

THE REPLETA GROUP
THE REPLETA GROUP

PRELIMINARY
DISSERTATION

The writer wishes to make grateful acknowledgment
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PART I

COMPARATIVE THE REPLETA GROUP CHROMOSOMES

Material and Methods

INTRODUCTION

The evidence accumulating on the nature of the evolutionary process in the genus Drosophila has assumed two broad aspects. The first is concerned with the nature and effect of chromosome balance and organization in Drosophila; the second phase, undertaken in the light of the first, is a study of the problem of speciation. Fundamental investigations along both of these lines are being carried out at the University of Texas, and the cumulative results of these studies offer a convincing body of facts.

The study of the repleta group, which is presented in this paper, employs both of the aforementioned lines of analysis. This complex group, representing a large number of divergent, yet closely related, species is peculiarly suited to a comparison of chromosome morphology. Of some twenty-eight described, and one undescribed member of the group, twenty-four are included in this analysis which constitutes the first division of the present study. The second section considers the species Drosophila repleta, its intraspecific and interspecific relationships.

PART I

(6) D. molayensis Patterson and Crow (redescribed by Patterson and Wheeler 1942). This stock was collected at Death Valley, California.

COMPARATIVE MORPHOLOGY OF THE CHROMOSOMES

Material and Methods

(7) D. longicauda Wheeler (1942), stock 314.5a, collected by Patterson at Aldrich Place,

Sturtevant (1940, 1941, 1942) has discussed chromosome morphology in the genus Drosophila, and has pointed out the probable nature of the changes which have modified the basic haploid number of chromosomes, which he regards as five rods and a dot, or six elements.

The analysis given here, however, deals with the comparative morphology of chromosomes within the repleta group, and is comprised of a study of the metaphase and salivary chromosomes of the following species:

- (1) D. repleta Wollaston, stock 235.3b, collected by Patterson at Elgin, Texas, 6/4/39. Stocks from Japan (obtained from Chino), and Guatemala (obtained from Sturtevant) were also checked.
- (2) D. mulleri Sturtevant, stock tested was collected at Aldrich Place, Austin, Texas, by Patterson.
- (3) D. aldrichi Patterson and Crow (completely described by Patterson and Wheeler 1942). The stock tested was derived from a female trapped by Patterson near Austin, Texas, in the summer of 1940.
- (4) D. arizonensis Patterson and Wheeler (1942). The stock tested was established from a female trapped in Arizona, September 1940, by Mainland.
- (5) D. buzzatii Patterson and Wheeler (1942). Stocks collected in Cordoba, Argentina and Trapani, Sicily were checked.

(18) D. nigrocyana Patterson and Wheeler (1942), stock 1232.9b, collected in the Chisos Mountains, Brewster County, Texas, 8/14/41, by Mainland and Wagner.

- (6) D. mojaviensis Patterson and Crow (redescribed by Patterson and Wheeler 1942). This stock was collected by Spencer at Mesquite Springs, Death Valley, California.
- (7) D. longicornis Patterson and Wheeler (1942), stock 514.5a, collected by Patterson at Aldrich Place, Austin, Texas, 12/17/39.
- (8) D. meridiana Patterson and Wheeler (1942), stock 1229.3, collected by Mainland and Wagner at a roadside park in Kinney County, Texas, 8/11/41.
- (9) D. sp. (meridiana-like) undescribed, stock 394.3d, collected at Aldrich Place, Austin, Texas, by Patterson 10/26/40.
- (10) D. peninsularis Patterson and Wheeler (1942), stock 1148.7, collected at Lake McKethan, Florida, by Mainland and Wheeler 6/19/41.
- (11) D. hamatofila Patterson and Wheeler (1942), stock 539.4a, collected by Patterson at Uvalde, Texas, 1/22/40.
- (12) D. bifurca Patterson and Wheeler (1942), stock 911.7m, collected by Mainland in Wild Rose Canyon, Texas, 9/22/42.
- (13) D. brevicarinata Patterson and Wheeler (1942). The stock tested was collected in San Josecito, Mexico, and was sent to us by Sturtevant.
- (14) D. ritae Patterson and Wheeler (1942), stock 911.5c, collected by Mainland in Wild Rose Canyon, Texas, 9/22/40.
- (15) D. linearepleta Patterson and Wheeler (1942) is a stock obtained by us from Sturtevant; it was collected by Dobzhansky at Antigua, Guatemala.
- (16) D. nigrospiracula Patterson and Wheeler (1942), stock 1254.3a, collected by Mainland and Wagner in Magdalena, Mexico, 8/23/41.
- (17) D. hydei Sturtevant, stock 914.2, collected in Limpia Canyon, Texas, 9/22/40, by Mainland.
- (18) D. nigrohydei Patterson and Wheeler (1942), stock 1232.9b, collected in the Chisos Mountains, Brewster County, Texas, 8/14/41, by Mainland and Wagner.

- (19) D. leonis Patterson and Wheeler (1942), was obtained by this laboratory from Sturtevant, and was collected at San Josecito, Mexico.
- (20) D. hydeoides Patterson and Wheeler (1942), was obtained by this laboratory from Sturtevant, and was collected at San Josecito, Mexico.
- (21) D. mercatorum Patterson and Wheeler (1942), stock 93b.7b, collected by Mainland at Santa Barbara, California, 8/30/40.
- (22) D. fuliginea Patterson and Wheeler (1942), stock 1283.10, collected seventeen miles from Silver City, New Mexico, 10/19/41, by Mainland and Wheeler.
- (23) D. neorepleta Patterson and Wheeler (1942), was obtained from Sturtevant, and derived from a stock collected by Dobzhansky at Sacapulas, Guatemala.
- (24) D. melanopalpa Patterson and Wheeler (1942), stock 1244.11, collected at Cave Creek, Arizona, 8/18/41, by Mainland and Wagner.

In addition to these members of the repleta group, the following interesting species not belonging to the group were studied:

- (1) D. orbospiracula Patterson and Wheeler (1942), stock 1232.1, collected in the Chisos Mountains, Brewster County, Texas, 8/14/41, by Mainland and Wagner.
- (2) D. polychaeta Patterson and Wheeler (1942), stock 119.6a, collected by Ray in Galveston, Texas, 10/21/38.
- (3) D. spinofemora Patterson and Wheeler (1942), derived from a stock sent from Hawaii by Zimmerman.
- (4) D. montana Patterson and Wheeler (1942), collected by Mainland and Wheeler in the summer of 1941.

In making salivary chromosome preparations, the usual smear technique was employed, using acetic-orcein as the

stain. The same type of stain was used for preparing brain smears from which the metaphase chromosome configurations were determined.

RESULTS

A study of the metaphase chromosomes of these species revealed the following facts, which are diagrammatically represented in Figures 1-5: Eight species, D. repleta, D. mulleri, D. arizonensis, D. aldrichi, D. buzzatii, D. mojavensis, D. longicornis, and D. meridiana show the basic number of six chromosome elements, consisting of five rods and a dot. The X chromosome is longer than the autosomes and the Y chromosome is considerably shorter than the X, although the extent of this discrepancy varies somewhat in the different species. D. sp. meridiana-like apparently differs from D. meridiana only in the fusion of two of its autosomes, thus reducing the chromosome elements to five: a long rod, which is the X; two shorter rods; a large V-shaped chromosome; and a dot. Two of the species, D. peninsularis and D. hamatofila, have six chromosome elements, a long rod-shaped X, four shorter rods, and a dot. In these two species, however, the Y chromosome is a small V-shaped body.

Four of the species, D. bifurca, D. brevicarinata, D. ritae, and D. linearepleta differ from the first group

in that the X and Y chromosomes are of equal length. D. bifurca is distinctive in that it has a definite constriction near the tip of each rod-shaped chromosome. D. nigrospiracula has five rods and a dot, but the X chromosome has a constriction near its tip which the Y does not have, thus making the latter appear somewhat shorter. The dot-like chromosomes are very large in metaphase preparations, but are not correspondingly large in the salivary cells. D. hydei has six chromosome elements consisting of four rod-shaped autosomes, a V-shaped X chromosome and a dot. The Y of this species is J-shaped, the short arm being very small. D. nigrohydei, D. leonis, and D. hydeoides each has six rod-like elements, the dots being absent. They differ from each other in several respects. D. nigrohydei has one very short autosome; its X chromosome is constricted near the tip, and the Y chromosome is very short, being about equal in size to the proximal constriction of the X. D. leonis has a pair of very thin autosomes, with a constriction near the centromere; in this species the Y is only slightly shorter than the X. In D. hydeoides, the Y is shorter than the X, and no constrictions were noted; one of the autosomes is rather short. D. mercatorum has only five chromosome elements: two autosomal rods, a rod-shaped X chromosome with a proximal

constriction, a large V-shaped chromosome derived from fusion, and a small V-shaped chromosome. This species is remarkable in that the Y chromosome is lacking; the female is XX, the male, XO. The dot-like element is absent in the metaphase. *D. fuliginea* showed the number of elements reduced to four, consisting of two large V-shaped chromosomes probably derived from fusion, a small V-shaped chromosome, and a long rod-shaped chromosome. The X and Y are of equal length; the dot-like chromosome was not observed.

D. neorepleta and *D. melanopalpa* each has six elements; the former has four rods, one of which is very short, a J-shaped autosome, and a short Y, corresponding in size to the "short arm" of the X. *D. melanopalpa* differs only in that it has a V-shaped rather than a J-shaped autosome. The dot-like element does not appear to be present in metaphase preparations of either of these stocks.

The following species which do not belong to the *repleta* group were examined: *D. orbospiracula* has six chromosome elements consisting of four rod-shaped autosomes, a rod-like X with a constriction at its tip, and a very small dot. No Y chromosome was observed in the metaphase preparations of the male larval brain. The female is XX, the male, XO, in this species.

D. polychaeta has six chromosome elements consisting of two rods, two J-shaped chromosomes, one V-shaped chromosome, and a dot; the X chromosome has a proximal constriction, and the Y chromosome is slightly shorter than the X.

D. spinofemora has only four chromosome elements: one long rod, one short rod, a large V-shaped chromosome, and a dot.

D. montana has six chromosome elements consisting of four rods, a J-shaped chromosome, and a dot (Stone, Griffen and Patterson, 1942). Examination of salivary preparations of the members of the repleta group revealed that each species has five long chromosome arms and the dot-like element. There is a striking similarity of salivary chromosomes within the group and the characteristic free chromosome ends are readily identifiable.

D. orbospiracula also has five long chromosome arms and the dot-like element. D. spinofemora, having four long arms and the dot-like chromosome, shows a decrease in the number of euchromatic arms. D. montana, on the other hand, shows an increased number of euchromatic arms, having six arms and a dot. D. polychaeta likewise shows an increased number of chromosome arms in the salivary preparation, having seven euchromatic arms and a dot-like element.

DISCUSSION

The data accumulated in the present study make possible an analysis of the occurrence of such events to bring about gross differences in the metaphase chromosome morphology of *Drosophila*. The nature and effect of changes which alter the number and linkage of chromosomes in the genus Drosophila has been the basis of much speculation and some experimental investigation. Salivary gland chromosomes have remained similar. Navashin (1932) advanced the "dislocation" hypothesis to explain observed increases and decreases in chromosome number. Dubinin (1934, 1936) succeeded in altering chromosome numbers in both directions, producing D. melanogaster with three and five chromosome pairs, through the use of suitable translocation stocks. He did not alter the genic balance system however. Stone and Griffen (1940) reported the experimental achievement of a change in the genome of D. melanogaster, producing true breeding stocks in which genic balance and chromosome number were changed; also, X chromosome material was, in effect, converted to autosomal, and vice versa. Sturtevant (1941, 1942) suggested different types of events which have contributed to the morphological variations observed in the metaphase chromosomes of *Drosophila*:

- (1) the acquisition of a non-terminal centromere by a rod;
- (2) the attachment of rod shaped elements to form a V-shaped chromosome;
- (3) the fusion of the dot-like chromosome with a rod.

centromeres, as Painter and Stone (1935) have sug-

The data accumulated in the present study make possible an analysis of the occurrence of such events to bring about gross differences in the metaphase chromosome morphology of species belonging to a single large group. Although the various members of the group may have acquired different chromosome rearrangements and gene mutations, the free ends of the salivary gland chromosomes have remained similar and are easily identified.

Each of the repleta species shows five long arms and the dot-like chromosome in salivary preparations. It is clear at the outset that the morphology of the metaphase chromosomes does not necessarily offer a reliable indication of the closeness of the relationships between members of the group. In fact, melanopalpa and repleta, which cross in one direction with some readiness, are extremely different as to metaphase chromosome morphology. They have a different number of arms, and the dot-like chromosome of melanopalpa has either undergone fusion, or has somehow acquired extra heterochromatin, so that it is not recognizable in the metaphase cells.

Let us consider the various changes which have occurred in the repleta series. Meridiana-like offers a clear case of autosomal fusion. It is impossible to deduce the exact nature of the change. Fusion may represent the amalgamation of two terminally located centromeres, as Painter and Stone (1935) have sug-

gested. It is most probable, however, that fusion represents a translocation in the (heterochromatic) region just distal to the centromere of one chromosome with the very short (heterochromatic) arm of the other chromosome. A similar type of exchange, producing a V-shaped chromosome has been demonstrated experimentally by Panshin (1935) and by Griffen and Stone (1940). Fuliginea has obviously undergone changes similar to that in meridiana-like in which four of its chromosomes are involved. It cannot be positively said that the rod-like chromosomes in D. fuliginea are the sex chromosomes, but cytological evidence indicates that this is probable. The male salivary preparations show only one haploid chromosome, the X; there are two possibilities: (1) that the X and Y are the rods, or (2) that the X and Y have become fused to the same autosome. The latter explanation is much less probable. In seven members of the repleta group the dot-like element is not detectable in metaphase preparations. In each of these cases where the dot is absent there is an actual increase in the number of chromosome arms in the metaphase. Since the dot-like element is observed to be present invariably in the salivary chromosome complex, we may assume that it has either fused with another chromosome, or has accumulated extra material, largely heterochromatic, thus forming an additional large body in the metaphase. This latter possibility is substantiated by the fact that increase

in chromosome arms in the metaphase is, with the single exception of D. hydei, achieved at the expense of the dot-like element. Also, it will be observed that nigrohydei, melanopalpa and neorepleta each has a pair of very short rods in the metaphase which may contain the dot-like chromosome. D. leonis has a pair of peculiarly thin autosomes with constrictions near their centromeres. Perhaps the slenderness of these rods is due to the relatively less coiled state of heterochromatic material, and possibly the constrictions near their tips represent the dots. D. fuliginea and D. mercatorum both have a small pair of V-shaped chromosomes. None of their salivary chromosomes shows any inversion across the centromere, such as is observed in montana. Therefore, the small V-shaped chromosome probably represents the modified dot-like element in these two species. An additional argument for the retention of independent dot-like elements is that there is a distinct selective advantage in the ability to segregate freely. More combinations are possible than if the dot-like chromosome were fused near the centromere of one of the other chromosomes. The possibilities thus far mentioned in regard to the location of the dot are not exhaustive. Perhaps the dot-like body simply acquired additional heterochromatic material by translocation or change in gene action. Or, in the case of nigrohydei, melanopalpa and neorepleta, perhaps the constricted tip of the X represents the dot which has become, in effect, the Y chromosome. This

would involve a more complex change and is, therefore, somewhat less probable. rum, the number of centromeres is reduced. It is observed that in several of the species extra heterochromatic arms are present in addition to the basic number of euchromatic arms. One arm of the V-shaped X chromosome of D. hydei is heterochromatic. The species leonis, nigrohydei, mercatorum and fuliginea have one chromosome that is entirely heterochromatic unless it carries the dot-like chromosome. Neorepleta and melanopalpa have more than two extra heterochromatic arms. If the short autosome represents the dot plus heterochromatin, then the small arm of the X and one whole additional arm are heterochromatic. If the dot has become fused to the X, then two large arms are heterochromatic. (Jones and Griffen, 1949) and doubtless could occur. The apparent change in the amount of heterochromatin in the repleta series might be comparable to the fluctuation in frequency of the B chromosome in maize (Randolph, 1941) as far as its effect on genic balance is concerned. In maize large numbers of B chromosomes affect viability, however, and we do not have evidence of a similar deleterious effect in Drosophila. The constrictions found in the chromosomes of certain of the Drosophila species might also be comparable to those found in the B chromosomes in maize which seem to set off a heterochromatic region. (as an intermediate condition) The repleta group also shows variation in the number of centromeres, but all deviations from the basic number of six

centromeres represent a decrease. In the case of meridiana-like and D. mercatorum, the number of centromeres is reduced to five. In the species D. fuliginea, two fusions have decreased the number of centromeres to four. After a species which has undergone such a fusion becomes isolated from the parent forms with a higher centromere number, this loss is not easily reversible. Thus, with a single step, a profound change in linkage relationships and in recombination possibilities may be effected. Although no such case is yet represented, it must not be overlooked that an increase in centromere number is a distinct possibility in the event of a particular type of translocation which would produce a "free" centromere. This has been accomplished experimentally (Stone and Griffen, 1940) and doubtless could occur in nature.

The Y chromosome is also subject to a wide range of alteration in the repleta group. D. mercatorum shows an XO condition in the male. D. peninsularis and D. hamatofila have small V-shaped Y chromosomes. It is interesting to speculate that the small V-shaped chromosome in XO mercatorum may be the result of a fusion of the V type of Y chromosome with the dot-like element. In many of the species of the repleta group, the Y chromosome is extremely short, as in D. repleta. D. longicornis represents an intermediate condition of the Y chromosome, which is shorter than the X, but not so short as in some of the other species. In

D. leonis the Y chromosome is only slightly shorter than the X. The X chromosome of D. nigrospiracula has a definite constriction near its centromere which the Y chromosome lacks, making the latter somewhat the shorter of the two. In D. bifurca, as in four other members of the repleta group reported here, the X and Y chromosomes are of equal length. In D. hydei, the Y chromosome is a long J-shaped body, about half the size of the V-shaped X chromosome.

It is particularly interesting to find, in this closely related group, almost every variation of length and shape in the Y chromosome. Dobzhansky (1937) after a study of the variable Y chromosome in D. pseudoobscura suggested that comparative chromosome morphology does not furnish especially reliable data for the determination of phylogenetic relationships, since genic differentiation and change in chromosome structure are not necessarily parallel events. The present study supplies a substantial proof for this criticism.

In the species belonging to the repleta group, there has been a consistent retention of five long chromosome arms and a dot-like element in the salivary chromosomes in spite of the gross alteration of metaphase chromosome morphology. This indicates that there has been little shifting of the euchromatic material aside from intrachromosomal changes and fusion.

A study of species not belonging to the repleta group, but which are reported here, contribute certain additional

and salient facts with reference to the alteration of chromosome morphology:

In montana and polychaeta there has been an increase in the number of euchromatic arms due to the occurrence of inversion across the centromere. A single event of this nature has given montana six, rather than five, long euchromatic arms, as may be seen in salivary chromosome preparations; two such events have given D. polychaeta seven euchromatic arms. Two J-shaped chromosomes in polychaeta have euchromatic arms which are much shorter than the other three long arms in the salivary gland nuclei. Therefore it seems more probable that they originated by inversion, as in montana, although they may have derived from mutual translocation. There has been no detectable increase of euchromatic material, nor has there been any addition of centromeres.

D. spinofemora has a reduced number of centromeres, there being only four in this species; it shows only four long chromosome arms in salivary preparations. One of these arms, however, is of extreme length, and has obviously been derived from the union of two chromosomes. This may have occurred in either of two ways: It could have resulted from the translocation of one of the chromosomes to the tip of the other; or it may have involved two steps, an initial translocation or fusion of the two chromosomes at the centromere region, followed by a pericentric inversion.

D. orbospiracula is a particularly interesting XO type. Unlike the XO mercatorum, there is no heterochromatic arm present which might conceivably bear the Y chromosome material. The essential Y genes must, therefore, be carried in the X chromosome or in the autosome.

In the light of such evidence, it is obvious that were it not for the check made possible by salivary chromosome analysis, any deductions concerning the evolution of metaphase chromosome complexes, or the phylogenetic relationships of the individuals possessing these complexes, would be extremely precarious. In other genera, plant or animal, where the critical analysis of large chromosomes such as the salivary chromosomes in Drosophila is not possible, the variability of the metaphase chromosome morphology might make it a distinctly unreliable tool in establishing phylogenetic relationships.

The variation in metaphase configurations occurring in the repleta series, unaccompanied by any appreciable variation in the salivary chromosomes, together with the evidence offered by the variation of the B chromosome in maize, makes questionable the actual extent of so-called aneuploidy in species where the number of chromosomes varies from simple multiples of the n number. A considerable variation in chromosome number might represent only a slight variation in the number of genes.

d. The usual centromere number in the repleta group,

SUMMARY

1. An analysis of the metaphase and salivary chromosomes of twenty-four species of the repleta group, together with four other species of Drosophila not belonging to this group, provided material for the study of the comparative morphology and organization of the chromosomes.

2. The changes observed in this study are:

a. Fusion of two long rod-shaped chromosome arms to form a V-shaped chromosome.

b. Fusion or translocation of the dot-like element, accompanied by an increase in the number or size of chromosome arms seen in the metaphase preparations. With one exception (D. hydei), the in-

crease in the number of chromosome arms in the metaphase occurred at the expense of the dot-like element. It is impossible to determine, without genetic tests, the new position of the dot-like body after its disappearance in the metaphase configuration, but several possibilities are suggested.

c. In some cases additional heterochromatic arms are observed in metaphase preparations. This is frequently associated with the disappearance of the dot.

d. The usual centromere number in the repleta group,

5. Since Drosophila, is six, but variants of five and four centromeres are reported for this series. Reduction of the number of centromeres is always accompanied by fusion. It is pointed out that an increase in the centromere number is a possibility, although no such instance has as yet been analyzed.

e. The Y chromosome exhibits a wide range of morphological variability within the repleta group, culminating in an XO type, D. mercatorum. The additional XO case, D. orbospiracula, is in another species group. These differ in that orbospiracula has no heterochromatic autosomal arm which might represent the Y.

3. Although no member of the repleta group shows an increase in the number of euchromatic arms, D. montana and D. polychaeta do show an increase. This was accomplished by inversion across the centromere in montana. In polychaeta, there were either two such inversions, or two independent mutual translocations involving the same chromosomes, which is unlikely.

4. D. spinofemora has one very long chromosome, composed of two of the originally separate euchromatic chromosomes of the basic complement of five long arms and a dot. This was accomplished by a simple translocation, or by a fusion, followed by a pericentric inversion, which seems more probable.

5. Since gross chromosome morphology, as seen in ordinary somatic or germinal metaphases is inherently variable, it is not a reliable guide to inter- or intraspecific relationships.

Certain isolating mechanisms are observed to operate between species. This naturally gives rise to the question of whether the same genes that separated species, which have since diverged in other respects, may act to produce similar isolation within species. Sexual isolation is one of the mechanisms separating species. *D. repleta* offers some evidence on this problem, as sexual isolation here separates certain strains within the species.

MATERIAL AND METHODS

The *repleta* stocks used in these tests include the following:

Fredricksburg 89.4a, Elgin 235.3b, Eagle Pass 506.9b, Galveston 494.4a, Livingston 247.5f, Rosenberg 250.4, and Brownsville 688.2 are stocks which were collected in Texas by Patterson. The stocks from New Haven and Guatemala were obtained from Sturtevant, and the stock from Ankara, Turkey, was obtained from Buzzati-Traversa.

D. melanopalpa was collected by Mainland and Wagner in Cave Creek, Arizona. *D. neorepleta* was collected by Dobzhansky at Sacapulas, Guatemala, and was sent to us by Sturtevant.

PART II

Certain isolating mechanisms are observed to operate between species. This naturally gives rise to the question of whether the same genes that separated species, which have since diverged in other respects, may act to produce similar isolation within species. Sexual isolation is one of the mechanisms separating species. D. repleta offers some evidence on this problem, as sexual isolation here separates certain strains within the species.

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D. melanopalpa was collected by Mainland and Wagner in Cave Creek, Arizona. D. neorepleta was collected by Dobzhansky at Sacapulas, Guatemala, and was sent to us by Sturtevant.

In the initial intraspecific fertility tests (Table I) five pairs of flies per vial were used in the cross. These flies had been aged for one week. Fertility was checked four weeks after the time of crossing. All tests were reciprocal.

A further test (Table II) consisted of making the various crosses using twenty-five pairs of flies per bottle. Flies used in this test were aged eight to twelve days, as *repleta* matures slowly. In cases where the cross was fertile, offspring were counted throughout the heavy hatching period (about six days). These crosses were kept for five weeks.

Controls were run in both of these tests. In each instance where the cross was sterile, females were dissected for the presence of sperm. Wherever the cross was fertile, the salivary chromosomes were checked for the presence of chromosome rearrangements.

Many attempts were made to obtain quantitative data through the use of pair matings, but *repleta* does not breed well under such conditions, and in no case were the controls sufficiently fertile to indicate that the amount of sterility observed was representative of genetic differences.

F_1 and F_2 crosses were made, using twenty-five pairs per bottle whenever a sufficient number of flies were available from the P_1 and F_1 crosses. No count of offspring was made in these tests. Backcrosses were made in several instances.

Interspecific crosses between D. neorepleta, D. melanopalpa and some of the repleta stocks were made. D. repleta strains tested to neorepleta and melanopalpa were: New Haven, Eagle Pass, Rosenberg, and Guatemala. Ten crosses in vials using ten pairs of flies per vial, were used in each of these interspecific tests. The initial test crosses of five pairs of flies in vials immediately indicated certain differences in the several strains. Therefore, a second set of crosses, using mass matings of twenty-five pairs in bottles, was made in order to obtain a further test of the sterility which appeared in the first crosses. An additional stock, Ankara, was also used in this test. The following facts were observed:

Fredricksburg females were sterile to Elgin males, but the reciprocal cross went reluctantly, producing twenty-one offspring. Fredricksburg females were sterile to New Haven males, but the reciprocal cross was quite fertile, producing over three hundred offspring. Fredricksburg females were practically sterile to Ankara, producing only fourteen offspring, but the reciprocal cross went readily, producing over four hundred F_1 flies.

Elgin females went reluctantly to Guatemala males, and the reciprocal cross was sterile. New Haven females went reluctantly to Guatemala males, and the reciprocal cross was sterile. Guatemala females were sterile to Eagle Pass males, but the reciprocal cross was fertile, producing over two hundred progeny.

The results of the second tests (Table II) were consistent with those of the initial tests (Table I) with a

RESULTS

The initial test crosses of five pairs of flies in vials immediately indicated certain differences in the several strains. Therefore, a second set of crosses, using mass matings of twenty-five pairs in bottles, was made in order to obtain a further test of the sterility which appeared in the first crosses. An additional stock, Ankara, was also used in this test. The following facts were observed:

Fredricksburg females were sterile to Elgin males, but the reciprocal cross went reluctantly, producing twenty-one offspring. Fredricksburg females were sterile to New Haven males, but the reciprocal cross was quite fertile, producing over three hundred offspring. Fredricksburg females were practically sterile to Ankara, producing only fourteen offspring, but the reciprocal cross went readily, producing over four hundred F_1 flies.

Elgin females went reluctantly to Guatemala males, and the reciprocal cross was sterile. New Haven females went reluctantly to Guatemala males, and the reciprocal cross was sterile. Guatemala females were sterile to Eagle Pass males, but the reciprocal cross was fertile, producing over two hundred progeny.

The results of the second tests (Table II) were consistent with those of the initial tests (Table I) with a

TABLE II. The results obtained from mass matings

TABLE I. Initial fertility tests

	Fred.	Elgin	N.H.	Guat.	E.P.
♂♂					
♀♀					
Fredricksburg (89.4a)	fertile	sterile	sterile	fertile	fertile
Elgin (235.3b)	fertile (slightly)	fertile	fertile	fertile (slightly)	fertile
New Haven	fertile	sterile	fertile	fertile	fertile
Guatemala	fertile	sterile	sterile	fertile	sterile
Eagle Pass (506.9b)	fertile	sterile	fertile	fertile	fertile

TABLE II. The results obtained from mass matings of twenty-five pairs

	Fred.	Elgin	N.H.	Guat.	E.P.	Ank.
♂♂						
♀♀						
Fredericksburg (89.4a)	++	sterile	sterile	++ (420)	++ (496)	+ (14)
Elgin (235.3b)	+ (21)	++	++ (149)	+ (12)	++ (164)	++ (154)
New Haven	++ (352)	sterile	+	+ (49)	++ (156)	+ (71)
Guatemala	++ (169)	sterile	sterile	++	sterile	++ (104)
Eagle Pass (506.9b)	++ (321)	++ (134)	++ (107)	++ (238)	++	++ (204)
Ankara	++ (440)	++ (230)	++ (200)	++ (117)	++ (121)	++

single exception. Eagle Pass females, which at first appeared to be sterile to Elgin males, proved to be fertile in the larger mass mating of the second cross.

In order to determine whether the females of the sterile crosses had been fertilized, they were dissected and examined for the presence of sperm. In no case were sperm present. Mating apparently did not take place.

The F_1 larvae salivary chromosomes were checked in each case where the cross was fertile, and no rearrangements were observed. Inbreed tests of F_1 and F_2 flies proved them to be quite fertile whenever there were enough flies to make adequate tests. The same was true of backcrosses.

Certain other P_1 crosses exhibited sexual isolation: Fredricksburg crossed to Rosenberg very reluctantly in either direction, failing to produce enough progeny to make adequate inbreed or backcross tests. Fredricksburg crossed very reluctantly to Brownsville in either direction. Guatemala was somewhat fertile to Galveston males, but the reciprocal cross did not go. Livingston females were fertile to New Haven males, but the reciprocal cross was practically sterile.

The interspecific crosses have not yet been tested extensively, but the results thus far obtained are as follows: D. melanopalpa females were slightly fertile to Eagle Pass repleta males, producing a few male and female offspring. The reciprocal cross was sterile. D. melanopalpa

females were slightly fertile to Rosenberg repleta males, producing very few male and female offspring. The reciprocal cross did not go. D. melanopalpa females were fairly fertile to New Haven repleta, producing a number of male and female offspring. The reciprocal cross was sterile.

D. melanopalpa females were slightly fertile to Guatemala repleta males, producing male-like, female-like, and extremely mixed type intersexes, as well as several phenotypically normal male and female offspring.

The F_1 from each of these crosses have failed to prove fertile when inbred. Male and female offspring have not yet been tested in backcrosses. Rearrangements were observed in the salivary chromosomes of the hybrids, but have not yet been analyzed.

D. melanopalpa and D. neorepleta crossed reciprocally, being quite fertile to each other, although the cross goes somewhat more vigorously when melanopalpa females are used to repleta males, but the reciprocal cross was practically sterile. No rearrangements were observed in the salivary chromosomes of the hybrids.

Apparently D. neorepleta is much more reluctant to cross with repleta than is melanopalpa. Although identical tests were made to repleta using neorepleta and melanopalpa, neorepleta hybridized only with the strain from Guatemala, producing one or two abnormal offspring.

D. aldrichi females were DISCUSSION fertile to D. arizonensis

males, but the reciprocal cross did not go. D. arizonensis females were fertile to D. aldrichi males, but the reciprocal cross was sterile in the other direction. Limpia Canyon D. limpiensis females, however, were sterile to D. aldrichi males, while the reciprocal cross was fertile. Mainland found a situation just the opposite to Dobzhansky's case of isolation between populations closely situated geographically, and the lack of isolation between the geographically distant species. He observed that in some D. macrospina x D. subfucosa crosses, the more closely situated geographically, the more likely were these populations to be fertile to each other.

Dobzhansky and Koller (1938) reported sexual isolation between D. pseudoobscura and D. miranda, and also between D. azteca and D. athabasca. They reported a certain degree of sexual isolation between races of D. miranda. In some cases strains of D. pseudoobscura which were in close juxtaposition to a race of D. miranda showed more sexual isolation to that race than did other strains of D. pseudoobscura which were more remote geographically. With another race of D. miranda, however, this relationship of geographical distribution to sexual isolation did not hold.

The virilis group shows sexual isolation (Patterson, Stone and Griffen, 1940). D. virilis females cross readily to D. americana males, but the reciprocal cross was practically sterile. D. virilis Henly was almost completely sexually isolated from the several wild forms. D. montana, on the other hand, which crossed very reluctantly, if at all, to most of the virilis group, is less isolated from Henly. If the genes which caused sexual isolation between the various stocks were identical, this would be indicated by some consistent cross-sterility relationships when the strains were interbred. Such was not the case, however.

The mulleri group (Patterson and Crow, 1940) exhibited sexual isolation in one direction in several instances.

D. mulleri females crossed to males of all other species of the mulleri group, but the reciprocal crosses were sterile.

D. aldrichi females were slightly fertile to D. arizonensis males, but the reciprocal cross did not go. Central Texas macrospina females (Mainland, 1942) were fertile to subfunnebris males, but the cross was sterile in the other direction. Limpia Canyon limpiensis females, however, were sterile to subfunnebris males, while the reciprocal cross was fertile. Mainland found a situation just the opposite to Dobzhansky's case of isolation between populations closely situated geographically, and the lack of isolation between the geographically distant species. He observed that in some macrospina x subfunnebris crosses, the more closely situated geographically, the more likely were these populations to be fertile to each other. Sexual isolation figures in the divergence of all species thus far studied in this laboratory. D. repleta is interesting in that sexual isolation was manifest between many of the strains tested.

The genetic heterogeneity of the repleta populations, and the complexity with which the sexually isolating genes were manifested, suggested that several genes were involved. If the genes which caused sexual isolation between the various stocks were identical, this would be indicated by some consistent cross-sterility relationships when the strains were interbred. Such was not the case, however, (Table I). For example, Fredricksburg and Eagle Pass crossed readily in either direction, but Eagle Pass males were sterile to

Guatemala females, while Fredricksburg crossed readily to Guatemala in either direction. Fredricksburg females were sterile to New Haven males, but Eagle Pass crossed reciprocally with New Haven. Also, Elgin and New Haven males were sterile to Fredricksburg females and to Guatemala females. Elgin and New Haven females were only slightly fertile to Guatemala males. Yet New Haven females were sterile to Elgin males.

Furthermore, there was apparently no correlation of the genes which controlled sexual isolation in repleta with the geographical distribution of the strains tested. Fredricksburg and Elgin, which are near to each other geographically, showed very different cross-sterility relationships when interbred with other stocks. Ankara, which is geographically a great distance from other strains tested, showed sexual isolation to Fredricksburg females; otherwise, Ankara showed very little isolation to other repleta strains.

The mutations which contribute to sexual isolation must have arisen by chance within the various populations. They occurred within different geographical strains and are present at random in the repleta species. If, by chance, two populations should become reciprocally isolated, so that no gene exchange occurred between the two strains, then their course of evolution might be independent, and the situation necessary for divergence could be established. Elgin and Guatemala approach this condition. Rosenberg

and Fredericksburg were very reluctant to cross in either direction also. Such populations might not diverge, however, if they could exchange genes through some intermediate population. Here the element of population size and distribution enters.

Sexual isolation between two strains does not imply that their genotypes are incompatible. Wherever a cross was fertile in only one direction between two repleta strains, the F_1 and F_2 crosses were frequently more fertile than either the P_1 or control crosses, even though the reciprocal P_1 cross was sterile.

The repleta strains are exceedingly stable as to gene arrangement, and even widely separated geographic strains (from Japan, Ankara, Guatemala, Texas, etc.) failed to show any large chromosome rearrangements when interbred. The differences between the stocks are genic.

Sexual isolation also exists between species in the repleta group. D. melanopalpa has thus far crossed with every repleta stock to which it has been tested. However, the cross has gone in only one direction, i.e., where melanopalpa was used as the female parent. Several interesting results have been obtained in these interspecific crosses. D. melanopalpa females, when crossed to Guatemala repleta males, produced offspring of several types: phenotypically normal males and females, the fertility of which has not yet been adequately tested; male-like intersexes;

female-like intersexes, and mixed type intersexes. These intersexes were analyzed and drawn by Dr. W. W. Newby (Figure 6).

The male-like intersex had very small, rudimentary claspers. The vaginal plates of the female-like intersex were greatly reduced and crossed. The extremely mixed type intersex had very badly formed anal valves, only one vaginal plate, and a large "genital knob". Newby (unpublished) says that this "genital knob" represents a chitinized and highly pigmented structure which forms about the undeveloped female genitalia.

Sturtevant (private communication to Patterson) reported that in a cross of D. neorepleta females to a repleta strain hybrid offspring were produced: "sterile males, and females slightly fertile but with anal plates suggesting intersexuality." We have no further information concerning his investigation of this cross.

New Haven repleta males, when crossed to melanopalpa females produced fairly numerous hybrid offspring of both sexes which were phenotypically normal. This cross went more readily than any of the other interspecific crosses. New Haven repleta males were slightly fertile to Guatemala repleta females, although the reciprocal cross was sterile. The F_1 and F_2 produced in the crosses were normal and fertile. Yet there is a difference in sex balance in these New Haven and Guatemala repleta strains which became evident in

the interspecific crosses to melanopalpa females. New Haven males to melanopalpa produced phenotypically normal offspring of both sexes, while Guatemala repleta males produced only a few offspring, some of which were intersex, as described.

The D. neorepleta hybridized much less readily with repleta strains than did melanopalpa. A few phenotypically abnormal offspring were obtained in a cross of neorepleta to Guatemala repleta males. Hybrids of neorepleta with other repleta strains have not been obtained. Nor is the fertility of melanopalpa x repleta hybrids adequately tested.

Sexual isolation is a descriptive term in which is concealed numerous and quite different reactions which conduce to the failure of mating between strains or species. In some cases, such isolation may have a simple cause, depending upon the action of a few genes. Other cases are doubtless much more complex. When the problem of providing favorable laboratory breeding conditions for repleta is solved, so that quantitative measures can be made, with adequate control, many such problems may be elucidated.

Another interesting point should be mentioned here. Silow (1941) commented on two different genes found by Hutchinson, Cp_a and Cp_b, which were scattered at random in one strain of the genus Gossypium. In numerous other geographic strains, only the gene Cp_b was carried. Neither Cp_a nor Cp_b had any visible phenotypic effect when present without the other. When strains which carried Cp_a were

crossed to strains carrying Co_b, however, the effect was deleterious in the event that the two genes occurred in the same genotype. The phenotypic distortion, "crumpled", appeared and the hybrids also proved sterile in most cases.

The degree of the effect in hybrids containing these two relationships in the interbred strains indicated that genes was conditioned by modifiers present in the strains which were involved in the crosses. Here a physiological effect is observed to be governed by two different genes

3. The genes which controlled sexual isolation were occurring at random in different populations. These genes, when separated, have no deleterious effect in their own genome. They serve as an isolating mechanism by rendering sterile the hybrids of strains containing them.

No direct analogy is drawn here between the isolation effected in F₁ crosses in the Gossypium case and the sexual isolation occurring in P₁ crosses of repleta strains. Nonetheless, it may be pointed out that different genes which are scattered at random in the repleta populations are responsible for P₁, or sexual isolation. Such isolation

varies in degree and kind between any two strains, according to the genes involved, the physiological effects which those

genes produce, and the system of modifiers with which they occur. Sexual isolation is one step removed from the isolation produced by F₁ sterility, but the factors involved must not be assumed to be of an entirely different order or derivation.

producing phenotypically normal hybrids of both sexes.

Since F₁ and F₂ progeny of Guatemala x New Haven repleta

are phenotypically normal SUMMARY fertile, the sex balance of the three stocks is assumed to be different.

1. Numerous strains of repleta showed sexual isolation when interbred.

2. The failure to find any consistent cross-sterility relationships in the interbred strains indicated that several different genes may cause sexual isolation between various strains.

3. The genes which controlled sexual isolation were not correlated with the geographic distribution of the repleta strains tested.

4. Since the mutations which were responsible for sexual isolation were several and different, they probably occurred by chance, and were scattered at random in the populations where they arose.

5. Sexual isolation, operating between strains, might conceivably establish the separation necessary to further divergence.

6. Sexual isolation between strains does not imply an incompatibility of genotypes.

7. Certain interspecific crosses were obtained, using melanopalpa and neorepleta females and repleta males of various strains. D. melanopalpa females crossed to Guatemala repleta males produced intersexes of varying degree. New Haven repleta males crossed to melanopalpa females, producing phenotypically normal hybrids of both sexes. Since F₁ and F₂ progeny of Guatemala x New Haven repleta

are phenotypically normal and fertile, the sex balance of the three stocks is assumed to be different.

8. Similar genetically controlled isolation of P_1 stocks (sexual isolation) occurs both within and between species.

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FIGURE 1









Species	Metaphase of ♀	Metaphase of ♂	Salivary chromosomes long arms	Salivary chromosome dots
<u>D. repleta</u> (235.3b)			five long arms	dot-like chromosome present
<u>mulleri group</u> <u>mulleri</u> <u>arizonensis</u> <u>aldrichi</u> <u>buzzatii</u> <u>mojavensis</u>			five long arms	dot-like chromosome present
<u>D. longicornis</u> (514.5a)			five long arms	dot-like chromosome present
<u>D. meridiana</u> (a) (1229.5)			five long arms	dot-like chromosome present

FIGURE 2









Species	Metaphase of ♀	Metaphase of ♂	Salivary chromosomes long arms	Salivary chromosomes dots
<u>D. meridiana</u> (b) (394.3d)			five long arms	dot-like chromosome present
<u>D. peninsularis</u> (1148.7)			five long arms	dot-like chromosome present
<u>D. hamatofila</u> (539.4a)			five long arms	dot-like chromosome present
<u>D. bifurca</u> (911.7m)			five long arms	dot-like chromosome present

FIGURE 3









Species	Metaphase of ♀	Metaphase of ♂	Salivary chromosomes long arms	Salivary chromosomes dots
<u>D. brevicarinata</u> (Sturtevant)			five long arms	dot-like chromosome present
<u>D. ritae</u> 911.5c			five long arms	dot-like chromosome present
<u>D. linearepleta</u> (Sturtevant)			five long arms	dot-like chromosome present
<u>D. nigrospiracula</u> 1254.3a			five long arms	dot-like chromosome present

FIGURE 4

















Species	Metaphase of ♀	Metaphase of ♂	Salivary chromosomes long arms	Salivary chromosomes dots
<p><u>D. hydei</u> (Limpia Canyon) 914.2</p>			<p>five long arms; ∴ one arm hetero- chromatic</p>	<p>dot-like chromosome present</p>
<p><u>D. nigrahydei</u> (1232.9b)</p>			<p>five long arms; ∴ one arm hetero- chromatic</p>	<p>dot-like chromosome present; ∴ fused</p>
<p><u>D. leonis</u> (Sturtevant)</p>			<p>five long arms; ∴ one arm hetero- chromatic</p>	<p>dot-like chromosome present; ∴ fused</p>
<p><u>D. hydeoides</u> (Sturtevant)</p>				

FIGURE 5

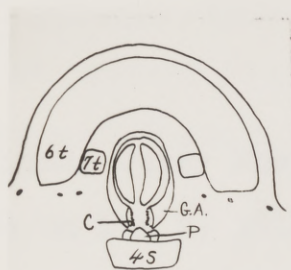
Species	Metaphase of ♀	Metaphase of ♂	Salivary chromosomes long arms	Salivary chromosomes dots
<u>D. mercatorum</u> (935.7b)			five long arms; ∴ one arm hetero- chromatic	dot-like chromosome present; ∴ fused
<u>D. fuliginea</u> (1285.10)			five long arms; ∴ one arm hetero- chromatic	dot-like chromosome present; ∴ fused
<u>D. neorepleta</u> (Sturtevant)			five long arms; ∴ two arms hetero- chromatic	dot-like chromosome present; ∴ fused
<u>D. melanopalpa</u> (1244.11)			five long arms; ∴ two arms hetero- chromatic	dot-like chromosome present; ∴ fused

EXPLANATION OF FIGURE 6*

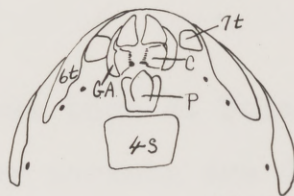
- I. 1a, 1b, 1c = normal male, external genitalia.
- A. t = tergite
 - B. s = sternite
 - C. G.A. = genital arch
 - D. P. = penis
 - E. C. = clasper
- II. 2a, 2b, 2c = male-like intersex, external genitalia.
- A. Claspers very reduced
- III. 3a, 3b, 3c = normal female, external genitalia.
- A. A.V. = anal valve
 - B. V.P. = vaginal plate
- IV. 4a, 4b, 4c = female-like intersex, external genitalia.
- A. Vaginal plates reduced and crossed
- V. 5a, 5b, 5c = extremely mixed intersex.
- A. Poorly formed anal valves
 - B. Only one vaginal plate
 - C. G.K. = "Genital knob"

* Figures drawn by Dr. W. W. Newby.

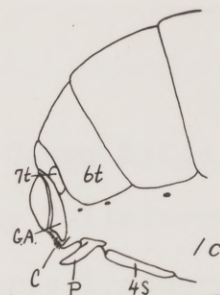
FIGURE 6



Normal Male 1a



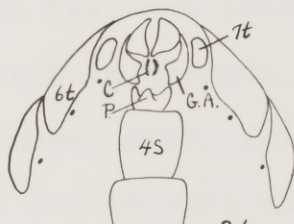
1b



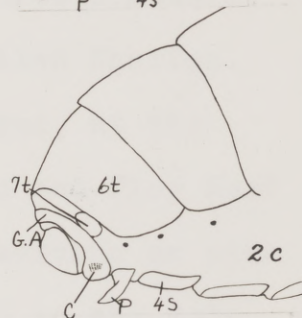
1c



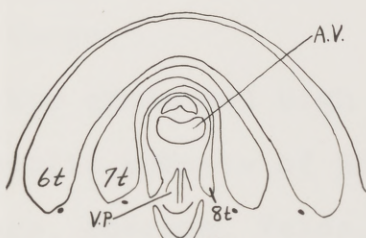
Male-like Intersex 2a



2b



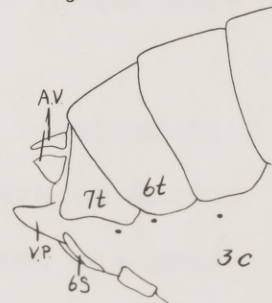
2c



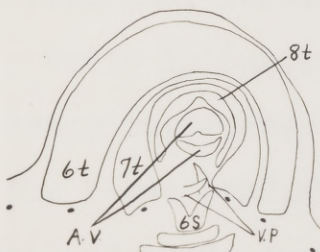
Normal Female 3a



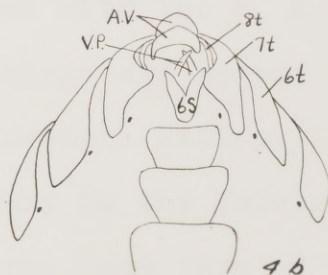
3b



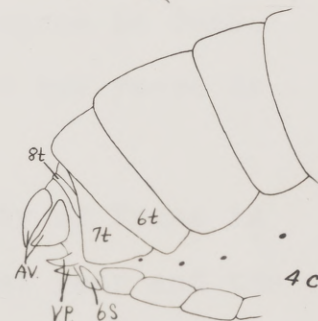
3c



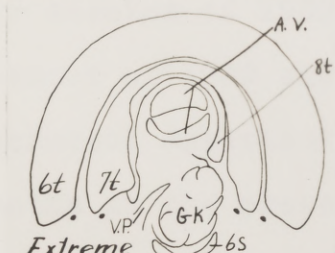
Female-like Intersex 4a



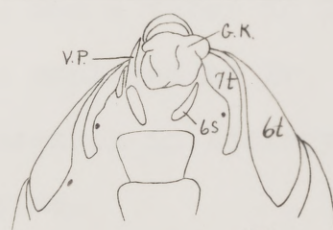
4b



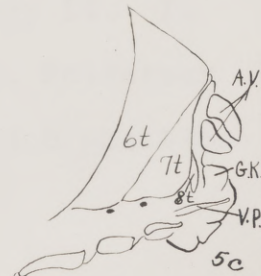
4c



Extreme Intersex 5a



5b



5c

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