings, reduce the balancers, diminish the number of posterior scutellar bristles, deform the thorax, and sterilize both sexes. Clipped and Scalloped, both on chromosome II, probably represent the dominant vestigial deficiencies (IIR). Approximated and dachs (IIL) are close parallels. These homologies indicate that the second chromosome of *willistoni* includes elements B and C, as in *melanogaster*. The location of Knot, which bears some resemblances to Delta (IIIR), on the second chromosome casts doubt on this conclusion. In either case, however, chromosome II must be the autosomal V; this is supported by the slightly greater number of mutants in II than in III (11 in II as compared to 8 in III).

Neither the genetic nor the cytological evidence gives a clue as to the location of element F in *willistoni*, but it is to be noted that the salivary

gland chromosomes have not yet been studied in this species. The most likely place to look for F would appear to be as a short arm on the apparently rod-shaped chromosome (III, element E?).

HOMOLOGOUS GENES IN THE VARIOUS SPECIES

Table 6 lists the homologous genes in the species discussed, to which some previously unpublished cases have been added. Unpublished data of other workers, available particularly in Drosophila Information Service, tend to strengthen the suggested homologies given in the table.

The occurrence of "sex-ratio" in *melanica*, a member of the subgenus Drosophila, seems worthy of special mention. It has been previously noted only in the subgenus Sophophora (*obscura*, *pseudoobscura*, *affinis*, *athabasca*, and *azteca*) and in those cases analyzed, is located in element D, which is part of the X chromosome. It seems likely, then, that the X of *melanica* is a V, one arm of which is element D. The cytological observations of METZ (1916) indicate that a V-shaped chromosome of the required size is present, although no identification of the X has been made.

LENGTHS OF THE ELEMENTS IN THE SALIVARY GLAND NUCLEI

The percentage each element comprises of the total euchromatic length of the salivary gland chromosomes is listed in table 2. These values have been derived from the published lengths for *ananassae*, *melanogaster*, and *virilis*, from measurements of the published drawings for *algonquin* and *azteca*, and from a combination of two sets of drawings plus our estimate for element D for *pseudoobscura*. The figure given for element B of *azteca* has been obtained by assuming that it bears the same ratio to the other autosomal elements as do the homologous elements in *algonquin*.

TABLE 2

Relative lengths of the elements in the salivary gland nuclei.

	A	B	C	D	E	AUTHORITY
algonquin	16.4	18.5	19.7	20.0	25.4	MILLER 1939
ananassae	19.3	19.1	18.0	18.4	25.1	KIKKAWA 1938
azteca	15.1	18.8	19.0	21.4	25.8	DOBZHANSKY and SOCOLOV 1939
melanogaster	18.9	18.5	21.0	18.0	23.6	BRIDGES 1935
pseudoobscura	14.0	19.9	16.3	24.0	25.8	TAN 1936; DOBZHANSKY and TAN 1939
virilis	19.3	19.3	19.5	18.3	23.5	FUJII 1936
virilis	19.3	20.1	19.0	18.2	23.4	HUGHES 1939

Since these lengths are a function of both the elasticity of the chromosomes and the personal equation of the observer, the comparative lengths of the homologous elements are quite variable, with one striking exception: in every case element E is considerably longer than any of the others. This

identification of the longest salivary gland element with element E may be of practical value in those instances where the correlation may be impracticable for other reasons. SLIZYNSKA and SLIZYNSKI (1941) report that in *funebris*, one of the elements is considerably longer than any of the others; it therefore probably corresponds to element E.

It is also to be noted that element D is about the same length as A when it is autosomal, but is distinctly longer in the three species in which it is part of the X. It seems clear from the table that the lengths of element A and D tend to show a negative correlation with each other. Numerous speculations are suggested by these relations, but they can scarcely be profitably discussed without more information than is now available.

SEQUENCE OF CORRESPONDING GENES IN DIFFERENT SPECIES

In general there is little similarity in the sequence of corresponding loci within each element, except when such closely related species as *melanogaster* and *simulans* are compared. In discussing *melanogaster* and *pseudoobscura*, STURTEVANT and TAN (1937) state: "The mathematical properties of series of letters subjected to the operation of successive inversions do not appear to have been worked out, so that we are so far unable to present a detailed analysis. It does appear, however, that the five arms (taken together) are definitely more alike in the two species than could result from chance alone." These statements now require some modification.

With the help of PROF. MORGAN WARD, a beginning has been made in the study of the mathematical consequences of successive inversions. Complete catalogs have been prepared, showing all the possible different arrangements of 2, 3, 4, 5, and 6 loci, respectively, together with the minimum number of successive inversions required to change each arrangement into a single arbitrarily chosen one. Actually, numbers were used, and the required arbitrary sequence was the ordinal one (1, 2, 3, 4, etc.). Table 3 shows the mean number of inversions required, together with the standard deviations for the respective populations.

TABLE 3

Mean number of inversions required to transform random arrangements of numbers into ordinal series.

NUMBER OF LOCI	MEAN	STANDARD DEVIATION
1	0	0
2	.500	.50
3	1.167	.69
4	1.750	.66
5	2.392	.70
6	3.036	.71
8	4.367 ± .092	.71 ± .06
9	4.975 + .13	.82 ± .09

In the cases where more than six loci were involved, it became impracticable to make complete catalogs. Accordingly, for the two rows shown (eight and nine loci), random series of numbers (60 and 40 sequences, respectively) were chosen, and for each such sequence there was determined the minimum number of successive inversions required to reduce it to the ordinal sequence chosen as "standard." For numbers of loci above nine the determination of this minimum number proved too laborious, and too uncertain, to be carried out.

The table as it stands, however, gives a solution that seems safe to use for any numbers of corresponding loci likely to be encountered in such studies as these, since a plot of the values shows that there is an approximately linear relation between the number of loci considered and the average number of inversions required to reduce to ordinal sequence. For each additional locus considered, there is an increase of about .64 in the average number of inversions required. The curve is more irregular near its point of origin; and the values are exact up to six loci. Accordingly, for values above six it seems safest to start with the value 3.036 (for 6) and add to it $.64(n-6)$, where n is the number of loci concerned.

The standard deviations shown in the table give a less regular curve when plotted, but it is clear that they are increasing only slowly. It may probably be assumed that σ will not be greater than 1 for values up to $n = 15$—beyond which it is unlikely that the present problem will require a solution for many years. This means that the spread is not great—in other words, that any random sequence is unlikely to require a number of inversions much different from the calculated one. If a number much less is in fact encountered, it may be concluded that there is a significant degree of resemblance. This method has been applied to the sequence differences between *melanogaster* and *pseudoobscura*, with the result shown in table 4. Evidently the two species are not more alike than could easily result from chance alone.

TABLE 4

Comparison of the required and calculated numbers of inversions to change the melanogaster into the pseudoobscura sequences.

ELEMENT	A	B	C	D	E	TOTAL
Loci	13	6	6	6	7	
Inversions required	7	2	4	3	3	19
Inversions calculated	7.6	3.0	3.0	3.0	3.7	20.3

In any series of successive loci, each locus has two neighbors, and each of these may be either "right" or "wrong"—that is, may or may not be one that lies adjacent to the given locus in the arbitrarily chosen standard

ordinal sequence. For a terminal locus there is only one adjacent locus, but the terminal position itself may be taken as constituting a "connection" and then may be treated in the same way, a "right connection" here meaning that the terminal locus is not only terminal in the chosen standard sequence but also lies at the same end (proximal or distal). There are then n + 1 "connections" in any sequence of n loci. It may be shown that in any series of random arrangements the average number of "right" connections is two, regardless of the value of n. It is also evident that any single inversion changes two and only two connections, since it has two ends, each of which must fall in a connection. These two relations are very helpful in working out the consequences of successive inversions. The conception of "right connections," however, is responsible for the incorrect conclusion drawn by STURTEVANT and TAN. One of the right connections appearing in their comparison has now disappeared, as a result of the revision of the *pseudoobscura* sequence recorded above (it may be noted that this revision does not change the number of inversions required to transform one sequence into the other). The result now is that there are 13 "right" connections (that is, identical in the two species) where ten are expected (two in each of the five elements) on chance alone. The difference is probably not a significant one.

This analysis does not take into account the distances concerned—a connection may be assumed to be like in two elements regardless of the amount of crossing over shown. Thus, in element B, jaunty and hook are adjacent in the series of corresponding genes both in *melanogaster* and in *pseudoobscura;* but the crossover values are 5.2 and 23.1, respectively. In such a case, it is probable that further corresponding loci will show that the sequence resemblance is an accidental one. For certain pairs of loci that lie quite close together, the situation is different, as may now be shown.

Yellow and scute are quite near each other in *melanogaster*, *simulans*, *pseudoobscura*, *virilis*, *ananassae*, and *willistoni;* they are separated by other loci in *affinis*. (Unpublished data of DR. W. P. SPENCER indicate that they are also separated in *hydei*.) Notch and white are from one to four units apart in *melanogaster*, *simulans* (Notch is not known here, but facet serves to identify its locus), *ananassae*, *montium*, *hydei*, *virilis*, and *pseudoobscura*. Miniature and dusky give few if any crossovers in *melanogaster*, *ananassae*, *pseudoobscura*, and *virilis*. It may be noted that these are all short distances, not only in terms of crossing over, but also in terms of the salivary gland chromosome maps of *melanogaster*, which is the only species for which element A is adequately known in the salivary glands.

It may be concluded that inversions with end-points falling within these short sections have not become established during the differentiation of

these species, except for those between yellow and scute in *affinis* and in *hydei*. It may be observed that all three sections appear to exist intact in both of the subgenera studied, since *virilis* and *hydei* belong to Drosophila while all the other forms named are members of the subgenus Sophophora. Evidently, then, these sections represent associations of loci that have been in their present conditions for a very long period of time.

DISCUSSION

The essential argument showing the improbability of the incorporation of a translocation into a population has been published a number of times and has been prevalent in genetic thought since an earlier date; nevertheless, it seems opportune to recapitulate it briefly here, with specific reference to the Drosophilas.

Translocations have been observed to occur spontaneously under laboratory conditions (all the Drosophila translocations discovered prior to the advent of the X-ray technique fall in this category) and, in the few cases mentioned below, have been found in natural populations. It seems not unreasonable to assume therefore that they continually arise in nature with some very low frequency. Once having arisen, such a translocation would exist in the heterozygous state and thereafter chiefly in the heterozygous rather than homozygous state. The extensive studies on such rearrangements in Drosophila have amply demonstrated the selective disadvantage which their heterozygotes must suffer as a consequence of their production of unbalanced gametes. Thus a certain number of translocated chromosomes along with a number of normal chromosomes is lost in every generation; the percentage loss of the former may be considerable because of its low total frequency, whereas the percentage loss of the latter is usually insignificant. In this way selection discriminates against the less prevalent arrangement, progressively decreasing its frequency until it is wiped out.

It follows that the sole opportunity for a translocation to become established lies in its attaining a percentage frequency sufficiently great so that the discrimination of selection is not adequately expressed before chance changes the ratio of the frequencies in favor of the newer arrangement, whereupon the original is eliminated. A high percentage frequency can be reached only when the total number of chromosomes is low—that is, when the population size (N) is small. Furthermore, the population size must be small for chance to upset the ratio of the two arrangements in favor of the newer type.

For reciprocal translocations in which only the completely balanced types survive, WRIGHT (1940) has shown that fixation is difficult unless N is very low. Certain types of translocations might be expected not to

encounter such drastic adverse selection, by virtue of the viability and fertility of individuals possessing an unbalanced (that is, heterozygous for a duplication or a deficiency or both) complement of chromosomes (STURTEVANT 1938b). The effect of this condition is to decrease the selection against the new arrangement and so to increase the maximum population size into which it might be incorporated. PROFESSOR WRIGHT has given us his kind permission to present here some more exact probabilities which he has calculated as an extension to his previous statements (WRIGHT 1940). For a translocation whose unbalanced products are completely inviable, the probabilities of fixation are of the order of 10^{-3} if $N=10$, 2×10^{-6} if $N=20$, and 3×10^{-14} if $N=50$. In the most favorable case, when the heterozygous unbalanced products are both viable and fertile (only the homozygous deficiencies being eliminated), the probabilities are roughly 3×10^{-3} in populations of 20, 4×10^{-6} in populations of 50, and 3×10^{-10} in populations of 100. For the case of a small insertional translocation whose homozygous duplication product is not always accompanied by a homozygous deficiency and consequently may be viable and fertile, the probabilities must be somewhat greater than those given for the last case. Nevertheless, they are still very small and cannot greatly increase the maximum values indicated for N in the last case.

The various factors involved in the incorporation of a translocation into a population may be summarized as follows:

1. The translocation must occur in at least one of the individuals of a subpopulation. As N becomes smaller, the probability of this event becomes smaller, the relation being an approximately linear one.

2. The translocation must become incorporated into the whole subpopulation. N must be very small (see figures above); as N increases, the probability decreases rapidly.

3. The subpopulation must not become extinct. As the value of N increases, the probability that the subpopulation will survive increases.

4. The translocation must maintain itself in the descendants of the subpopulation, in competition with normal chromosomes that may be introduced by interbreeding with other subpopulations. As N increases, this probability also increases, since each introduced chromosome has a smaller effect on the composition of the subpopulation.

The third factor might be omitted, since it could be treated merely as decreasing the total number of subpopulations concerned. It is included here to emphasize the point that, if there is variability in the size of the subpopulations, the smaller ones are more likely to become extinct than the larger ones.

The final probability of the establishment of a translocation is the product of the four probabilities just enumerated. The first, third, and

fourth components increase with an increase in N, the second decreases very rapidly with an increase in N. The result is that the product of the four must always be very small. The only situation in which the establishment of a translocation seems likely to occur with any considerable frequency—even if a long period of time be assumed—is that in which hermaphroditic individuals frequently self-fertilize—that is, when there are many subpopulations in which $N=1$. It would thus seem desirable to have suspected cases of translocation in Drosophila supported by more conclusive evidence than has been considered necessary in the past.

For a translocation to be observed in a natural population, it must be found in the relatively short interval between its origin and its elimination. It seems significant that, despite the extensive genetic and cytological analyses of natural populations of Drosophila, not one translocation has been reported.

In the grasshopper Trimerotropis, CAROTHERS (1931) found a single individual heterozygous for a translocation among fifteen specimens from one locality. A more substantial case from the population standpoint is that reported by WHITE (1940). In 1934 he found a specimen of the grasshopper Metrioptera heterozygous for a translocation and in 1937 recognized the same translocation in some more individuals from the same locality. We are not familiar with the genetic properties of this translocation, with the statistical data for this case, or with the population mechanics of this genus; it therefore seems unwise to discuss the situation further.

Among hermaphroditic plants there are many instances of translocations that have become established. Here, however, the population size may frequently reach the theoretical minimum value of one and remain essentially that for several generations.

A class of "neutral" translocations which might be expected to give non-random segregation in the heterozygote includes those found in *virilis americana* and *virilis texana*, where two elements, ordinarily separate, have fused near the centromere. Such a fusion would seem likely to result, in the heterozygote, in almost completely regular disjunction of the individual rods from their attached homologs. If, in this particular case, there is some slight irregularity in disjunction, it would seem to be more than compensated for by the small sizes of the populations in which these two subspecies are found (PATTERSON, STONE, and GRIFFEN 1940).

RHOADES (1940) has demonstrated the instability inherent in a telocentric chromosome in maize; both the X and fourth chromosomes of *melanogaster*, once considered to have terminal centromeres, have been shown to have two arms (KAUFMANN 1934; GRIFFEN and STONE 1939, 1940). The view of NAWASCHIN (1916) and LEWITSKY (1931) that telo-

centric chromosomes do not exist is considerably strengthened. On this basis, apparent rods in Drosophila have a minute heterochromatic second arm. This arm would then serve as an anchor on which another element, less its centromere, could become attached by translocation, forming a V.

The reverse change, the formation of two rods from a V, demands an additional centromere. STURTEVANT and TAN (1937) suggest that "perhaps the spindle attachment of the Y chromosome is somehow utilized, since the properties of this chromosome allow it to be present as an extra or a fragment without damage to the organism." Means are thus provided for increasing the chromosome number. The one clear instance where such an increase has occurred, in *obscura* Gershenson, involves the breakage of a V-shaped element into two rods.

From the genetic data and metaphase chromosome configurations in *melanogaster*, *willistoni*, *immigrans*, *virilis*. and *pseudoobscura*, it is apparent that they all differ in the associations of their elements. Regardless of the metaphase chromosome configuration that may be assumed ancestral to that of all the Drosophilas, both fusion and fragmentation must have taken place. Although not in complete agreement with the systematic relationships, perhaps the simplest interpretation is that the ancestral type corresponded to the *virilis* configuration, five pairs of rods and a pair of dots. With slight modifications, mostly in the distribution of heterochromatin, this is the most common in the subgenus Drosophila. All other types can be derived by simple fusions of elements and changes in the positions of the centromeres within the elements by inversions, with the exception of *obscura* Gershenson and *ananassae*, as noted above. Within *virilis* itself, a single translocation of this kind has evidently given rise to the configuration found in subspecies *texana*; and two translocations from the *texana* arrangement have produced that of subspecies *americana*. The sequences found within the elements are in agreement with this conclusion (PATTERSON, STONE, and GRIFFEN 1940).

It seems clear that, in general, decrease in chromosome number is more easily brought about than is increase. Accordingly lower chromosome numbers may be regarded as probably (though not necessarily in every case) more recent than higher numbers. This conclusion will hold, however, only in forms—such as Drosophila—in which chromosomes frequently have nearly terminal centromeres, with one arm made up of heterochromatin.

A NOTE ON THE RELATIONS BETWEEN THE SPECIES GROUPS OF SOPHOPHORA

It was suggested by STURTEVANT (1940) that the *willistoni* (AD, BC, EF) and *melanogaster* (A, BC, DE, F) chromosome configurations have been derived from the type now found in *pseudoobscura* (AD, B, C, E, F).

If this be so, the first event was probably the union of elements B and C, to give a hypothetical arrangement AD, BC, E, F. From this, a union of E and F presumably gave the *willistoni* type, while a translocation between AD and E gave the *melanogaster* one.

The hypothetical arrangement, which seems likely to have existed even if the sequence of events was different from that outlined above, has not been reported. In the absence of the identification of the X, it would be listed as the *melanogaster* type; accordingly we have been led to examine the descriptions of the phenotypic characters of the few Sophophoras for which such a report exists. One of them, *D. suzukii*, turns out in fact to be intermediate between the *melanogaster* and *obscura* species groups, as shown in table 5. We are inclined to surmise that this species (recorded by KIKKAWA and PENG (1938) from Japan and China) will be found to have the hypothetical configuration.

TABLE 5

Comparison of some phenotypic characters of the melanogaster and obscura species groups and D. suzukii.

	COLOR	COSTAL INDEX	4TH VEIN INDEX	SECOND ORAL	MIDDLE ORBITAL
melanogaster group	yellow	1.2–2.2	2.2–2.7	long	short
suzukii	yellow	4.0*	2.2	long	long
obscura group	black	2.7–3.0	1.7–2.1	short	long

* The description given by KIKKAWA and PENG lists the costal index as 4.0; their illustration (Plate 32) suggests that this is perhaps a misprint for 3.0, which would make *suzukii* fall within the range of variation of the *obscura* species group.

THE EFFECT OF PERICENTRIC INVERSION ON THE ELEMENTS

None of the changes discussed above interferes with the integrity of the elements. There remains, however, one mechanism which may exchange genic material from one element to another. If two elements are united to form a V, an inversion across the centromere (pericentric) will shift genes from one to the other, and *vice versa*. Heterozygotes for this type of inversion are at a selective disadvantage because single crossovers within the inverted section produce inviable duplication-deficiency zygotes, thus leading to a situation similar to that of translocations. However, if crossing over be hampered, either by the nature of the inversion or by the presence of a crossover suppressor, the new arrangement may become established in the population. The products of a subsequent separation of these two limbs will simulate the effects of a reciprocal translocation.

MILLER (1939) has recorded such an inversion in *algonquin*, occurring with a high frequency in eight out of nine localities studied between Quebec, Canada, and Wooster, Ohio. In this case, only one element (E) was

TABLE 6

Summary of the corresponding mutant types in the different species.

ELEMENT A

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
yellow	yellow	yellow	yellow	yellow	yellow	*busckii, willistoni*
scute	scute	scutellar	scute	scute	scute	*busckii, takahashii, willistoni*
prune	prune					
white	white	white	white	white	white	*busckii, hydei, montium*
facet	facet					
Notch		Notch		Notch	Notch	*busckii, funebris, hydei,* [*montium*
echinus		echinus		echinus?		
bifid						*willistoni* (triple)
ruby	ruby			magenta?		
crossveinless	crossveinless			crossveinless		
vesiculated	vesiculated			vesiculated?		
cut	cut	beaded	cut	ragged?	cut	
singed	singed	forked		singed	singed?	*busckii, willistoni* (forked)
ocelliless	ocelliless					
lozenge	lozenge	glazed		glazed, rugose		[*montium? willistoni*
vermilion	vermilion	vermilion	vermilion	vermilion	vermilion?	*busckii? funebris, hydei?*
miniature		miniature	miniature	miniature	miniature	*busckii, takahaskii*
dusky	dusky	dusky		dusky	dusky	*algonquin*
garnet	garnet			apricot?		
rudimentary	rudimentary			rudimentary		*willistoni* (square)
forked	forked	singed		forked	forked?	*busckii, funebris? willistoni*
Beadex		Pointed		Beadex	Beadex	(stubby)
fused	fused					
bobbed	bobbed	bobbed	bobbed	bobbed	on chrom. IV	*hydei, funebris*

ELEMENT B

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
dachsous				dachsous		
net		tangled	net	plexus	plexus	
Star		Rough		Star		
Curly		Curly				
dumpy	Truncate		truncate	Clipped		
dachs						*willistoni* (approximated)
abrupt		incomplete				
black	black			black?		
jaunty		jaunty	jaunty			*montium* (curled)
reduced				reduced		
hook		hook				

ELEMENT C

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
straw	straw		straw	straw		
apterous						*willistoni*
cinnabar		orange	cinnabar	scarlet?		*athabasca, algonquin, funebris*
vestigial	vestigial-nick	Jagged		vestigial		*willistoni* (Clipped, Scalloped)
gap					gap	
curved		curved				
arc	arc					
plexus		plexus		branched?		*montium*
brown		purple		eosinoid		
Plum					Plum	
lanceolate		narrow	narrow			
	polychaete	polychaete, Scute				

ELEMENT D

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
veinlet		short	veinlet	short veins		*willistoni* (short)
javelin	javelin	slender				
Henna				Garnet		
sepia	sepia	sepia				
hairy			hairy			
tilt		tilt	tilt			
scarlet	scarlet	scarlet	scarlet	cinnabar?		
ascute		ascute	ascute	hunch		
r. incompletus	r. incompletus	snapt				*willistoni* (stump)
		compressed		telescoped		*willistoni* (deformed, serrate)
		sex-ratio	sex-ratio			*athabasca, azteca, melanica, obscura*

ELEMENT E

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
pink	peach	pink, claret?	pinkish, claret?			
curled		upturned				*montium* (curled-b)
Stubble		Stubble				
bithorax		bithorax	bithorax			*athabasca*
aristapedia	aristapedia	aristapedia				
glass		glass		varnished		
Delta	Delta	Smoky	Delta	Confluent, Delta	Plexate	*busckii, montium* (Confluent)
Hairless	hairless					*funebris, montium*
cardinal		cinnabar			cardinal	*azteca*
crumpled		crumpled	crumpled	concave		
claret	claret	claret, pink?	pinkish, claret?	brick?		
		Bare			Off?	
		pauciseta	pauciseta			
				Puffed	Puffed	

ELEMENT F

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
abdomen rotatum			abdomen rotatum	abdomen rotatum		
grooveless		grooveless				
shaven	Shaven		reduced?	stubby	Shaven	
Minute-4	Minute-4					

involved (this element being a V in this species), and another associated inversion acted as a crossover suppressor. The fact that this inversion has been found to have a high frequency makes more probable the occurrence of similar inversions in other species where a V is composed of two different elements.

It is self-evident that the scheme of the integrity of the elements must break down for the more distantly related species of the Diptera. Where this will first happen is not clear. The common metaphase chromosome configuration among the higher Diptera has six chromosomes; whether these are the Drosophila elements with a few alterations or whether they represent quite different gene combinations can be answered only after a more thorough analysis of their genetics is made.

SUMMARY

The six chromosome arms of *D. melanogaster* (X, IIL, IIR, IIIL, IIIR, IV) retain their essential identity among the species of Drosophila so far studied. They are here called "elements" and are designated by the letters A to F in the order just stated.

The corresponding mutant genes on which this conclusion is based are summarized in table 6 of this paper; and the relation of the letter designations to the various chromosome terminologies previously used is summarized in table 1.

Element E is consistently the longest of the six, when studied in the salivary gland chromosomes; element F is always very short. The others are so nearly the same in size that their lengths are not diagnostic.

Analysis of the mathematical properties of sequences subjected to repeated inversions indicates that, in the specific case of *melanogaster* and *pseudoobscura*, the sequences are not significantly more alike than might result from chance.

Three pairs of loci—yellow and scute, Notch and white, miniature and dusky—apparently represent sections which have remained intact over a long period, since the two members of each pair are associated in numerous species and in representatives of both subgenera studied. Yellow and scute are separated, however, in *affinis* and in *hydei*.

The conclusion that the elements remain intact within the group means that few or no translocations have become established in the history of these two subgenera. Analysis of the conditions necessary for such establishment leads to the conclusion that the supposed instances of its occurrence as between species are not adequately established—with the exception of the attachment of part of element A to element F in *ananassae*.

The various ways in which the elements are attached to each other may be supposed to result from a special type of translocation in which both breaks occur in heterochromatin near the centromeres. Under the conditions found in most Drosophila species, this type of translocation more easily decreases chromosome number than increases it. It may be surmised that the configuration found in *virilis* (where all six elements are separate) is the most primitive one among those yet analyzed in Drosophila.

Summary

1. By means of nine X-ray induced translocations, the genetic linkage groups and the salivary gland chromosomes of Drosophila affinis have been correlated.

2. A salivary gland chromosome analysis of strains of D. athabasca from thirteen localities has revealed the presence of nineteen different chromosome sequences. The nature of some of these sequences with respect to each other and to their geographical distribution has made possible an interpretation of them in terms of phylogeny. A theory of the mechanism by which certain of these sequences have arisen is proposed.

3. The genetic analysis of anomalous sex ratios in D. affinis has shown that both sex chromosomes and autosomes are involved. The role of such aberrant sex ratios in natural populations has been evaluated.

4. A comparison of the mutant types known in the various species of Drosophila has led to the conclusion that certain groups of genes, or elements, remain linked from species to species. The absence of translocations differences between species has been explained in terms of the conditions necessary for their establishment.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to a number of persons whose assistance and cooperation have made this work possible. He is particularly indebted to Professor A. H. Sturtevant whose stimulating ideas and invaluable criticisms have strongly influenced the course of the research. He also wishes to acknowledge the helpful cooperation of other members of the Biology Department, especially Dr. J. Schultz and Dr. D. D. Miller.

The writer also wishes to thank Mr. C. H. Ellis for his kindness in preparing the photographs. Many other persons, including Prof. T. Dobzhansky of Columbia University, Prof. J. T. Patterson of the University of Texas, Prof. W. P. Spencer of the College of Wooster, Dr. C. V. Beers of the University of Southern California, and Dr. P. T. Ives of Amherst College, have aided in the prosecution of the research by contributions of material and by helpful advice.

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