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II. GENE VARIATION AND EVOLUTION

Directed by

J. T. PATTERSON
Professor of Zoology
The University of Texas

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The benefits of education and of useful knowledge, generally diffused through a community, are essential to the preservation of a free government.

Sam Houston

Cultivated mind is the guardian genius of Democracy, and while guided and controlled by virtue, the noblest attribute of man. It is the only dictator that freemen acknowledge, and the only security which freemen desire.

Mirabeau B. Lamar

Studies in the genetics of Drosoph
(U.T. pub. 4228, July 22, 1942)

Gene variation and evolution

CONTENTS

PREFACE

The articles appearing in this bulletin constitute number II of the series of publications to be issued from our genetics laboratory. The number I publication appeared in August, 1940, under the same general title. It dealt primarily with the effects of aneuploidy and chromosomal abberation on the phenotype, viability, and fecundity of *Drosophila,* and contained but two articles on the subject of gene variation and evolution. The present publication contains ten papers, all of which, either directly or indirectly, are concerned with the latter topic. They were prepared by various members of the group working in the laboratory during the present year, including Professor W. W. Newby, on sabbatical leave from The University of Utah.

We again wish to express our appreciation to The University Research Institute, which, under the direction of Dean A. P. Brogan, has supplied funds for meeting the expenses of publication.

J. T. PATTERSON.

Austin, Texas,
July 1, 1942.

I. INTERSPECIFIC HYBRIDIZATION IN THE GENUS *DROSOPHILA*

J. T. PATTERSON

INTRODUCTION

Interspecific hybrids offer excellent genetic material, especially when either one or both sexes are fertile. They are indispensable for the study of such problems as those of comparative genetics and phylogeny. Until recently the genus *Drosophila* has yielded but very few cases of hybridization. As late as 1934 only two cases of hybrids had been recorded for the genus. Since then the list of established cases has been gradually increasing. This increase is due to the use of more intensive and better methods of collecting and to an improved technique for the detection of hybridization.

A few years ago we began collecting the wild strains of *Drosophila* with the view of studying the problem of the genetics of evolution. Over forty undescribed forms have been found among the specimens collected and classified. These new forms together with those already known can be arranged into a series of groups, each composed of one or more known species. In making tests for the detection of possible cases of hybridization, we have found it better to begin by selecting members from the same group, rather than to engage in haphazard cross-testing of forms which at best can only be regarded as remotely related. In handling any new form brought into the laboratory, we therefore follow the plan of first making a detailed study of its morphological and physiological characters, and in this way it is usually possible to place it in its proper species group. Once a new form has thus been assigned, it is carried through a rather definite series of cross tests with the other members of the group.

In carrying out the tests between two forms we have followed the plan of first making reciprocal crosses in small mass cultures in vials (about ten pairs to the vial). If larvae do not appear in the cultures within a few days, the flies are transferred to fresh food vials, and if necessary this procedure is repeated until it can be determined whether or not this method is adequate as a test of the cross fertility of the two forms. If the results are negative, the experiment is repeated by using larger mass cultures in half-pint bottles (50–100 pairs to the bottle), and if the results are still negative, it may be assumed that the two species are very probably cross-sterile. If the results are positive, the experiment is continued by using pair matings and carrying the tests through to the F_3 generation. This procedure and a study of the salivary gland chromosomes of the F_1 larvae will show whether the two forms under test belong to the same or to different groups.

We present below a list of the known cases of interspecific hybrids for the genus *Drosophila*. There are included in this list a few cases about which there may be some question, because of the fact that the

parent forms have not been ranked above "races" or "sub-species." A case in point is represented by races A and B of *D. pseudoobscura.* In phenotype these two forms are practically indistinguishable, so that their separation into races is based on genetic results from cross-matings. As Dobzhansky (1937) has pointed out, these two races should be regarded as distinct species. Hence their hybrids may be termed interspecific. The same is true for the other similar crosses in the list, although in most instances the difference in phenotype of the parent forms is quite distinct.

Records from Other Laboratories

Melanogaster-simulans: The first discovered case of interspecific hybridization in *Drosophila* was reported by Sturtevant in 1920, and involved crosses between *D. melanogaster* and *D. simulans.* The hybrids are invariably sterile and their sex-ratios vary according to the way the cross is made. The cross *melanogaster* ♀ X *simulans* ♂ gives only female offspring. The reciprocal cross, *simulans* ♀ X *melanogaster* ♂, goes less well and usually produces only males; occasionally a few females. Sturtevant has shown that the hybrids survive only if they carry a *simulans* X chromosome, and that survival is not usual in the presence of *simulans* egg cytoplasm and a *melanogaster* X, even though a *simulans* X is also present. Several studies have been made on the salivary gland chromosomes of the hybrid larvae (Pltau, 1935; Kerkis, 1936, 1937; Horton, 1939). The last named investigator found ten chromosomal rearrangements, of which six are classed as inversions and four as changes of one or a few bands at the free ends of certain chromosomes. He also found fourteen short areas where the chromosomes do not ordinarily synapse.

Pseudoobscura-miranda: The second case of interspecific hybridization was not found until nearly a decade later. In 1929 Lancefield reported that *D. pseudoobscura* (then listed as *D. obscura*) was represented by two "races," A and B, and that of the offspring from the reciprocal crosses between the two races, the males are sterile but the females are partially fertile when backcrossed to either parent male. Tan (1935) showed that races A and B differ in their salivary chromosomes in six inverted sections; four in the X and one each in the second and third chromosomes.

Six years later Dobzhansky (1935) added a third member to the group and described it as *D. miranda.* This new species hybridizes with both races of *D. pseudoobscura,* thus producing the third and fourth cases of hybrids. The cross *miranda* ♀ X *pseudoobscura* ♂ produces males and females in equal numbers, while the reciprocal cross, *pseudoobscura* ♀ X *miranda* ♂, produces mostly females. It was at first stated that all of the hybrids were completely sterile, but later it was found that female hybrids derived from crosses with certain strains of *miranda* are slightly fertile (Macknight, 1939). *D. miranda* differs from *D. pseudoobscura* A and B in a large number of rearrangements in the salivary chromosomes (Dobzhansky and Tan, 1936).

Affinis group: Three different cases of interspecific hybridization have been reported for this group. The first case was recorded by Sturtevant and Dobzhansky in 1936, and involved the cross between *D. athabasca* and *D. azteca*. Both reciprocal crosses produce hybrids, but that of *athabasca* ♀ X *azteca* ♂ is more difficult to obtain. All of the hybrids have rudimentary gonads and are sterile. More recently Miller (1939, 1941) has reported two other cases of interspecific hybridization for this group. He found that *D. athabasca* will hybridize with *D. affinis* and *D. algonquin*. Hybrids were obtained in the cross *algonquin* ♀ X *athabasca* ♂. The male hybrids are sterile, but the female hybrids are fertile in the back-cross to *algonquin* males. The hybrid larvae show but little synapsis in their salivary gland chromosomes. In the other combination, *D. affinis* ♀ X *D. athabasca* ♂, the hybrid production was found to be low, although the frequency of insemination of the *affinis* females was quite high. Both male and female hybrids from this cross are sterile.

Virilis-americana: In 1936 Spencer discovered a new form in Ohio which he classified as a subspecies of *D. virilis* and named it *D. virilis americana*, the original species then becoming *D. virilis virilis*. Later (1938, 1940a) Spencer showed that cross-matings give a few hybrid offspring. In the cross *virilis* ♀ X *americana* ♂, which is more successful than the reciprocal mating, only about two per cent of the eggs develop. Both male and female hybrids from the two crosses are partially fertile when mated inter se or back to either parent form. Hughes (1939a) has shown that the metaphase plate in the female of *americana* has two pairs of V-shaped chromosomes, a pair of rods and a pair of dots. In the male the plate shows a pair of V's, a single V paired with two rods, a pair of rods and a pair of dots. This condition is in sharp contrast to the one found in *virilis*, in which the metaphase plate of either male or female has five pairs of rods and one pair of dots.

It has been shown independently and almost simultaneously by three different investigations (Patterson, Stone, and Griffen, 1940; Chino, 1940; Stalker, 1940) that the V-shaped chromosomes in *americana* are the result of "fusions." One of the pair of V's of *americana* is the equivalent of rods 2 and 3 of *virilis*. The X is fused with chromosome 4, so that the female has two pairs of V's and one pair of rods, while the male has but three V's, due to the fact that there is no Y–4 fusion. The salivary gland chromosomes of the *virilis-americana* hybrid larvae have inversions in the X, 2, 4, and 5 chromosomes (Hughes, 1939b; Patterson, Stone, and Griffen, 1940). For further details of this case the reader may be referred to the last article in this publication.

Palustris-subpalustris: Spencer has reported the discovery of a second case of hybridization between two forms which were collected in a swamp in Ohio. In connection with his preliminary report on this case, he states that cross-matings produce partially fertile hybrids and quotes Stalker to the effect that, "both reciprocal crosses are made if large numbers of individuals are used. The hybrid salivaries present a tangled picture, with long regions of loosely paired chromosomes (1940b, p. 306)." In a

letter dated March 23, 1941, Professor Spencer states that, "there appears to be considerable variation in the ease with which various strains of the two species cross."

Occidentalis-suboccidentalis: Recently, in a paper describing six new species of the quinaria group, Spencer (1942) states that *occidentalis* and *suboccidentalis* will hybridize, but he does not indicate whether the hybrids are fertile.

Records from the Texas Laboratory

Melanopalpa Group: One of the subdivisions of the very large *repleta* species group is what may be called the melanopalpa group. Dr. Linda T. Wharton has carried out a series of tests involving crosses between *D. melanopalpa* Patterson and Wheeler, *D. neorepleta* Patterson and Wheeler, and *D. repleta* Wollaston. The degree to which hybrids are produced depends upon the strain of *repleta* used in the cross. Her results are as follows:

1. *melanopalpa* ♀ X *repleta* ♂ (Japan, Eagle Pass, Elgin, New Haven, Guatemala strains) gives a few male and female hybrids, which apparently are completely sterile when inbred; reciprocal mating is cross-sterile. In the cross to the strain of *repleta* from Guatemala, male- and female-like intersexes are produced in addition to phenotypically normal offspring.
2. *melanopalpa* ♀ X *neorepleta* ♂ gives fertile male and female hybrids; reciprocal cross gives same result, but goes less readily.
3. *neorepleta* ♀ X *repleta* ♂ (Guatemala strain) gives a few abnormal offspring; reciprocal mating cross-sterile. This species fails to hybridize with all other strains of repleta.

The species of the melanopalpa sub-group form a series of three different cases of hybridization, of which one produces fertile hybrids. Details of these tests will be found in Article III of this publication.

Mulleri group: An interesting series of interspecific hybrids has been obtained in crosses between members of what we have called the mulleri group. In reality, these forms constitute a sub-group of a larger taxonomic unit known as the repleta group. In preliminary papers (Patterson and Crow, 1940; Crow, 1941) some members of this sub-group were classified as subspecies, but more recent studies would seem to justify ranking them all as distinct species. On the basis of morphological differences alone, one can easily distinguish six species of mulleri-like forms among our collection of wild species (Patterson and Wheeler, 1942). One of these *(D. meridiana)* does not produce offspring when mated to other members of the group.

Drosophila mulleri Sturtevant occurs mainly in Texas, although we have collected two specimens on the Florida Keys and two others near Shreveport, Louisiana. We have also received two individuals from the state of Coahuila, Mexico. *Drosophila aldrichi* also occurs in Texas, where it is

fairly common. A single specimen has been taken in Oklahoma, and our collecting crew trapped a total of fifty-nine specimens in cactus patches near Hermosillo, Mexico. *Drosophila mojavensis* is found in the deserts of California. The type material of *D. arizonensis* came from Tucson, Arizona, and recently this species, or a very closely related form, has been collected in the southeastern corner of Arizona and in Sonora, Mexico. *Drosophila buzzatii* is represented by single stocks from Argentina and Sicily. Mass-matings between these five species have thus far produced eight different cases of hybridization, as follows:

1. *mulleri* ♀ X *aldrichi* ♂ gives sterile male and female hybrids; reciprocal mating is cross-sterile.
2. *mulleri* ♀ X *mojavensis* ♂ gives fertile female and sterile male hybrids; reciprocal mating is cross-sterile.
3. *mulleri* ♀ X *arizonensis* ♂ gives sterile male hybrids; reciprocal mating is cross-sterile.
4. *mulleri* ♀ X *buzzatii* ♂ gives abnormal flies which usually die in pupal stage; reciprocal mating is cross-sterile.
5. *aldrichi* ♀ X *mojavensis* ♂ gives sterile female hybrids; reciprocal mating is cross-sterile.
6. *aldrichi* ♀ X *arizonensis* ♂ gives sterile female hybrids; reciprocal mating is cross-sterile.
7. *mojavensis* ♀ X *arizonensis* ♂ gives fertile male and female hybrids; reciprocal cross gives fertile female and sterile male hybrids.
8. *arizonensis* ♀ X *buzzatii* ♂ gives hybrid larvae which die by mid-larval stage; reciprocal mating is cross-sterile.

In several of the combinations it was necessary to use large mass matings in order to obtain any hybrids, and even then the number produced was small. In the cross *arizonensis* ♀ X *buzzatii* ♂ a mass mating of seventy-five pairs usually yields about a half dozen larvae. Every effort to bring these larvae to the imago stage has failed. They never reach the pupal stage and usually die shortly after hatching from the egg. In the other successful cross with *D. buzzatii*, *mulleri* ♀ X *buzzatii* ♂, the larvae reach the pupal stage, but die in this stage. Only a single hybrid fly has been obtained, and this was an abnormal, sterile female with rudimentary ovaries. Salivary gland preparations of the hybrid larvae show the chromosomes to be rather poorly synapsed, but with few or no rearrangements.

It will be observed from the data listed above that, *mulleri* females are cross fertile with males of the other four species, *aldrichi* females with *mojavensis* and *arizonensis* males, *arizonensis* females with *mojavensis* and *buzzatii* males, *mojavensis* females with *arizonensis* males, and that *buzzatii* females are cross-sterile with all other types of males. A large majority of the hybrids are sterile. The only exceptions are the hybrids from *mulleri* females to *mojavensis* males, and those from the reciprocal crosses between *mojavensis* and *arizonensis*. From the facts stated above,

it is clear that *D. meridiana* is completely separated from all other members of the group, with *buzzatii* and *aldrichi* next in order. The most closely related species are *mojavensis* and *arizonensis*. These two forms would have been ranked as subspecies by us were it not for the fact that morphologically they are easily distinguishable, even in pinned specimens. Furthermore, the results obtained in the crosses to other members of the group show that considerable differences exist in their genotypes.

The outstanding result obtained in the tests listed above was the failure of *mulleri* males to produce hybrids when mated to females of all the other species, although *mulleri* females produced some sort of hybrids in crosses with the males of all of these species.

Melanica group: Five forms are now known for this species group, as follows: *Drosophila melanica* Sturtevant, which is found scattered throughout the southern states from Florida to Arizona, and even down into Mexico; *D. nigromelanica* Patterson and Wheeler, which occurs both in the north and the south of eastern United States; *D. micromelanica* Patterson, which is found in some of the southern states; *D. melanica paramelanica,* a new subspecies (to be described elsewhere), which seems to be confined to the northern part of the country, from Wisconsin to the Atlantic seaboard; and *D. melanissima* Sturtevant, which is found in the southern states, from Florida to east Texas.

Drosophila melanissima has never been bred in the laboratory, and Dr. A. B. Griffen's tests have shown that *micromelanica* does not cross with any of the other forms. His results indicate that there is considerable variation in the degree of cross fertility between the other three forms, with success often depending on the geographical strains used in the tests. In the first place, they show that *melanica* is cross-sterile to all strains of *nigromelanica,* and this is one of the main genetic differences between *melanica* and *paramelanica,* which will hybridize with *nigromelanica.* His results, which are presented in Article V of this publication, are as follows:

1. *nigromelanica* ♀ X *paramelanica* ♂ gives a few fertile male and female hybrids; reciprocal mating gives the same result.
2. *melanica* ♀ X *paramelanica* ♂ gives a few fertile male and female hybrids; reciprocal mating gives the same result.

The number of fertile crosses is usually less than one per cent. The first cross above represents a case of interspecific hybridization; the second one of interracial hybridization.

In some of our previous publications, the name "submelanica" was applied to the southern strains, but it now seems clear that these strains really belong to *D. melanica* Sturtevant. The use of the term submelanica should, therefore, be discontinued, since it was applied to a species already described. Professor Sturtevant first called the writer's attention to the fact that the type material for *melanica* came from southern Alabama, and he suggested that the northern form was probably the undescribed one. Griffen's results show that this is the case, and we are using the

subspecific name of *paramelanica,* suggested by Sturtevant, for the northern form.

Macrospina group: The macrospina group is composed of three known species. These are the cosmopolitan form *D. funebris* Fabricius, *D. subfunebris* Stalker and Spencer, and *D. macrospina* Stalker and Spencer. Apparently, *D. funebris* is completely cross-sterile to the other two species and for that reason may be disregarded in this account of interspecific hybridization. *Drosophila subfunebris* is known only from the vicinity of Pasadena, California. *Drosophila macrospina* has been divided into three subspecies, as follows: *D. macrospina macrospina* Spencer based on the type material from central Texas; *D. macrospina ohioensis* Spencer from Ohio; and *D. macrospina limpiensis* Mainland from west Texas, New Mexico, southern Utah, Arizona, and Sonora, Mexico. All of these subspecies are represented by numerous geographical strains which tests have shown often differ from one another.

The crosses between *subfunebris* and *limpiensis* or *macrospina* may be regarded as interspecific in character, while those between *limpiensis* and *macrospina* would be interracial. In the following list of crosses the first three represent cases of interspecific hybridization, and the fourth a case of interracial hybridization. For a detailed account of these hybrids the reader is referred to Article VI of this publication.

1. *subfunebris* ♀ X Utah or N. M. *limpiensis* ♂ gives fertile female and sterile male hybrids; reciprocal cross gives the same result.
2. *subfunebris* ♀ X west Texas *limpiensis* ♂ gives fertile female and sterile male hybrids; reciprocal mating is cross-sterile.
3. *subfunebris* ♂ X central Texas *macrospina* ♀ gives fertile female and sterile male hybrids; reciprocal mating is cross-sterile.
4. *limpiensis* ♀ X *macrospina* ♂ gives fertile female and sterile or semisterile male hybrids; reciprocal cross is fertile and gives fertile male and female hybrids.

Virilis group: We have found here in the Southwest and elsewhere a series of forms belonging to the virilis group which consists of five known species. These are *Drosophila virilis* Sturtevant, *D. americana* Spencer, and three new forms named *D. texana, D. novamexicana* and *D. montana* (see Patterson and Wheeler, 1942). Because of difference in pupa color, we have been referring to the various strains of *D. virilis* as the "gray forms" and to the other species as the "red forms." On the basis of additional information concerning differences in habitats, we deem it best to refer to the strains of *D. virilis* as the domestic forms, and to the others as the wild forms.

When mated together the strains of the domestic form, *virilis,* are highly cross fertile, and although the results from domestic-wild crosses demonstrate that the various strains of *virilis* may differ from one another, yet they should be classified as a single species. In a previous publication (Patterson, 1941) the four forms then known were ranked as subspecies,

following the lead of Spencer in designating *americana* from Ohio as *D. virilis americana.* The results derived from cytological analysis and genetic tests now make it clear that these five forms should be regarded as separate species.

The metaphase plates of the five species differ from one another in one or more respects. The constitutions of the metaphase plates of *virilis* and *americana* have already been given in the preceding section. In *texana* there are three pairs of rods, one pair of V's, and a pair of dots, with the V-shaped element derived by a fusion of chromosomes 3 and 4. In *montana* there are four pairs of rods, one pair of small V's and a pair of dots. In this species the V-shaped element is not the result of a fusion of rods, but is due to an inversion in chromosome 2 which has placed the centromere near the middle of the long euchromatic arm. Finally, in *novamexicana* there are five pairs of rods and a pair of dots, the same as in *virilis,* but the salivary chromosomes of the two species differ in respect to several inversions.

Interspecific crosses between the different wild forms, and between them and *D. virilis,* have given a total of eight different cases of hybridization. With the exception of the crosses between *texana* and *americana,* the number of hybrids produced in the different combinations is relatively small. The list is as follows:

1. *virilis* ♀ X *americana* ♂ gives fertile male and female hybrids; reciprocal mating gives the same result.
2. *virilis* ♀ X *texana* ♂ gives fertile male and female hybrids; reciprocal mating gives the same result.
3. *virilis* ♀ X *novamexicana* ♂ gives few fertile female and sterile male hybrids; no information on reciprocal mating.
4. *virilis* ♀ X *montana* ♂ gives few fertile female and sterile male hybrids; reciprocal mating gives same result.
5. *americana* ♀ X *texana* ♂ gives fertile male and female hybrids; reciprocal mating gives the same result.
6. *americana* ♀ X *novamexicana* ♂ gives fertile male and female hybrids; no information on reciprocal mating.
7. *montana* ♀ X *americana* ♂ gives few offspring, thus far sterile; reciprocal mating cross-sterile.
8. *montana* ♀ X *texana* ♂ is cross-sterile; reciprocal mating gives fertile male and female hybrids.

SUMMARY

In summarizing the data from the two sources of records on interspecific hybrids, we shall count as one case of hybridization each successful combination irrespective of whether or not the cross goes one or both ways. On this basis there have been reported a total of thirty-one cases of hybridization, of which twenty-two different types of hyrids are known to be fertile. This number is certain to be increased in the near future,

so that eventually there will be a considerable body of material available for a critical analysis of the genetics of evolution in the genus.

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II. HETEROSIS IN DROSOPHILA HYDEI

WILSON S. STONE

Drosophila hydei is a cosmopolitan species with a large population in the various sections of the United States in which we have collected. It has next to the largest population of Drosophila in Texas and was found in almost all collections made at different points in the State. This species is very vigorous and fertile when tested under laboratory conditions. For these reasons it was chosen to determine the relations between this type of dense population and the extent of genetic and cytological variability, measured in terms of fertility and fecundity.

MATERIALS AND METHODS

Stocks from different sections of the United States, as well as from several other regions of the world, were used in making the tests. Each stock employed was derived from a single female fertilized in nature, or else from a single pair. We wish to thank Dr. W. P. Spencer for a stock from Yucatan, known as *D. hydei yucatanensis;* Dr. Eloff for the Rand stock from South Africa; and Dr. Buzzati-Traverso for the Milano stock from Italy. The places of origin of the several stocks are indicated in the tables.

Fertility was determined from pair matings, and usually 100 pairs were tested, although only 50 to 60 pairs were checked for the F_1 x F_1 results displayed in Table 1. The term fertility as used here means the per cent of the pairs tested that produced offspring. The fecundity of *hydei* was too high to be determined from these tests because overcrowding prevents hatching. Special tests were therefore run with four stocks, *yucatanensis*, Rand, Limpia, and Goleta. Pairs of flies were mated on the day of their emergence, and thereafter daily transfers were made until their offspring began to appear. Hatch counts were made on some of the days from a number of different pairs for each cross. The term fecundity as here used means the number of offspring per pair per day. The gene arrangement present in these and several other stocks was checked in the salivary glands and larval ganglion. Only a few showed differences in gene order, but this will be reported later.

RESULTS

The results from the P_1 and F_1 tests are given in Table 1. All except the Goleta stock from California seemed to be sufficiently similar in genotype to cross very readily. The Goleta stock consistently gave fertility values below 85 per cent if used as the male parent, but no other stock gave such values in these tests. Hatch counts are not given in Table 1. No F_1 test for any stock gave a fertility lower than the parents. Such

fluctuations as exist are most probably the result of chance. On the contrary, heterosis is evident from the fact that almost universally the number of pairs of F_1 hybrids that were fertile was greater than the number of their parents.

Table 2 shows the results of the more critical tests of fertility and fecundity. These tests all involved 100 or more pairs in the test for fertility. The fecundity test gives the average number of individuals from one pair from one day's egg laying. The hatch counts were made from a consecutive series of four to eight days between the 10th and 20th days after the flies were mated, and from a number of pairs of flies for each cross. There was sufficient food in the vials so that the lack of food had little or no effect on the hatch. This table shows that hybrid vigor is effective in increasing fecundity as well as fertility. Certain other data on fertility of *virilis* (Patterson, Stone, and Griffen, 1942) are included for comparison in Tables 3, 4, and 5.

Discussion

Strains of *hydei* from several parts of the world are sufficiently similar to cross readily with one another. The Goleta stock showed some consistent reduction in cross-fertility. Furthermore, the fertility of the F_2 was reduced in some crosses with this stock. With this exception, crossing usually produces heterosis. Dr. Crow's calculations (see appendix) show that hybrid vigor is the rule not only in *hydei*, but also in both asiatic and southwest domestic *virilis* as well as two of the wild forms of *virilis*. This consistent heterosis is of interest in view of the marked difference in habitat and population density that exists between these species. Patterson (1942) has reported the origin of the few hundred specimens of *virilis* which have been captured. In contrast to this, 133,460 specimens of *hydei* have been recorded from Texas alone.

The comparisons in fertility of the control, P_1, F_1, and F_2 means averaged from Tables 1 through 5. They show consistently that heterosis is present in crosses between strains of these several species.

Hydei from Table 1		Hydei from Table 2	
Control mean	= 89.6	Control mean	= 87.7
P_1 mean	= 90.0	P_1 mean	= 74.7
F_1 mean	= 98.1	F_1 mean	= 88.1
		F_2 mean	= 84.6

Wild virilis from Table 3

	T x T	A x T	Both
Control mean	= 56.0	59.3	58.6
P_1 mean	= 62.1	56.1	59.2
F_1 mean	= 80.8	91.2	86.0

Asiatic Domestic Forms from Table 4		Southwest Domestic Forms from Table 5	
Control mean	= 85.3	Control mean	= 95.0
P_1 mean	= 89.9	P_1 mean	= 91.6
F_1 mean	= 94.7	F_1 mean	= 96.9

Heterosis increases fecundity as well as fertility (Table 2). This can best be seen in the average values calculated from the original data used in Table 2. These values are:

$$\text{Controls} \quad \frac{4326 \text{ flies}}{104 \text{ days}} = 41.6 \text{ flies per day.}$$

$$P_1 \quad \frac{16803}{373} = 45.0 \text{ flies per day.}$$

$$F_1 \quad \frac{21901}{364} = 60.2 \text{ flies per day.}$$

$$F_2 \quad \frac{38238}{651} = 58.7 \text{ flies per day.}$$

In addition to these measured effects, size and viability seem to be increased, although no exact measurements were made of these factors.

Heterosis extends through the F_1 to the F_2, so that we can calculate the cumulative effect. This calculation must be based on both fertility and fecundity for each generation. These values are:

	Per Cent of Pairs Fertile		Flies/Day	= Net fecundity of population
Control	87.8	×	41.6	= 36.5 flies/day
P_1	74.7	×	45.0	= 33.6 "
F_1	88.1	×	60.2	= 53.0 "
F_2	84.6	×	58.7	= 49.7

This cumulative effect through these three generations gives rise to the following differences:

Control (av.) $36.5 \times 36.5 \times 36.5 = 48627$
$P_1 \quad F_1 \quad F_2$
Crosses (av.) $33.6 \times 53.0 \times 49.7 = 88606$
$88606/48627 = 1.82.$

Therefore these differences, without taking into account the effect of hybrid vigor on viability, makes the average of the crosses between strains 1.82 times more efficient in reproduction than the controls.

In order to approximate these differences in terms of actual numbers of flies, it is necessary to make certain assumptions. The reproductive cycle (from egg to egg) is about twenty days with only fourteen days of normal egg laying. We must neglect mortality as we cannot measure it, and assume a normal sex ratio. If we start with one representative pair

for each type of experiment and run through three 20-day cycles, the number of descendants from the control pair would number some thirty-three million, while the number for the crosses would be some sixty million. While such ideal conditions would never hold in nature, yet *hydei* does show nearly comparable increases at certain seasons of the year, particularly in the citrus dumps of south Texas.

The general problem of heterosis has been discussed at length by East (1936), Powers (1941), and Jones (1942). The material and evidence they discuss was designed to show how the several genes in the particular genome interacted. These experiments were not designed to study the reason for heterosis, but rather to test for heterosis in natural populations of *Drosophila.*

The extent of hybrid vigor here is not quite as great as that shown in corn yield (Lindstrom, 1939). Nevertheless, it is quite marked and is shown here to exist in several types of natural populations. The fact that F_2 retained such a marked effect must be due to the limitations imposed on recombinations by linkage. The several cumulative and completely or partially dominant genes for vigor from each stock must be so distributed in the chromosomes that they can seldom be accumulated by crossingover and segregation into one gamete.

The presence of these several different gene combinations for vigor in these natural populations of a single species affords some indirect evidence for the mutation and fixation of dominant beneficial genes in the various local populations.

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APPENDIX: STATISTICAL COMPARISONS*

The comparison of the fertility percentages was made by means of analysis of variance, using a table of F distribution (Snedecor, 1937). Since, in percentages the theoretic variance is a function of the proportion p, there may be serious errors incurred by the use of this technique when percentages are very high or low (i.e., over 85% or under 15%). Bliss (1937) has recommended the use of the transformation $p = \sin^2\theta$.

*Dr. J. F. Crow, now of Dartmouth, was kind enough to make these calculations.

The use of the angular value θ makes the estimated variance independent of the true value of θ if the sample is large. As p varies from 0 to 100%, θ changes from 0° to 90°. This transformation was used in the following comparisons which are between the P_1 and F_1.

P_1 and F_1.

Wild *virilis* (T x T) : $F = 8.82$, D.F. $= 1$ and 9, $P = .05 - .01$
(T x A) : $F = 42.9$, D.F. $= 1$ and 9, $P < .01$
(Total) : $F = 36.6$, D.F. $= 1$ and 19, $P < .01$
Asiatic domestic *virilis* : $F = 14$, D.F. $= 1$ and 35, $P < .01$
Southwest " " : $F = 17$, D.F. $= 1$ and 31, $P < .01$
hydei (table 1) : $F = 92$, D.F. $= 1$ and 79, $P < .01$

Although there was no constant difference among the several stocks used, the difference between the P_1 and F_1 was highly significant in each case.

TABLE 1.

Fertility in *hydei*.

	Control		Galveston, Texas		La Lajita Mexico		Rand S. Africa		Limpia Canyon, Texas		yucatanensis, Mexico	
CONTROL		Per Cent	92		88		98		94		64	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	Per Cent		Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent
Conroe, Texas	87	P_1	93	89	98	95	96	95	94	91	91	97
		F_1	98	96	100	100	100	96	100	94	89	92
Goleta, California	95	P_1	99	69	94	80	87	80	94	56	93	68
		F_1	94	98	98	100	87	98	98	100	98	100
Tucson, Arizona	75	P_1	93	84	86	93	91	95	87	97	87	90
		F_1	100	98	100	94	100	98	96	96	100	100
Milano, Italy X Y Galveston, Texas	93	P_1	98	97	96	95	89	98	98	87	83	97
		F_1	96	94	96	100	100	100	100	100	100	100
Columbus, Mississippi	93	P_1	98	89	100	95	80	90	90	96	59	97
		F_1	100	98	98	96	98	98	84	96	100	94
Cliff, N. Mexico	100	P_1	88	93	95	97	93	99	90	96	76	92
		F_1	100	100	98	100	100	100	96	96	100	100
Minneapolis, Minnesota	88	P_1	89	75	82	88	96	86	91	73	84	76
		F_1	100	100	92	100	96	100	100	100	98	100
Milano, Italy X Marfa, Texas	98	P_1	98	97	83	93	89	93	99	98	98	98
		F_1	100	100	100	100	100	98	98	100	98	100

TABLE 2.

Fertility and Fecundity in *hydei*.

	♂ / ♀	Limpia, (Texas)			Goleta, (California)			yucatanensis, (Mexico)			Rand, (South Africa)		
		P_1	F_1	F_2	P_1	F_1	F_2	P_1	F_1	F_2	P_1	F_1	F_2
Limpia	per cent fertile	94.1			55.6	100	87.5	75.0	49.5	78.0	49.0	76.4	89.8
	average hatch/day	51.0			67.5	68.5	58.4	32.7	43.4	35.9	38.9	73.1	57.6
Golenta	per cent fertile	94.3	98.2	67.9	94.8			92.9	98.2	63.2	86.5	86.5	84.7
	average hatch/day	57.7	56.1	65.3	46.8			52.5	44.1	63.4	55.3	53.0	54.3
yucatanensis	per cent fertile	63.3	81.1	89.6	68.0	100	90.6	64.2			83.6	98.0	84.2
	average hatch/day	33.6	55.5	64.5	35.3	55.7	61.4	31.4			30.4	63.2	61.3
Rand	per cent fertile	71.1	85.9	97.9	79.8	98.1	90.3	77.5	82.8	91.9	97.9		
	average hatch/day	40.8	65.5	68.9	50.4	65.8	51.6	29.4	66.2	54.4	35.4		

TABLE 3.

Cross-fertility of wild *virilis*.

	Control		821.12b (texana)	821.12c (texana)	825.13c (texana)	841.10 (texana)	849.11 (texana)
Control			44	55	67	72	44
Americana ♀	74	P_1	81	35	22	66	68
		F_1	100	94	86	96	96
Americana ♂	74	P_1	66	89	45	25	66
		F_1	94	90	78	88	90
Texana ♀ (84.7)	54	P_1	71	55	45	57	78
		F_1	92	86	54	74	90
Texana ♂ (84.7)	54	P_1	86	23	62	54	90
		F_1	90	96	64	64	98

TABLE 4.

Fertility of Asiatic domestic *virilis*.

	Control		Hanchow	Hiroshima	Kirin	Mukden	Otaru	Peking	Sendai	Shengking	Tokyo
Control			92	94	79	85	75	88	86	89	52
V ♀	100	P_1	89	91	93	90	85	99	71	91	96
		F_1	92	98	90	98	100	98	88	98	100
V ♂	100	P_1	80	97	94	88	92	99	96	94	94
		F_1	100	88	100	100	100	100	74	98	98
H ♀	98	P_1	92	92	89	94	80	93	78	93	87
		F_1	100	82	96	88	98	98	100	92	84
H ♂	98	P_1	93	87	91	92	97	98	57	92	93
		F_1	98	82	100	100	90	98	96	92	94

TABLE 5.

Fertility of Southwest domestic *virilis*.

	Control		Beaumont 754.9a	Blanco 290.3	Galveston 472.4	Galveston 494.5a	San Antonio 725.9f	Victoria 718.7a	California 863b	California 863e
Control			95	95	100	98	91	100	80	93
V ♀	100	P_1	94	95	95	96	93	98	87	79
		F_1	100	100	94	100	100	94	100	100
V ♂	100	P_1	97	99	95	96	86	94	91	93
		F_1	100	100	96	98	98	100	98	100
H ♀	98	P_1	100	95	85	97	84	99	87	96
		F_1	100	98	94	96	98	96	100	98
H ♂	98	P_1	94	98	78	96	90	97	82	65
		F_1	98	98	98	58	96	98	98	100

III. ANALYSIS OF THE REPLETA GROUP OF DROSOPHILA

LINDA T. WHARTON

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 genic balance and chromosome organization in *Drosophila;* the second phase, undertaken in the light of the first, is a study of the problem of speciation. The study of the repleta group, which is presented in this paper, employs both of these 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, and genic balance in the heterozygotes.

PART I

COMPARATIVE MORPHOLOGY OF THE CHROMOSOMES

Material and Methods

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

The analysis given here 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. *Drosophila 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. *Drosophila mulleri* Sturtevant, stock tested was collected at Aldrich farm, Austin, Texas, by Patterson.
3. *Drosophila aldrichi* Patterson and Crow (1940); (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. *Drosophila arizonensis* Patterson and Wheeler (1942). The stock tested was established from a female trapped in Arizona, September, 1940, by Mainland.

5. *Drosophila buzzatii* Patterson and Wheeler (1942). Stocks collected in Cordoba, Argentina, and Trapani, Sicily, were checked.
6. *Drosophila mojavensis* Patterson and Crow (1940); (redescribed by Patterson and Wheeler, 1942). This stock was collected by Spencer at Mesquite Springs, Death Valley, California.
7. *Drosophila longicornis* Patterson and Wheeler (1942), stock 514.5a, collected by Patterson at Aldrich farm, Austin, Texas, 12/17/39.
8. *Drosophila 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. *Drosophila* sp. *(meridiana-like)* undescribed, stock 394.3d, collected at Aldrich farm, Austin, Texas, by Patterson 10/26/40.
10. *Drosophila peninsularis* Patterson and Wheeler (1942), stock 1148.7, collected at Lake McKethan, Florida, by Mainland and Wheeler 6/19/41.
11. *Drosophila hamatofila* Patterson and Wheeler (1942), stock 539.4a, collected by Patterson at Uvalde, Texas, 1/22/40.
12. *Drosophila bifurca* Patterson and Wheeler (1942), stock 911.7m, collected by Mainland in Wild Rose Canyon, Texas, 9/22/40.
13. *Drosophila brevicarinata* Patterson and Wheeler (1942). The stock tested was collected in San Josecito, Mexico, and was sent to us by Sturtevant.
14. *Drosophila ritae* Patterson and Wheeler (1942), stock 911.5c, collected by Mainland in Wild Rose Canyon, Texas, 9/22/40.
15. *Drosophila linearepleta* Patterson and Wheeler (1942) is a stock obtained by us from Sturtevant; it was collected by Dobzhansky at Antigua, Guatemala.
16. *Drosophila nigrospiracula* Patterson and Wheeler (1942), stock 1254.3a, collected by Mainland and Wagner in Magladena, Mexico, 8/23/41.
17. *Drosophila hydei* Sturtevant, stock 914.2, collected in Limpia Canyon, Texas, 9/22/40, by Mainland.
18. *Drosophila nigrohydei* Patterson and Wheeler (1942), stock 1232.9b, collected in the Chisos Mountains, Brewster County, Texas, 8/14/41, by Mainland and Wagner.
19. *Drosophila leonis* Patterson and Wheeler (1942), was obtained by this laboratory from Sturtevant, and was collected at San Josecito, Mexico.
20. *Drosophila hydeoides* Patterson and Wheeler (1942), was obtained by this laboratory from Sturtevant, and was collected at San Josecito, Mexico.
21. *Drosophila mercatorum* Patterson and Wheeler (1942), stock 935.7b, collected by Mainland at Santa Barbara, California, 8/30/40.
22. *Drosophila fuliginea* Patterson and Wheeler (1942), stock 1283.10, collected seventeen miles from Silver City, New Mexico, 10/19/41, by Mainland and Wheeler.

23. *Drosophila neorepleta* Patterson and Wheeler (1942), was obtained from Sturtevant, and derived from a stock collected by Dobzhansky at Sacapulas, Guatemala.
24. *Drosophila 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. *Drosophila orbospiracula* Patterson and Wheeler (1942), stock 1232.1, collected in the Chisos Mountains, Brewster County, Texas, 8/14/41, by Mainland and Wagner.
2. *Drosophila polychaeta* Patterson and Wheeler (1942), stock 119.6a, collected by Ray in Galveston, Texas, 10/21/38.
3. *Drosophila spinofemora* Patterson and Wheeler (1942), derived from a stock sent from Hawaii by Zimmerman.
4. *Drosophila 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 Plates 1–5:

Eight species, *repleta, mulleri, arizonensis, aldrichi, buzzatii, mojavensis, longicornis, 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.

Drosophila sp. *meridiana-like* apparently differs from *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, *peninsularis* and *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, *bifurca, brevicarinata, ritae,* and *linearepleta* differ from the first group in that the X and Y chromosomes are of equal length. *Drosophila bifurca* is distinctive in that it has a constriction near the centromere of each rod-shaped chromosome.

Drosophila 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 the metaphase preparations, but are not correspondingly large in the salivary cells.

Drosophila 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.

Drosophila nigrohydei, leonis, and *hydeoides* each has six rod-like elements, the dots being absent. They differ from each other in several respects. *Drosophila 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. *Drosophila 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 *hydeoides,* the Y is shorter than the X, and no constrictions were noted; one of the autosomes is rather short.

Drosophila mercatorum has only five chromosome elements: two autosomal rods, a rod-chaped X chromosome with a proximal constriction, a large V-shaped chromosome derived from fusion, and a small V-shaped chromosome. At least one strain of 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.

Drosophila 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.

Drosophila neorepleta and *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. *Drosophila 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:

Drosophila orbospiracula has six chromosome elements consisting of four rod-shaped autosomes, a rod-like X with a constriction at its top, 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.

Drosophila 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.

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

Drosophila 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.

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

Discussion

The nature and effect of changes which alter the number and linkages of chromosomes in living forms has been the basis for much speculation and some experimental investigation.

Navashin (1932) advanced the "dislocation" hypothesis to explain observed increases and decreases in chromosome number. Dubinin (1934, 1936) succeeded in increasing and decreasing chromosome numbers, producing strains of *Drosophila 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 experimental changes in the genome of *melanogaster,* producing true breeding stocks in which genic balance and chromosome number were altered. In some strains parts of the X chromosome were, in effect, converted to autosomal material and vice versa.

Sturtevant (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; (2) the attachment of rod-shaped elements to form a V-shaped chromosome; (3) the attachment of part of another chromosome element to the dot; (4) the fusion of the dot-like chromosome with a rod.

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 large natural 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 species of the repleta group 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 here offer a reliable indication of the closeness of the relationships between members of the group. In fact, *melanopalpa* (Plate 4) and *repleta* (Plate 1), which cross in one direction with some readiness, show the extreme difference of two chromosome arms in metaphase preparations. Furthermore, 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. Some of the ways in which the described changes may have been accomplished are diagrammatically represented in Plate 6.

Drosophila meridiana-like (Plate 1) 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 suggested. 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 and Khvostova (1938) and Griffen and Stone (1940). Fusion of of this nature is demonstrated in the virilis group of species. *Drosophila texana* has an autosomal fusion involving chromosomes 3 and 4. *Drosophila americana* has two fusions, involving 2–3, and X–4. It should be noted that the condition in *americana* is probably derived from the *texana* condition. Furthermore, Griffen (Patterson, Stone, and Griffen, 1940) has indicated that chromosome 4 in *Drosophila virilis* has a short arm which was involved in the original fusion of *texana*. *Drosophila fuliginea* (Plate 4) has obviously undergone changes simliar to that in *meridiana-like,* in which four of its chromosomes are involved. It cannot be positively stated that the rod-like chromosomes in *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 later explanation is much less probable.

Within the species of the repleta group the dot-like chromosome, as seen in the metaphase preparations, varies in size. In *longicornis* it appears to be very small, for example. In *meridiana,* it is of intermediate size, while in *nigrospiracula* it is quite large. This variation in size is due to changes in the amount of heterochromatin. In seven members of the repleta group the dot-like element is not detectable in metaphase preparations. 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 is, with the exception of *hydei* (Plate 3), achieved concomitantly with the disappearance of the dot-like elements. Also, it will be observed that *nigrohydei* (Plate 3), *melanopalpa* and *neorepleta* (Plate 4) each has a pair of very short rods in the metaphase which may contain the dot-like chromosome.

Drosophila leonis (Plate 3) has a pair of peculiarly thin autosomes with constrictions near their centromeres, for which there are at least two possible explanations. Perhaps the short region near the centromere represents the dots, and the slenderness of these rods is due to a relatively less coiled state of distally added heterochromatin. Another explanation is that the distal heterochromatin suffers from nucleic acid starvation giving an effect similar to that demonstrated by Darlington and LaCour (1940). If so, this localized starvation is genetically controlled, since the Y chromosome is normal in appearance.

Drosophila fuliginea and *mercatorum* (Plate 4) 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* (Plate 5) of the virilis group. 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 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 cases of *nigrohydei, melanopalpa* and *neorepleta,* the constricted tip of the X may represent the dot which has become, in effect, the Y chromosome. This would involve a more complex change and is, therefore, somewhat less probable.

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 *hydei* is heterochromatic. The species *leonis, nigrohydei, mercatorum* and *fuliginea* have one chromosome which is entirely heterochromatic, unless it carries the dot-like element. *Drosophila 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.

The question naturally arose as to whether the extra heterochromatic arms represented a true increase of heterochromatic material or simply resulted from the redistribution of this material. This was checked in the metaphase preparations from hybrid larvae of the interspecific cross between *melanopalpa* females and New Haven *repleta* males, where a difference of more than two heterochromatic arms exists. If the greater

number of heterochromatic arms in *melanopalpa* was due simply to a redistribution of the heterochromatin, then the paired metaphase chromosomes should vary in length due to shifting of material. The V-shaped autosome and the J-shaped X-chromosome of *melanopalpa,* the dot-like element (lacking in *melanopalpa*) and the very short Y-chromosome of *repleta* were distinguishable in the hybrid metaphase preparation. The other autosomes showed no difference in length. (See diagram and camera lucida drawing, Plate 5.) Therefore *melanopalpa* has acquired more than two extra heterochromatic arms.

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 *mercatorum,* the number of centromeres is reduced to five. In *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 form 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 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). Free centromeres are produced as complementary parts of the translocations which give the fusions. All changes in the chromosome complexes of the species here reported have resulted from the retention of the fusion, and the loss of the free centromere with a reduction of the chromosome number. The large fusion element has been retained due to its gene content. Even if the free centromeres occasionally became fixed in some strains, they have always been lost before they could be utilized to increase chromosome number. The selective disadvantage of translocations involving parts of chromosome arms opposes the utilization of a free centromere or a Y chromosome centromere to increase the chromosome number.

The Y chromosome has been subject to a wide range of alterations in the repleta group. *Drosophila peninsularis* (Plate 2) and *hamatofila* (Plate 2) have small V-shaped Y chromosomes, and the dot-like chromosomes. *Drosophila mercatorum* shows an XO condition in the male. It is interesting to speculate that the small V-shaped chromosome of the XO *mercatorum* may be the result of fusion or translocation involving the small V-type Y chromosome and the dot-like element. In many of the species of the repleta group, the Y chromosome is extremely short, as in *repleta* and *nigrohydei. Drosophila longicornis* (Plate 1) represents an intermediate condition of the Y chromosome, which is distinctly unequal in length to the X, but not so short as in some of the other species. In *leonis* the Y chromosome is only slightly shorter than the X. The X chromosome of *nigrospiracula* (Plate 3) has a definite constriction at its tip which the Y chromosome lacks, making the latter somewhat the shorter of the two. In *bifurca* (Plate 2), as in four other members of

the repleta group reported here, the X and Y chromosomes are of equal length. In *hydei*, the Y chromosome is a long J-shaped body, and is about half the size of the V-shaped X chromosome.

It is particularly interesting to find such a wide range of differences in Y chromosome morphology, as almost every possible variation in length and shape is encountered in this closely related group. Dobzhansky (1937b), after a study of the variable Y chromosome in *Drosophila 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 supports this suggestion.

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 intrachromasomal changes and fusions. The stringent selection against translocations, except those with breaks next to the centromere, has been so effective that no case has been reported in *Drosophila.* This strengthens the conclusion that no such change is present in the repleta group.

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* (Plate 5) and *polychaeta* (Plate 5) 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 (Stone, Griffen, and Patterson, 1942) ; two such events have given *polychaeta* seven long 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 been derived from mutual translocations. The latter possibility is negligible because of the selective disadvantage of such translocations due to aneuploid gamete formation. There has been no easily detectable increase of euchromatic material, nor has there been any addition of centromeres.

Drosophila spinofemora (Plate 5) 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. Such a pericentric inversion of a V-shaped chromosome has been reported in *Drosophila algonquin* by Miller (1939).

Drosophila orbospiracula (Plate 5) is a particularly interesting XO type. Unlike the XO *mercatorum,* there is no heterochromatic arm present which might conceivably bear the Y material. The essential functions of the Y genes must, therefore, be carried out by genes in the X chromosome or in the autosomes. In this case the constriction on the X chromosome may represent a separation into an X and Y part of a compound chromosome derived from an X-Y translocation. The situation in *hydei,* which has a V-shaped X with one heterochromatic arm, and a J-shaped Y with one very short arm, is certainly most easily explained in this fashion.

There are instances in *Drosophila* species where there have been changes in the frequency of certain genes due to their linkage with the chromosomal sex determining systems, with a resulting alteration of the genic balance. Such cases have not been encountered in the repleta group, although genic balance is known to differ both between certain strains and between species (see Part II of this paper). *Drosophila americana* of the virilis group has an X-4 fusion. As a result, it now carries a free, sex-limited fourth chromosome which is accumulating mutations that cannot be selected through crossingover and recombination. *Drosophila pseudoobscura,* which has an X-autosome fusion, has actually lost the sex-limited chromosome thus derived, so certain genes in the male have passed from the diploid to the haploid condition in the sequence of events, and the genic balance has been altered. In the case of *Drosophila miranda,* which was derived from *pseudoobscura,* the third chromosome has become incorporated in the Y, and most of the genes of 3 have been lost. The free third, or X^2, has become, for the most part, haploid (Dobzhansky, 1935, 1937a, and McKnight, 1939.) Each of these cases represents a shift of genic balance in the same direction, with the decrease in the genic material of the autosomal complex, and the compensating increase in the genic material of the sex chromosomes. Sturtevant (1940) and Sturtevant and Novitski (1941) have tentatively suggested that *melanogaster* has been derived from the *pseudoobscura* type, which they consider the more primitive. This represents two compensatory shifts of genic balance, in opposite directions, involving the XR element of *pseudoobscura* (which is equivalent to 3L of *melanogaster*). Sturtevant later (1942) stated that "Any phylogeny of the subgenus *Drosophila* must be very speculative at present." In view of the frequent occurrence of increases in heterochromatic arms and the several cases of fusion which have been detected in *Drosophila,* in this and other recent studies, it does not seem necessary to assume that the primitive type which gave rise to *melanogaster* had the X-autosome fusion. It seems more logical to assume the derivation of both *melanogaster* and *pseudoobscura* from a primitive type which did not have the X-autosome fusion, but which may have resembled *pseudoobscura* more closely morphologically.

The change in the amount and distribution of heterochromatin in the repleta series may be compared to the fluctuation in frequency of the B chromosome in maize (Randolph, 1941). The presence of the B chromosome is apparently neither necessary nor beneficial to maize, although

several of these elements may be present without impairing viability or fertility. Very considerable reduplication of the B chromosome, however, has a detrimental effect on the system, lowering the viability and fertility of the plant, as well as causing phenotypic effects. It has not been possible to relate the B chromosome to any member of the basic chromosome complement, nor is it known whether it carries out any genic function related to that of the normal complement. In this respect it is somewhat comparable to a virus, being parasitic, or even pathogenic in its effect. The B chromosome may serve as a free centromere, as translocations to it have been obtained. This function of the B element to increase chromosome number is minimized by the irregular disjunction of the body, which makes difficult the establishment of such translocations. The constrictions of the B chromosomes in maize set off heterochromatic regions; perhaps certain of the constrictions observed in chromosomes of the *Drosophila* species are comparable. There is no evidence that extra heterochromatic material has a deleterious effect in these *Drosophila* species.

It is obvious that the variation in amount and distribution of chromosome material, as observed in *Drosophila,* and the change in frequency of chromosomes, as observed in maize, are not paralleled by equivalent variations in the amount and distribution of genic or euchromatic material. In cases where chromosome numbers vary from simple multiples of the *n* number, the actual extent of aneuploidy is questionable, except in the instances where it can be demonstrated without doubt, by evidence such as that afforded by salivary chromosomes, trisomic associations, or crossover configurations. A considerable variation in chromosome number, or seeming quantitative differences, may be due to heterochromatin, and may often represent little or no discrepancy in the actual amount of genic material.

As has already been implied on the basis of this evidence, the derivation of phylogenetic schemes from metaphase chromosome numbers and configurations, in plants or in animals, is not justifiable in the absence of much more critical evidence, morphological and genetic.

SUMMARY OF PART I

1. An analysis was made of the comparative morphology and organization of the metaphase and salivary gland chromosomes of twenty-five members of the repleta species group, together with four species from other groups of the genus *Drosophila.*

2. The chromosome complement in the salivary gland nuclei of all members of the repleta group was five long arms and a very short arm (the dot-like element). The free ends of the chromosomes were sufficiently characteristic to be easily identifiable in every case. Therefore no extensive interchromosomal modification, translocation, nor pericentric inversion has occurred in the group.

3. The basic haploid number of chromosomes, as observed in metaphase preparations of the repleta group of species, is five rods and a dot, the

equivalent of the five long arms and one short arm of the salivary gland chromosomes. Modifications of this condition which have been found in the study of their several metaphase plates are as follows:

(a) The number of centromeres is reduced due to fusion of rod-shaped elements. This has occurred in *meridiana-like* and *mercatorum,* reducing the centromere number to five, and in *fuliginea,* reducing the centromere number to four.

(b) In the metaphase chromosome configurations of some members of the repleta group, extra heterochromatic arms were observed. The V-shaped X chromosome of *hydei* showed one heterochromatic arm which has possibly been derived from an X-Y chromosome fusion. *Drosophila mercatorum, fuliginea, nigrohydei, leonis, hydeoides, neorepleta* and *melanopalpa* have acquired extra heterochromatic material, and the characteristic appearance of the dot-like chromosome in their metaphase configurations has been lost. Several suggestions were made as to the location of the dot in each of these species, and the association of its disappearance with the acquisition of additional heterochromatic arms was discussed.

4. The Y chromosome varies widely in its morphology. In *hamatofila* and *peninsularis,* the Y is a small V-shaped body. In *ritae,* and other species, the Y is as long as the X, in *nigrospiracula* it is slightly shorter, in *longicornis* it is of intermediate length, in *repleta,* it is very short, and in one strain of *mercatorum* it has disappeared from the metaphase configuration. The additional XO case reported in this study is *orbospiracula,* which belongs to another species group. It differs from *mercatorum* in that *orbospiracula* has no heterochromatic autosomal arms which might represent the Y.

5. No member of the repleta group shows an increase in the number of euchromatic arms in salivary chromosome preparations. *Drosophila montana,* of the virilis group, and *polychaeta* have six and seven euchromatic arms respectively. The increase of euchromatic arms in *montana,* in which the salivary chromosomes are known, is due to an inversion across the centromere of one of the autosomes. *Drosophila polychaeta* has seven euchromatic arms due to the occurrence of a pericentric inversion in each of two autosomes or, though it is not likely, due to two independent translocations involving the same chromosomes.

6. The primitive chromosome complement has been modified in *spinofemora,* which is not of the repleta species group. Two of its rod-like chromosomes fused to form a V-shaped body. Also, this species has one extraordinarily long chromosome arm which has been formed by the union of two of the originally separate euchromatic arms. This was accomplished either by the simple translocation of one of the chromosomes to the tip of the other, or, more probably, by a fusion of the two arms to produce a V-shaped chromosome, followed by a pericentric inversion, which made a long rod.

7. There is no instance of an increase in centromere number in *Drosophila* species thus far reported, although it is pointed out that such an increase could occur either through the use of the centromere of the Y, or by translocation to a free centromere which is produced by a fusion, provided that such translocations were not eliminated through selection.

8. *Drosophila melanopalpa* and *repleta* of the repleta group, species which show the greatest diversity of metaphase chromosome morphology, cross fairly readily, and produce phenotypically normal offspring.

9. Since it is possible for metaphase chromosome morphology to be very considerably altered without affecting the genotype to a corresponding degree, the metaphase chromosome configurations of plants and animals are wholly unreliable guides to phylogenetic relationships in the absence of other more critical genetic, cytological and morphological data. Furthermore, some reported instances of aneuploidy may be more apparent than real, except where proved by such evidence as trisomic associations or crossover configurations.

PART II

Studies of various sexually reproducing plants and animals have revealed numerous mechanisms, both genetic and environmental, which separate different species. In certain favorable material the genetic factors have been analyzed. In order to appreciate the role of these factors in evolution, it is important to know whether interspecific and intraspecific variations differ in kind, or merely in degree. Sexual, or psychological isolation very often operates between animal species. Similarly, sexual isolation is operative between several of the strains of the single species *Drosophila repleta.*

Material and Methods

The repleta stocks used in these tests include the following: Fredericksburg 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-Traverso.

Drosophila melanopalpa was collected by Mainland and Wagner in Cave Creek, Arizona. *Drosophila 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. In each case reciprocal crosses were made.

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

TABLE 1.

Initial fertility tests

♀♀ \ ♂♂	Fredericksburg	Elgin	New Haven	Guatemala	Eagle Pass
Fredericksburg (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 2.

The results obtained from mass matings of twenty-five pairs

♀♀ \ ♂♂	Fredericksburg	Elgin	New Haven	Guatemala	Eagle Pass	Ankara
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)	++

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, the females were dissected and examined 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 or consistently 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 *neorepleta, melanopalpa,* and some of the *repleta* stocks were made. *Drosophila repleta* strains tested to *neorepleta* and *melanopalpa* were New Haven, Eagle Pass, Rosenberg, Guatemala, and Japan. Ten crosses in vials using ten pairs of flies per vial, were made in each of these interspecific tests.

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 cross-sterility which appeared in the first crosses. An additional stock, Ankara, was also used in this test. The following facts were observed:

Fredericksburg females were sterile to Elgin males, but the reciprocal cross went reluctantly, producing twenty-one offspring. Fredericksburg females were sterile to New Haven males, but the reciprocal cross was quite fertile, producing over three hundred offspring. Fredericksburg females were practically sterile to Ankara, producing only fourteen offspring, but the reciprocal cross went readily, yielding 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 2) were consistent with those of the initial tests (Table 1) with a 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: Fredericksburg crossed to Rosenberg very reluctantly in either direction, failing to produce enough progeny to make adequate inbreed or backcross tests. Fredericksburg 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: *Drosophila melanopalpa* females were slightly fertile to Eagle Pass *repleta* males, producing a few male and female offspring. The reciprocal cross was sterile. *Drosophila melanopalpa* females were slightly fertile to Rosenberg *repleta* males, producing very few male and female offspring. The reciprocal cross did not go. *Drosophila melanopalpa* females were fairly fertile to New Haven *repleta,* producing a number of male and female offspring. The reciprocal cross was sterile. *Drosophila 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 reciprocal cross was also sterile in this case. *Drosophila melanopalpa* females crossed to *repleta* from Japan, producing two offspring. The reciprocal cross did not go.

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. The salivary chromosomes of the hybrids usually synapse well, although occasionally they fail to do so, or they may synapse loosely. Only one of the long autosomes shows inversion (see map).

Drosophila melanopalpa and *neorepleta* crossed reciprocally, being quite fertile to each other, although the cross went somewhat more vigorously when *melanopalpa* females were used. No rearrangements were observed in the salivary chromosomes of the hybrids.

Drosophila 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 *repleta* strain from Guatemala, producing a few phenotypically abnormal offspring.

Discussion

In the various *Drosophila* groups where speciation has been studied, the phenomenon of sexual, or psychological isolation is commonly observed. Dobzhansky and Koller (1938) reported sexual isolation between *Drosophila pseudoobscura* and *Drosophila miranda,* and also between *Drosophila azteca* and *Drosophila athabasca.* They reported a certain degree of sexual isolation between races of *miranda.*

The virilis group showed sexual isolation (Patterson, Stone, and Griffen, 1940). *Drosophila virilis* females crossed readily to *Drosophila americana* males, but the reciprocal cross was practically sterile. *Drosophila virilis* Henly was almost completely sexually isolated from the several wild forms. *Drosophila montana,* on the other hand, which crossed very reluctantly, if at all, to most of the virilis group was less isolated from Henly. (Stone, Griffen, and Patterson, 1942.)

The mulleri group (Patterson and Crow, 1940; Crow, 1942) exhibited sexual isolation in one direction in several instances. *Drosophila mulleri*

females crossed to males of all other species of the mulleri group, but the reciprocal crosses did not go.

Central Texas *Drosophila macrospina* females were fertile to *Drosophila subfunebris* males, but the cross was sterile in the other direction. The Limpia Canyon stock of *Drosophila macrospina limpiensis* females, however, were sterile to *subfunebris* males, while the reciprocal cross was fertile (Mainland, 1942).

Sexual isolation figures in the divergence of all species thus far studied in this laboratory. *Drosophila repleta* is interesting in that sexual isolation was manifested between many of the strains tested.

The genetic heterogeneity of the *repleta* populations, and the complexity with which the sexually isolating genes were manifested in cross-fertility tests, 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. For example, Fredericksburg and Eagle Pass cross readily in either direction, but Eagle Pass males were sterile to Guatemala females, while Fredericksburg crossed readily to Guatemala in either direction. Fredericksburg females were sterile to New Haven males, but Eagle Pass crossed reciprocally with New Haven. Also, Elgin and New Haven males were sterile to Fredericksburg 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 between the point of origin of geographical strains and the degree of sexual isolation between them. For example, Fredericksburg and Elgin, which are quite near to each other geographically, showed very different cross-sterility relationships. Ankara, which is distant from all other strains, showed appreciable sexual isolation only to Fredericksburg. Dobzhansky and Koller (1938) suggested that "if sexual isolation is engendered by natural selection raising a barrier against the production of sterile or otherwise inferior offspring, one may expect the isolation to be most rigid between species that inhabit the same or adjacent territories." They found this expectation to hold in some instances but not in others. Some strains of *pseudoobscura* which were in close juxtaposition with a race of *miranda* showed more sexual isolation to that race than did other strains which were geographically more remote. Here it is possible that the genes causing sexual isolation were selected to prevent the production of inferior hybrids. With another race of *miranda* this relationship of geographical distribution to sexual isolation did not hold. Mainland (1942) observed that in some *macrospina* x *subfunebris* crosses, the more closely situated geographically, the more likely were these populations to be fertile to one another. In the virilis group (Patterson, Stone, and Griffen, 1942) the American domestic form showed sexual isolation to the American wild form. The genes controlling isolation may here have been selected to prevent undesirable hybridization. However, the Asiatic domestic form

of *virilis* also showed sexual isolation to the wild form of this country, a fact which is not explained by selection against hybridization, but must represent the chance fixation of mutations which cause sexual isolation. *Drosophila repleta* shows the very specialized condition of sexual isolation which exists to varying degrees between different strains of a single rather large natural population. The isolation follows no geographic pattern, but seems to have resulted from random mutation followed by fixation. Here sexual isolation does not function to prevent the production of weak or sterile hybrids. On the contrary, 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. *Drosophila repleta* represents a rather large population. Large sexually reproducing populations may suffer certain evolutionary disadvantages (see Wright, 1940). It seems reasonable to assume that sexual isolation between strains of the *repleta* species, which does not entail the complete cessation of gene exchange between the semi-isolated groups, functions to establish within the large population smaller and more effective breeding units which are more flexible for rapid evolutionary changes.

The mutations which contribute to sexual isolation occurred within different geographical strains and are present seemingly at random in the *repleta* species. If, by chance, two populations should become reciprocally isolated, so that no gene exchange occurred between the strains, then their course of evolution might proceed independently, 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. Strains which are isolated from each other might not diverge, however, if they could exchange genes through some intermediate population. Here again the element of population size and distribution enters.

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

Sexual isolation also exists between species in the *repleta* group. *Drosophila melanopalpa* has thus far crossed with every *repleta* stock to which it has been tested. However, the cross has gone only in one direction, i.e., where *melanopalpa* was used as the female parent. Several interesting results have been obtained in these interspecific crosses.

Drosophila 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 (Plate 7).

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 poorly formed anal valves, only one vaginal plate, and a large "genital knob," which Newby (1942) states represents a chitinized and highly pigmented structure formed about the undeveloped female genitalia.

Sturtevant (private communication to Patterson) reported that in a cross of *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 cross 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* females produced phenotypically normal offspring of both sexes, while Guatemala *repleta* males crossed to *melanopalpa* females produced only a few offspring, some of which were intersex types, as described above.

Drosophila 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, although they have not proved to be fertile when interbred.

The strains of *repleta* which have been tested differ in genotype, as shown by their cross-fertility relationships. Sexual isolation, then, is a descriptive term in which may be concealed numerous and quite different reactions which lead 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 measurements can be made, with adequate control, many such problems may be elucidated.

Sexual isolation operates both within and between species in the repleta group. The nature of the isolation may differ somewhat, and may serve different functions. It prevents reproductive wastage through interspecific crosses. In *repleta* it may increase the efficiency of the evolutionary process by subdividing the large population into smaller, semi-isolated breeding units.

SUMMARY OF PART II

(1) Sexual isolation, or failure to mate, is a mechanism which frequently separates species. In the single species *Drosophila repleta,* sexual isolation was observed to function between geographic strains. When

the various stocks of repleta were interbred, no consistent cross-sterility relationships obtained between the several stocks, so the genes which caused the isolation were several and different.

(2) There was no correlation between the point of origin of geographical strains and the sexual isolation between them. The mutations causing isolation apparently occurred at random in the *repleta* species and became fixed in the various geographical populations.

(3) Sexual isolation does not imply incompatibility of genotypes. Wherever a cross was fertile in only one direction between the *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.

(4) It is conceivable that sexual isolation, operating between strains, might establish the separation necessary to further divergence. The Elgin and Guatemala strains were almost completely sexually isolated from each other, for example. If strains thus isolated exchanged genes through some intermediate population, they might not diverge.

(5) The salivary gland chromosomes of the F_1 larvae from crosses between the various geographic strains of *repleta* failed to show rearrangements, even in the crosses between strains of remote geographic location. The differences shown by the interbreeding of the strains were genic.

(6) Sexual isolation was also shown to be operative between species of the repleta group. *Drosophila melanopalpa* females and *neorepleta* females crossed to males of certain of the *repleta* strains, but in no instance was the reciprocal cross fertile. *Drosophila neorepleta* crossed only to Guatemala *repleta,* whereas *melanopalpa* proved fertile, in varying degree, to each of the five repleta strains with which it was tested, so *neorepleta* and *melanopalpa* differed in the degree of their isolation to *repleta.*

(7) Certain interspecific crosses revealed differences in genic balance: *Drosophila melanopalpa* females crossed to Guatemala *repleta* males and produced male-like, female-like and extremely mixed intersexes, as well as a few phenotypically normal males and females. *Drosophila melanopalpa* females, when crossed to New Haven *repleta* males, produced only phenotypically normal hybrids of both sexes. Since the F_1 and F_2 progeny of the cross between Guatemala *repleta* females and New Haven *repleta* males were phenotypically normal and fertile, the sex balance of the three stocks was assumed to be different.

(8) Similar genetically controlled sexual isolation occurs both within and between species. Mutations causing sexual isolation occur within different genotypes, and may represent various reactions of greater or less complexity. Sexual isolation may serve different functions. In some cases it prevents undesirable hybridization which would produce inferior progeny. Sexual isolation between *repleta* strains does not serve this purpose, since the strains show hybrid vigor whenever they interbreed. It was suggested that in the *repleta* species sexual isolation may function to establish, within the large population, smaller and more effective breeding units which are more flexible for rapid evolutionary changes.

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DROSOPHILA SPECIES	METAPHASE ♀	METAPHASE ♂	SALIVARY CHROMOSOMES EUCHROMATIC ARMS
REPLETA (235.3b)			5 LONG ARMS 1 DOT
MULLERI ARIZONENSIS ALDRICHI BUZZATII MOJAVENSIS			5 LONG ARMS 1 DOT
LONGICORNIS (514.5a)			5 LONG ARMS 1 DOT
MERIDIANA (1229.3)			5 LONG ARMS 1 DOT
MERIDIANA-LIKE (394.3d)			5 LONG ARMS 1 DOT

PLATE 1

DROSOPHILA SPECIES	METAPHASE ♀	METAPHASE ♂	SALIVARY CHROMOSOMES EUCHROMATIC ARMS
PENINSULARIS (1148.7)			5 LONG ARMS 1 DOT
HAMATOFILA (539.4a)			5 LONG ARMS 1 DOT
BIFURCA (911.7m)			5 LONG ARMS 1 DOT
BREVICARINATA			5 LONG ARMS 1 DOT
RITAE (911.5o)			5 LONG ARMS 1 DOT

PLATE 2

DROSOPHILA SPECIES	METAPHASE ♀	METAPHASE ♂	SALIVARY CHROMOSOMES EUCHROMATIC ARMS
LINEAREPLETA			5 LONG ARMS 1 DOT
NIGROSPIRACULA (1254.3a)			5 LONG ARMS 1 DOT
HYDEI (914.2)			5 LONG ARMS 1 DOT
NIGROHYDEI (1232.9b)			5 LONG ARMS 1 DOT
LEONIS			5 LONG ARMS 1 DOT

PLATE 3

DROSOPHILA SPECIES	METAPHASE ♀	METAPHASE ♂	SALIVARY CHROMOSOMES EUCHROMATIC ARMS
HYDEOIDES			5 LONG ARMS 1 DOT
MERCATORUM (935.76)			5 LONG ARMS 1 DOT
FULIGINEA (1283.10)			5 LONG ARMS 1 DOT
NEOREPLETA			5 LONG ARMS 1 DOT
MELANOPALPA (1244.11)			5 LONG ARMS 1 DOT

PLATE 4

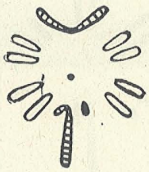
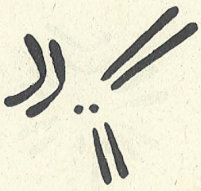
DROSOPHILA SPECIES	METAPHASE ♀	METAPHASE ♂	SALIVARY CHROMOSOME EUCHROMATIC ARMS
MELANOPALPA ♀ x NEW HAVEN REPLETA ♂ MALE HYBRID (SEE TEXT)			5 LONG ARMS 1 DOT
MONTANA			6 LONG ARMS 1 DOT
POLYCHAETA (119.6a)			7 LONG ARMS 1 DOT
SPINOFEMORA (HAWAII)			4 LONG ARMS 1 DOT
ORBOSPIRACULA (1232.1)			5 LONG ARMS 1 DOT

PLATE 5

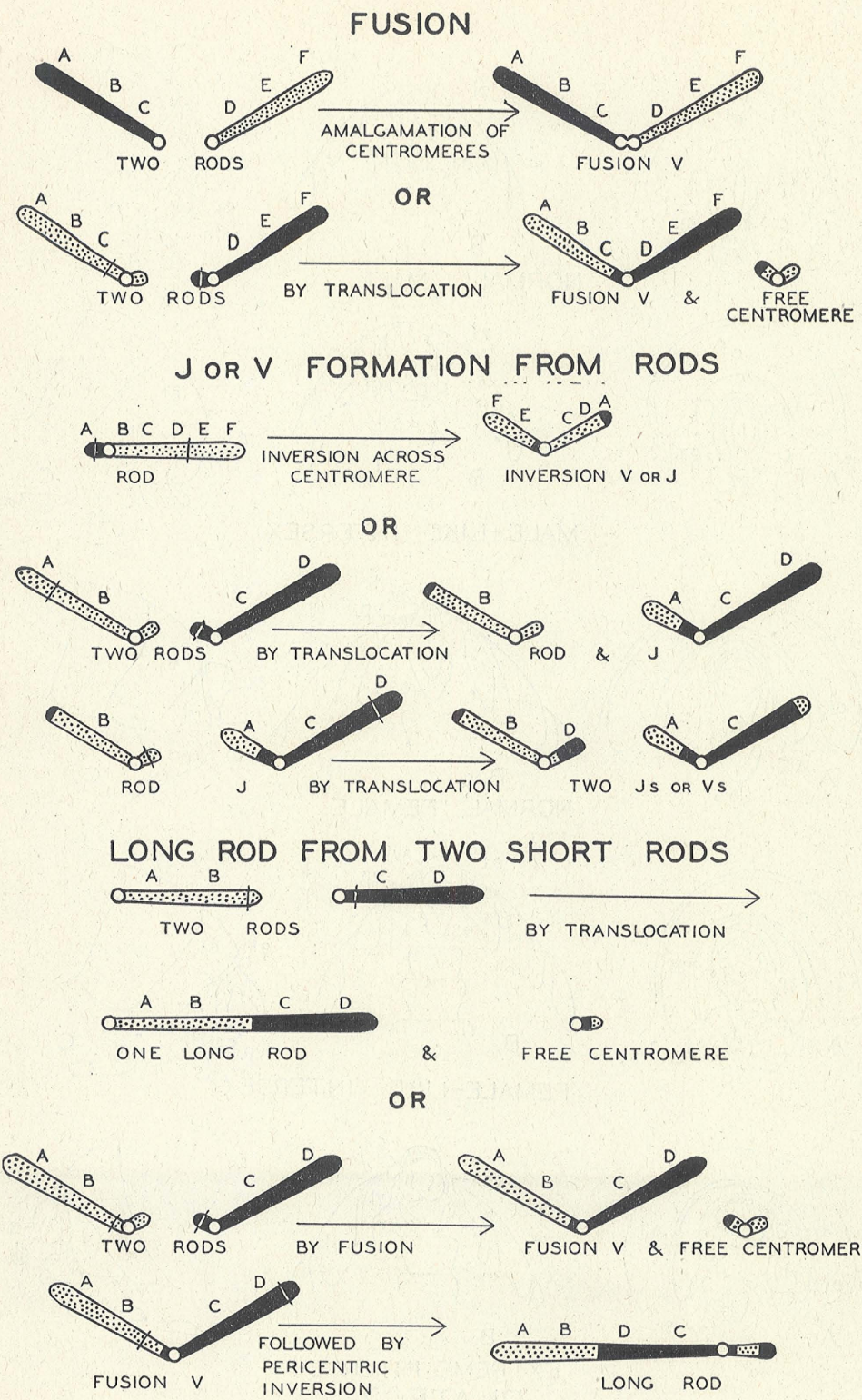
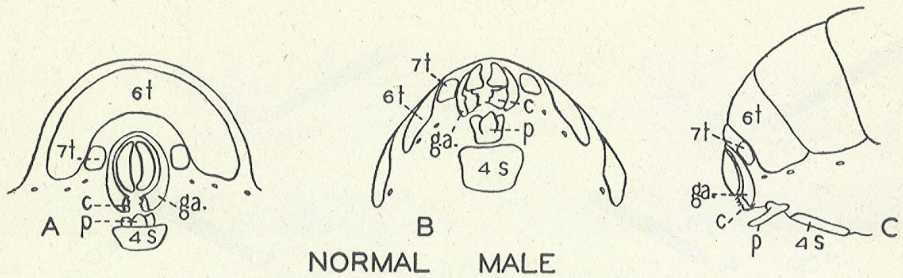
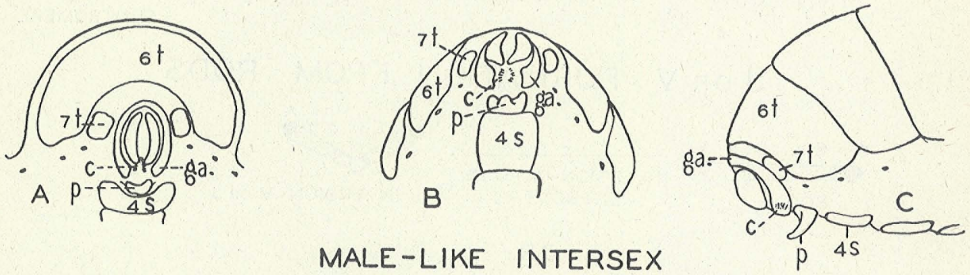

FUSION
A B C
F E D
TWO RODS
AMALGAMATION OF CENTROMERES
A B C D E F
FUSION V
OR
A B C
F E D
TWO RODS
BY TRANSLOCATION
A B C D E F
FUSION V &
FREE CENTROMERE
J OR V FORMATION FROM RODS
A B C D E F
ROD
INVERSION ACROSS CENTROMERE
F E C D A
INVERSION V OR J
OR
A B
D C
TWO RODS
BY TRANSLOCATION
B
ROD &
D
A C
J
B
ROD
D
A C
J
BY TRANSLOCATION
B D
TWO
A C
Js OR Vs
LONG ROD FROM TWO SHORT RODS
A B
C D
TWO RODS
BY TRANSLOCATION
A B C D
ONE LONG ROD
&
FREE CENTROMERE
OR
A B
D C
TWO RODS
BY FUSION
A B C D
FUSION V & FREE CENTROMERE
A B C D
FUSION V
FOLLOWED BY PERICENTRIC INVERSION
A B D C
LONG ROD

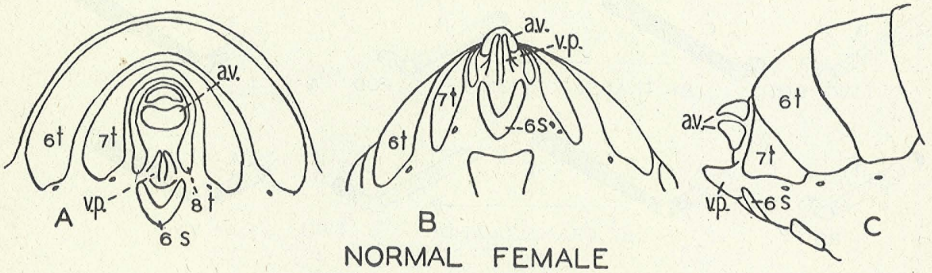
PLATE 6



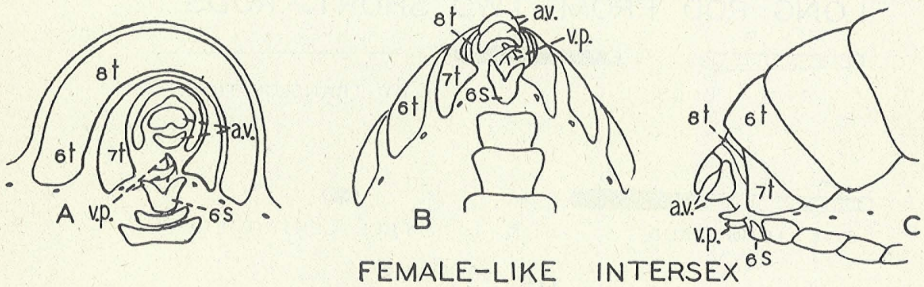
NORMAL MALE



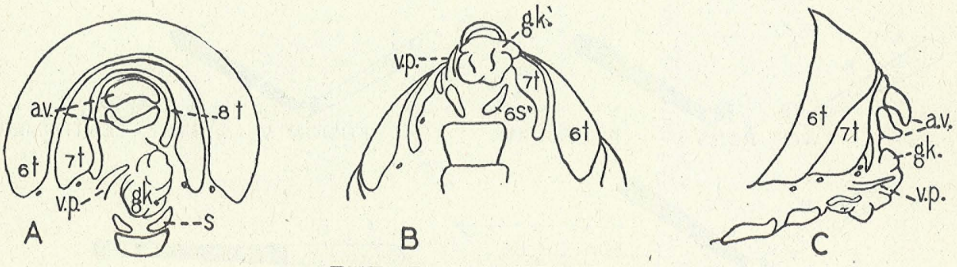
MALE-LIKE INTERSEX



NORMAL FEMALE



FEMALE-LIKE INTERSEX



EXTREME INTERSEX

PLATE 7

EXPLANATION OF PLATE 7*

posterior (A), ventral (B) and lateral (C) views of external genitalia

1. Normal male.
2. Male-like intersex, claspers much reduced.
3. Normal female.
4. Female-like intersex, vaginal plates reduced and crossed.
5. Extremely mixed intersex, poorly formed anal valves, only one vaginal plate, and a "genital knob."

Key to Abbreviations

t = tergite
s = sternite
g.a. = genital arch
p = penis
c = clasper
a.v. = anal valve
v.p. = vaginal plate
g.k. = genital knob

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

The salivary chromosome map of *Drosophila repleta* was made from an Elgin, Texas, strain. The system of band designation employed by Patterson, Stone, and Griffen (1940) for the *D. virilis* map has been used here. 1A2a, for example, refers to the X chromosome, 1; the first large section, A; the second division of this section, 2; and the first band in the division, a.

The X chromosome is readily identified. The free end flares out loosely and the band of heavily staining chromosomes (1A2a) is a clear landmark. The attached end of the X is a large heterochromatic mass which is frequently almost, or entirely, broken loose from the rest of the chromosome. A large puff near the base of the X is also quite characteristic. Other striking areas of the X are 1C3 with its four heavy bands, and 1D3b, a row of large darkly staining chromomeres which causes a small puff. Two regions of this chromosome are almost invariably distorted, and the structure of the bands is seldom seen clearly. These are 1B3 and 1B4, which include a double puff, and 1E1 and 1E2, where structurally weak points permit distortion when the chromosome is stretched.

Chromosome 2 is the only other chromosome of *repleta* which has a flared free end. Just behind the free tip are three darkly staining bands. The heavily staining bands in 2A2 and 2A3 also aid in identification. In other species of the repleta group, such as *bifurca, hydei* and *mulleri,* the free end of the chromosome 2 is compact rather than flared and the terminal bands, which appear faint in *repleta,* are often clearly seen in these species. The attached end of chromosome 2 is characterized by a compact puff with a constriction containing two dark bands just distal to the puff, followed by a wider region, the whole of the area reminding one of an hourglass (2G3, 2G4, 2G5). *Drosophila melanopalpa* x *repleta* shows an included inversion in this chromosome. The limits of the outer inversion are about 2C2–2De, of the inner inversion, 2C7a–2D4a. The result is two inverted sections of unequal length on either side of the uninverted region, and the hybrid chromosome shows two unsynapsed loops.

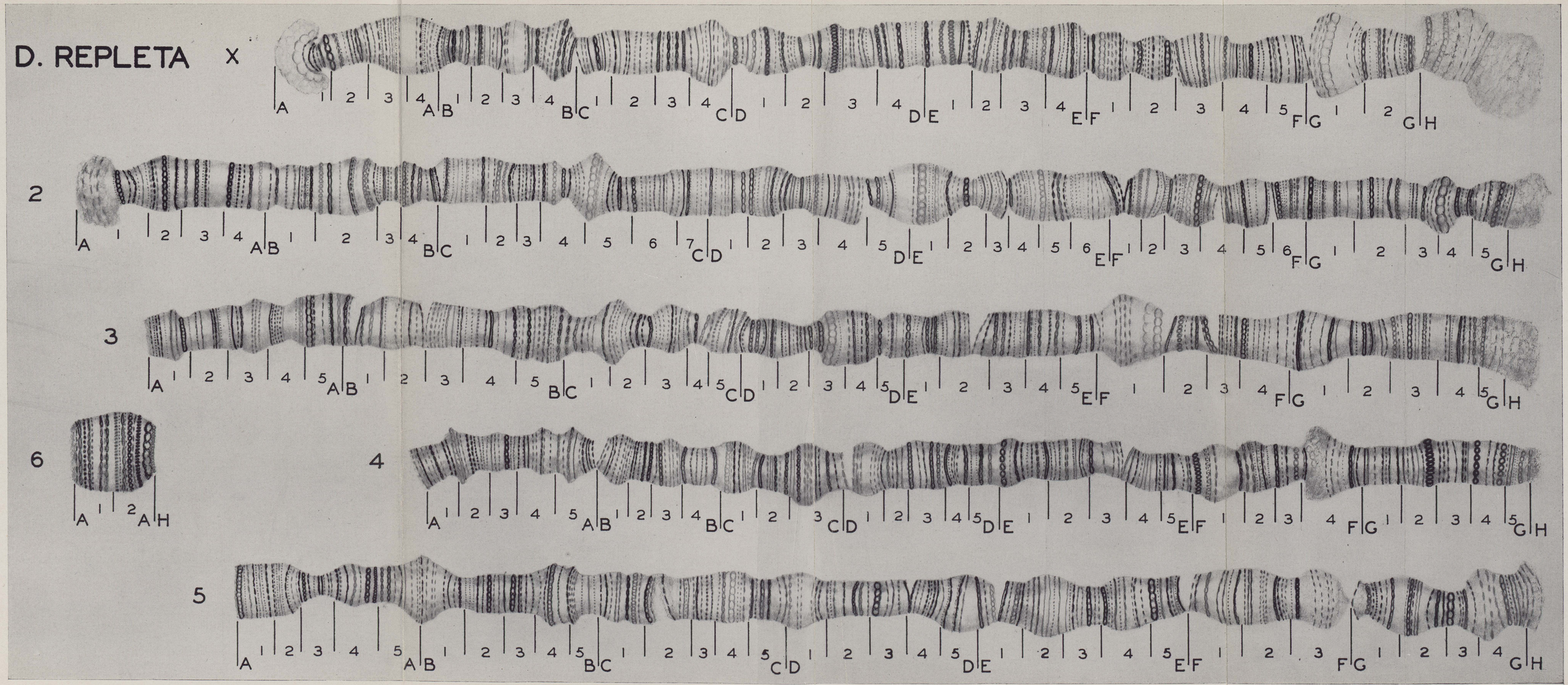
The free end of chromosome 3 has a blunt tip and a small compact puff preceded by four thin dark bands. The attached end is pestle-shaped. Band 3A5a, which is shown as a row of large, darkly stained chromomeres is usually stretched out of shape, and is not clearly seen. The large puff in region 3C is a good landmark. One of the most striking "repeats" of the *repleta* chromosomes is seen in 3D1. This repeat sometimes synapses or causes a knot in the chromosome.

Except for the michrochromosome (6), chromosome 4 is the shortest. The shape of its free end reminds one of the *melanogaster* X. There is a short straight section having several dark bands, a noticeable puff, followed by three darkly staining dotted bands. The attached end is quite free of heterochromatic material, and the large bell-shaped puff, 4F4, makes it easy to identify.

Chromosome 5 has a blunt, rectangular free end, which tapers into a constriction. The large puff 5A5, 5B1, aids in identification. The attached end is quite large, and sometimes appears so clear-cut in structure as to resemble a free end when it is broken away from the chromocenter. It is recognized by the puff, 5G4, followed by a slight constriction, and a slight flaring of the heterochromatic region. Region 5D is frequently stretched and distorted. Region 5F is bounded on either side by a structurally weak spot and is sometimes almost broken out of the chromosome; its structure is seldom clearly seen.

Chromosome 6 is characterized by large, fuzzily staining bands. Among the members of the repleta group its appearance in the salivary preparations is quite variable. For example, in *hydei* its structure is rarely seen clearly, and it seems to be very small, while in *repleta* it is a fairly large body.

The centromeres are seen occasionally, but they are not darkly staining bodies as in *virilis.* The chromosomes of *repleta* show striking structurally weak spots, where the chromosomes frequently stretch or break when smeared. These points have been designated in the mapping.


D. REPLETA X
A 1 2 3 4 AB 1 2 3 4 BC 1 2 3 4 CD 1 2 3 4 DE 1 2 3 4 EF 1 2 3 4 5 FG 1 2 GH
2
A 1 2 3 4 AB 1 2 3 4 BC 1 2 3 4 5 6 7 CD 1 2 3 4 5 DE 1 2 3 4 5 6 EF 1 2 3 4 5 6 FG 1 2 3 4 5 GH
3
A 1 2 3 4 5 AB 1 2 3 4 5 BC 1 2 3 4 5 CD 1 2 3 4 5 DE 1 2 3 4 5 EF 1 2 3 4 FG 1 2 3 4 5 GH
6
A 1 2 AH
4
A 1 2 3 4 5 AB 1 2 3 4 BC 1 2 3 CD 1 2 3 4 5 DE 1 2 3 4 5 EF 1 2 3 4 FG 1 2 3 4 5 GH
5
A 1 2 3 4 5 AB 1 2 3 4 5 BC 1 2 3 4 5 CD 1 2 3 4 5 DE 1 2 3 4 5 EF 1 2 3 FG 1 2 3 4 GH

IV. CROSS FERTILITY AND ISOLATING MECHANISMS IN THE DROSOPHILA MULLERI GROUP

J. F. CROW[1]

Thus far five members of the mulleri species group of *Drosophila* have been studied. These belong to a larger group that has become known as the repleta group of species. In the mulleri group there is great diversity in the degree of cross fertility between different members. The hybrid zygotes from some crosses fail to reach the pupa stage while other combinations produce normal adults. Two members of the mulleri series represent large dense populations which occupy the same area, while the other three are geographically isolated from these and from each other. A study of the cross relationships and isolating mechanisms of such a group should make possible a better knowledge of the genetic nature of species differentiation.

MATERIAL AND METHODS

The members of the mulleri group studied in this series of experiments are listed below with the origins of the particular stocks used.

1. *Drosophila mulleri* Sturtevant, 1921. Sturtevant's description is based on specimens taken at Houston, Texas. The stock used in these experiments has descended from a single female which had been fertilized in nature and was taken at McAllen, Texas.

2. *Drosophila aldrichi* Patterson & Crow, 1940. The two stocks used have descended from fertilized females trapped near Austin, Texas.

3. *Drosophila mojavensis* Patterson & Crow, 1940. A stock of this species was kindly furnished by Dr. Warren P. Spencer, who collected the original flies at Mesquite Springs, Death Valley, California.

4. *Drosophila arizonensis* Patterson & Wheeler, 1942. This species was obtained near Tucson, Arizona, by Mr. G. B. Mainland, and has since been taken in Sonora, Mexico.

5. *Drosophila buzzatii* Patterson & Wheeler, 1942. Two stocks were used, one from Sicily and the other from Argentina. Genetical and cytological tests indicate that, although the two differ somewhat, they are of the same species.

In previous publications (Patterson and Crow, 1940, and Crow, 1941) *mojavensis* and *arizonensis* were considered as sub-species of *mulleri*, but further studies have made it seem more desirable to consider them as separate species.

The geographical distribution of these forms is not completely known. Collection records of the two western forms, *mojavensis* and *arizonensis*, are not available for a very large area, although Prof. Spencer states

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[1]Now at Dartmouth College, Hanover, New Hampshire.

that *mojavensis* is very common in California deserts. *Drosophila mojavensis* has also been taken in the Chocolate Mountains, near the Salton Sea in southern California. Likewise, little is known of the distribution of *buzzatii*, other than that it has been taken in Argentina and Sicily.

Drosophila mulleri has been found over the whole of Texas, and in Louisiana and Florida. It is most common in the southern and central parts of Texas and is rare in the wooded eastern regions. The population is known to extend some distance into Mexico and Sturtevant (1921) reports having examined specimens from Florida, Cuba, Jamaica, and Honduras. *Drosophila aldrichi* has almost the same distribution in Texas and has recently been taken in Oklahoma and Sonora, Mexico.

There is considerable correlation between the amount of cactus (*Opuntia* species) present and the size of the *aldrichi* population. *Drosophila aldrichi* is generally taken in regions where *Opuntia* is present, while *mulleri* is often found feeding on other food.

Of about 14,000 flies of these species collected in Texas up to April, 1941, 26.1 per cent have been *aldrichi* and the rest *mulleri*. This percentage varies widely in the different ranges where collections have been made.

All the members of the *mulleri* series are very similar morphologically, although living individuals can be quite easily distinguished. It is quite doubtful, however, if these would have been classified as separate species from a study of pinned specimens. *Drosophila mulleri* and *aldrichi* are especially similar in appearance and were not established as distinct species until a series of cross tests demonstrated that two distinct types were present among the Texas collections.

The length of the life cycles of these species differs considerably. In *mulleri* the average period from the time the egg is laid until the imago emerges is about 11 days under ordinary laboratory conditions (temperature 22 degrees C.). In *aldrichi* the time is about 15 days, in *arizonensis*, 10–11 days, and in *mojavensis*, 12–13 days. In *mulleri-aldrichi* hybrids the life cycle seems to be comparable to that of the parent which takes the longer time to develop.

In the laboratory, when fed on regular banana-yeast agar, *mulleri*, *mojavensis*, and especially *arizonensis* grow very readily, but cultures of *aldrichi* are very difficult to keep alive. The fact that *aldrichi* specimens were nearly always found in regions where cactus was abundant suggested the use of cactus in the food, and when the fruit of the prickly pear *(Optunia lindheimeri)* was added much more vigorous cultures were obtained.

In the laboratory these species were first tested in mass cultures for cross fertility relationships. It is necessary that such tests be repeated a number of times, since most of the species are very reluctant to hybridize, and offspring sometimes occur from parental combinations that have repeatedly failed to produce hybrids. Sometimes, under very crowded conditions, offspring will be produced when ordinarily none would occur. In most crosses involving *aldrichi* cactus was added to the food.

For quantitative tests of degree of sexual isolation pair matings were used. Flies at least seven days old were mated in shell vials containing banana-yeast-agar and changed to fresh vials after seven days. After seven more days the parent flies were removed, and if both were still active, the female was dissected and examined for the presence of sperm in the spermathecae anad ventral receptacle. Since, in no case, were offspring found to have been produced by females in which sperm had not been detected by this method, it was assumed that the presence or absence of sperm in the female indicated whether or not successful copulation had taken place.

Cytological examination was made by means of acetocarmine smears.

Interspecific Crosses and Hybrid Fertility

The results obtained from the various possible crosses of the members of the mulleri group are shown in Table 1. From this it can be seen that most of the crosses did not produce hybrids reciprocally and that fertility of the hybrids differed widely. It is not improbable that the parent combinations listed as not producing offspring might hybridize under the proper conditions, but they have failed to do so in a number of trials in the laboratory. An account of the nature of the hybrids follows.

Drosophila mulleri female X *aldrichi* male. Sterile hybrids of both sexes are produced. These are similar to *mulleri* in eye color and abdominal pattern. The testes are very small and degenerate and the ovaries never completely develop. Very few offspring were produced even in mass matings and there was great reluctance for the parents to mate. Male hybrids of this type have been found in nature.

Drosophila mulleri female X *mojavensis* male. Sterile male and fertile female hybrids are produced. Their appearance is somewhat more like *mojavensis,* especially in body color, and the males have small testes. The females were quite fertile in backcrosses to males of the parent types. When the F_2 backcross males were backcrossed to the same stock to which the original backcross was made a small percentage was fertile, as would be expected. Location of sterility factors is complicated by the low fertility of the original cross and the lack of suitable genetic markers and crossover suppressors.

Drosophila mulleri female X *arizonensis* male. From numerous crosses involving about 2,000 flies in mass cultures, only nine offspring were obtained. These were all males with small testes and were sterile. Although this is a small sample, there is a probability of less than one in 250 of obtaining as large a chance deviation from the expected one to one sex ratio. This, in addition to the fact that in *mulleri* the females emerge first and occur in larger numbers than males, makes it seem quite certain that there is some factor reducing the percentage of females.

Drosophila aldrichi female X *mojavensis* male. From the numerous crosses made only twenty-four offspring have resulted. These were all females, of intermediate appearance and sterile.

Drosophila aldrichi female X *arizonensis* male. Only one of the crosses made produced any larvae and from these one sterile female emerged. She was definitely weak in appearance and had abnormal wings.

Drosophila mojavensis female X *arizonensis* male. The hybrids of both sexes were fertile, both among themselves and in backcrosses. A preliminary test indicated that the male hybrids are not fertile to *mulleri* females, although the test was not repeated often enough for the results to be conclusive. At least the hybrids do not seem to be conspicuously more fertile to *mulleri* than their parents.

Drosophila arizonensis female X *mojavensis* male. This cross produced hybrids much more readily than any other combination. The female hybrids were very fertile in backcrosses but the males were sterile.

Drosophila mulleri female X *buzzatii* male. A few larvae were produced, one of which matured. This was a sterile female with an abnormal abdomen.

Drosophila arizonensis female X *buzzatii* male. Larvae have been produced but none of these reached the pupal stage.

Degree of Sexual Isolation

Table 2 shows the quantitative results of the various crosses possible with the members of the North American group. The data were obtained from pair matings made as described previously. In each case the figure represents the per cent of females which had been inseminated in the 14-day period.

It will be noticed that some of the crosses known to produce hybrids are indicated as being completely isolated. This is in accordance with the expectation when one considers the difficulty of obtaining the hybrids even in mass cultures left for long periods of time. These results are from pair matings left together for only two weeks. The number of progeny and the number of females fertilized could probably have been increased in the crosses involving *aldrichi* by the addition of cactus to the food, but in order to keep the conditions as near constant as possible, all matings were made using the same kind of food.

Since the number of individuals dissected from each cross is fairly large (ca. 100), the mathematical standard deviations in per cents would be low. However, individual results are not reproducible on repetition within the mathematical expectation. Hence, a listing of standard deviations is omitted because this might give a false idea of the accuracy of the data, as most of their variability is due to factors other than sampling errors.

The degree of sexual isolation in the hybrids between *arizonensis* and *mojavensis* is shown in the following results. These were obtained from pair matings under the same conditions as those of the original parent crosses.

	Per Cent of Females Fertilized
mojavensis ♀ X *arizonensis* ♂	(77)
F_1 ♀ X *arizonensis* ♂	78
F_1 ♀ X *mojavensis* ♂	100
F_1 ♂ X *arizonensis* ♀	80
F_1 ♂ X *mojavensis* ♀	56
arizonensis ♀ X *mojavensis* ♂	(33)
F_1 ♀ X *arizonensis* ♂	89
F_1 ♀ X *mojavensis* ♂	100
F_1 ♂ X *arizonensis* ♀	0
F_1 ♂ X *mojavensis* ♀	0

Genes Affecting Hybrid Viability

In addition to the stocks already mentioned, a strain of *aldrichi,* which will be designated as *aldrichi 2,* has been tested and found to differ from the other *aldrichi* strains in certain inter-species relations. This strain is identical with other *aldrichi* cytologically and differs phenotypically only in the possession of a recessive scarlet eye mutant.

When males of this strain are crossed to *mulleri* females the offspring are predominantly male. A large number of these males show an abnormal abdominal pattern similar to that of the mutant bobbed. As the cultures become more and more crowded the percentage of males increases, suggesting that the viability of the females is impaired. The females that are produced are noticeably abnormal and usually have wing deformities.

The following results were obtained from a series of crosses:

Cross	Per Cent Males	
	Mass Culture	Pair Matings
aldrichi ♂ X *mulleri* ♀	46	
aldrichi 2 ♂ X *mulleri* ♀	91	
aldrichi 2 ♂ X *aldrichi* ♀		
F_1 ♂ X *mulleri* ♀	47	45
aldrichi ♂ X *aldrichi 2* ♀		
F_1 ♂ X *mulleri* ♀	88	65
aldrichi 2 ♂ X *aldrichi* ♀		
F_1 ♂ X F_1 ♀		
F_2 ♂ X *mulleri* ♀	(in pair matings)	see below

The percent of males in the cultures was distributed as follows:

Per Cent Males	Number of Cultures	
7.5—12.5	1	
12.6—17.5	0	
17.6—22.5	1	
22.6—27.5	0	
27.6—32.5	0	
32.6—37.5	2	
37.6—42.5	3	
42.6—47.5	5	Average per cent
47.6—52.5	4	males=57.1
52.6—57.5	3	
57.6—62.5	2	
62.6—67.5	5	
67.6—72.5	3	
72.6—77.5	2	
77.6—82.5	1	
82.6—87.5	0	
87.6—92.5	1	
92.5—97.5	1	

These data indicate that there is a gene, or possibly a series of genes, on the X-chromosome of *aldrichi 2* which has no noticeable effect within the species but acts as a dominant semi-lethal in interspecies crosses.

That this sex ratio is due to lethality of the female zygotes and not to some sort of sex reversal mechanism has already been suggested. The following results support this suggestion.

When F_2 males from the cross *aldrichi* female by *aldrichi 2* male are crossed to *mulleri* females in pair matings, half the cultures show the unusual sex ratio. The average number of offspring per vial of those with the abnormal sex ratio was 15.3, while in the others it was 22.7. It will be remembered that the sex ratio is normally about 46 per cent males, but 65 per cent males in the abnormal ratio stocks. If it is assumed that the difference in sex ratio is due to lethality of some of the potentially female zygotes, and the 15.3 is corrected on this basis the result is 15.3 X .65/.45, or 22.1 which is very near the result of 22.7 obtained in the normal sex ratio cultures. F_2 males from the P_1 cross *aldrichi* by *aldrichi 2* were used instead of males from pure stocks of *aldrichi* and *aldrichi 2* in order that other possible differences in the two strains would be minimized.

An effort was made to see if this *aldrichi 2* X-chromosome carries the same effect in crosses involving *mojavensis* and *arizonensis* as it does with *mulleri*. It is known that crosses between ordinary *aldrichi* females and *mojavensis* males produce only female offspring. If the gene (or genes) acts the same way as it does in crosses involving *mulleri*, the cross, *aldrichi 2* female by *mojavensis* male, should produce either no hybrids at all or a few weak females. In no case were offspring produced from this cross although numerous tests were made, and in some cases the females had been fertilized as evidenced by the presence of sperm in the ventral receptacle. However, since hybrids between *aldrichi* and *mojavensis* are so rarely obtained, these results are not absolutely conclusive, but may be taken as indicative of the fact that the same lethal effect is produced

by the *aldrichi 2* X-chromosome in crosses involving mojavensis as in those where mulleri was concerned.

Crosses between *aldrichi 2* females and *arizonensis* males have also failed to produce hybrids, but since the same number of tests could have been made using regular *aldrichi* females without obtaining hybrids, this cannot be considered as at all convincing. All that can be said is that it is not incompatible with the idea that the lethal effect is not confined to *mulleri-aldrichi 2* hybrids.

Hybrids between *mulleri* females and *aldrichi 2* males, in addition to having an abnormal sex ratio, often show an abnormal abdomen effect in the males. Phenotypically the effect is very similar to the mutant *bobbed*. The penetrance is low since only about 25 per cent of the males show the effect clearly. In crosses between *aldrichi* males and *mulleri* females the incidence of males with abnormal abdomens is less than one per cent. The percentage of abnormal males in the hybrids produced by various crosses is given below.

Cross	Per Cent of Abnormal Males, with Standard Deviation
aldrichi 2 ♂ X *mulleri* ♀	25.3 ± 4.9
aldrichi ♂ X *mulleri* ♀	less than 1
aldrichi 2 ♂ X *aldrichi* ♀	
F_1 ♂ X *mulleri* ♀	11.1 ± 3.6
aldrichi ♂ X *aldrichi 2* ♀	
F_1 ♂ X *mulleri* ♀	11.4 ± 2.1

These results indicate that the gene or genes causing or modifying the effect are autosomal. Early results seemed to indicate that the abnormal abdomen was being transmitted from the father to the male offspring, and it was so reported (Crow, 1941). Later results do not confirm this observation, however.

Cytological Results

The metaphase chromosomes of the five species are indistinguishable. Smears made from larval ganglionic tissue show that the chromosomes of these forms are all rod shaped with terminal spindle attachments. In each case the diploid set in the female consists of two long rods, eight shorter rods, and a pair of dot-like chromosomes. In the male one of the longer rods is replaced by another of about the same length as the autosomes indicating that the longer rod is the X chromosome.

The salivary gland chromosomes of these species are rather long but small in diameter as compared to most other *Drosophila*. There does not seem to be as definite a chromo-center as is usually found. Often three of the autosomes along with the dot-like sixth are attached at their centromere ends and the other two, the X chromosome and one of the autosomes, are connected with the nucleolus.

The *mulleri-aldrichi* hybrids show no large chromosome differences, but there is a definite tendency for the homologues to remain unsynapsed. In some cases almost a whole chromosome pair will fail to unite, while in others there is synapsis at various points along the length of the chromosome arms. Since these regions of non-synapsis are not the same in different cells, they cannot be due to major rearrangements.

In *mulleri-mojavensis* hybrids several inversions are evident on some of the autosomes, and synapsis is poor, though possibly better than in *mulleri-aldrichi* hybrids. Salivary chromosomes of *aldrichi-mojavensis* hybrids fail to synapse also. On the other hand, hybrids between *mojavensis* and *arizonensis* have chromosomes that synapse very closely although there are several inversions.

Discussion

The mulleri group is widely diverse in the ability of different members to hybridize with one another. *Drosophila mojavensis* and *arizonensis* cross readily, one combination (*arizonensis* male X *mojavensis* female) produces completely fertile hybrids, while the reciprocal produces fertile females and sterile males. On the other hand, *buzzatii* and *arizonensis* produce only aberrant larvae that never mature and *buzzatii* and *aldrichi* have failed to give any evidence of hybridization. Nevertheless, all the members of the group are related to some other members by some degree of cross fertility, although even the most closely related forms show definite isolating mechanisms. These mechanisms are of a variety of types.

How important geographical isolation is in the evolution of the group cannot be answered at present. Obviously, the particular individuals of *mojavensis* and *arizonensis* obtained for this study were geographically isolated from each other and from the *mulleri* and *aldrichi* population of Texas, but whether the populations overlap at some point is not known. Surely, geographical isolation has played a part in the evolution of the differences between the Texas and the Western forms.

There is ecological isolation operating in the case of *mulleri* and *aldrichi*, due to their different food preferences. Hence, even if there were no mating preferences, there would certainly be non-random mating of the individuals of the two groups due to the fact that *aldrichi* tends to stay near cactus while *mulleri* is found on decaying fruit and vegetables.

The quantitative data show clearly that sexual isolation is an important factor in the speciation of the *mulleri* group. Except in the crosses between *mojavensis* and *arizonensis*, only a very small per cent, if any, of the females were fertilized in pair matings. These crosses represent more or less forced matings since males and females of the same species were not allowed to be together.

A brief test of mating preference was made and showed the same results. The offspring produced when *mulleri* females were placed in

bottles with *aldrichi*, *mojavensis*, and *mulleri* males in equal numbers were over 99.5 per cent *mulleri*.

The results seem to indicate that the most effective barrier to cross breeding in the mulleri group, disregarding geographical isolation which cannot be compared with the others, is sexual isolation. It has often been mentioned that this is the most efficient form of isolating mechanism since reproductive effort is conserved and there is no competition from hybrids.

Throughout the genus *Drosophila* there are numerous cases where sexual isolation plays a part. Sturtevant (1920) has shown that in mixed cultures *Drosophila melanogaster* and *Drosophila simulans* prefer members of their own species for mating partners. Lancefield (1929) showed that the same preference is shown by the A and B races of *Drosophila* pseudoobscura. Dobzhansky & Koller (1938) found the same differential preference between *Drosophila pseudoobscura* and *Drosophila miranda*. In the virilis group there is definite sexual isolation, both between species and between strains within a species (Patterson, Stone, and Griffen, 1940, and Stalker, 1941). In *D. repleta* there is sexual isolation, apparently without any other mechanism of separation, between various strains (Wharton, 1942). This may indicate that sexual isolation is sometimes the first step in species differentiation, preceding the other forms. Even individual mutants in *melanogaster* have been said to show definite preferences for their own type (Spett, 1932, and Diederich, 1941).

The percentage of zygotes which fail to reach the adult stage in these crosses has not been satisfactorily determined. Egg hatch counts in the mulleri group are very difficult to obtain and even in pure strains the hatch is low and inconsistent. However, in many of the hybrid crosses the number of offspring produced by females known to have been fertilized by males of another species is considerably lower than the control values. This is particularly true in the cross *mojavensis* female by *arizonensis* male. Here, 77 per cent of the females were fertilized but only 3 per cent produced offspring, as opposed to the reciprocal cross where 33 per cent were fertilized and 75 per cent of these produced hybrids. Other crosses give less convincing data, but it seems quite certain that in some of the crosses the fecundity of fertilized females was considerably lower when inseminated by a male of another species. In the above mentioned case (*mojavensis* female by *arizonensis* male) the lowered fecundity seems to be due largely to the failure of the female to lay eggs.

Various degrees of hybrid sterility are present, most often in the males. In six out of the seven crosses which produce hybrids the males are either absent or sterile, and only one cross (*mulleri* female by *arizonensis* male) produces more males than females. Haldane (1922) has stated that in hybrids the heterozygous sex is more likely to be weak, rare, or sterile than the homozygous. If each of the parent species has a particular balance between the X-chromosome and the autosomes, this balance would still be maintained in the female hybrids but would be upset in the males.

The mulleri series upholds the hypothesis. Much of the hybrid sterility and lethality may then be due to genic unbalance, although a Y-autosome unbalance may be effective as well as an X-autosome relation.

Isolation of this type would seem to be built up incidental to the process of evolution of the separate genotypes rather than being caused by specific genes selected as isolating factors. That isolating factors as such may not have positive selective value solely for this reason is suggested by the fact that they are found in species geographically isolated as well as those which live in the same locality.

The case of *aldrichi 2* cross-lethal factor is especially interesting. Here, a species, already completely isolated genetically from *mulleri* because of the complete sterility of the hybrids and a strong sexual isolation, has an additional isolating mechanism in certain strains. The elimination of all the hybrid zygotes which receive this sex-linked factor removes the competition from the hybrids, and consequently would be an advantage to both parent populations if hybridization were frequent.

No other case of this type has been reported in animals, but a parallel case has been found in plants of the genus *Crepis* by Hollingshead (1930). Certain strains of *Crepis tectorum* carry a dominant gene which has no effect within the species, but in crosses with *C. capillaris* causes the hybrid to die in the cotyledon stage. It was found to be present in some localities and absent in others. The gene was found to be effective against *C. leontodontoides* and *C. bursifolia* but not in hybrids with *C. setosa* and *C. taraxacifolia.*

A similar case in cotton has been reported by Silow (1941). In crosses between certain strains of *Gossypium arboreum* a lethal or semi-lethal type of abnormality known as "crumpled" appeared. This was shown to be due to the interaction of two complementary genes, Cp_a and Cp_b, neither of which had a detectable phenotypic expression without the other. The Cp_a gene was found in one strain of *arboreum* while Cp_b was found in 25 of 41 tested strains of *arboreum* and *herbaceum.* In many respects this is very similar to the case in *mulleri-aldrichi-2* crosses where a semi-lethal condition results from crossing certain strains.

No complete study of the frequency of this factor in *aldrichi* populations has been made. A specimen of *aldrichi* taken in Fayette County, Texas, was found to have the same lethal effect in hybrid crosses.

Whether the gene would have appreciable selective advantage under existing conditions may be doubtful since the sexual isolation is strong. It is possible, however, that the cross-lethal gene was introduced into the population at a time when there was relatively low sexual isolation but high sterility of the F_1 hybrids or subsequent hybrid generations. In such a case the gene would have some selective advantage to both *mulleri* and *aldrichi* populations due to the reduction of competition from hybrids, and, being dominant, might increase its frequency at a rapid rate but would not be likely to reach a stage of fixation in a large population. Later, genes making for sexual isolation between the two species

would, as they became increasingly prevalent, reduce the selective advantage of the cross-lethal gene by eliminating hybrid competition as well as conserving reproductive effort of the parents. Thus, the cross-lethal factor as it now exists in the *aldrichi* population may be only a remnant of a formerly useful gene.

An alternative and equally plausible explanation of the case may be that the mutant has occurred recently, since the other isolating mechanisms have been formed, and has spread through a part of the population by chance or has selective value for some other reason. Whether or not in this case the cross-lethal factor confers enough advantage to have selective value it still demonstrates that such mutations, acting as lethals or semi-lethals in hybrids but having no noticeable effect within the species, are occurring in Drosophila populations and could be effective in preventing gene exchange under certain conditions.

It is probable that the mutant causing the female lethality is not specific for *mulleri-aldrichi* 2 hybrids, but is also effective in crosses involving *mojavensis* and *arizonensis*. This cross-lethal factor would seem to act in genic environments not normal for it, rather than behave as a lethal in a specific hybrid environment.

It is also probable that a similar gene or set of genes is effective in preventing any female hybrids from surviving in crosses involving *mulleri* females and *mojavensis* males. This also appears to be a sex linked gene acting as a lethal in the hybrid environment.

The high incidence of abnormal abdomens in the hybrid males is significant. It is possible that this is a manifestation of a gene, such as *bobbed,* which is acted on by recessive modifiers in the relatively homozygous population but in a hybrid is unsuppressed, another case of genic unbalance. A gene having little or no detrimental effect in its normal environment may cause pathological conditions in a genic environment in which it is not properly balanced, similar to the situation in certain fish species (Gordon, 1937, 1938).

The mulleri series illustrates a number of mechanisms of isolation and shows very clearly that in nature the same result may be attained by widely different methods. That the sum of the isolating mechanisms in certain crosses is effective in nature is indicated by the very small number of hybrids obtained in wild populations. Out of several thousand *mulleri* and *aldrichi* taken in Texas only 26 hybrid males were recorded.

There is an interesting relationship between the proportion of *aldrichi* in the population and the presence of *mulleri-aldrichi* hybrids. The hybrid males can be detected by their small testes and have been recorded as collected. In populations containing both species, but where no hybrids were taken, the *aldrichi* specimens comprise 13 per cent of the *mulleri-aldrichi* populations. In populations where hybrids were found *aldrichi* represented 56 per cent of those collected. In one case where nine hybrid males were found in a collection of 277 male flies the population sample was 79 per cent *aldrichi.*

The Texas populations of *mulleri* and *aldrichi* are comparatively large and dense, and all individuals tested from this area fall clearly into one or the other group. The population of *Drosophila hydei,* which is also large and dense, has this same uniformity (Stone, this bulletin). All the individuals tested have been found to be perfectly fertile to each other and to be quite free from chromosomal changes. Contrasted to this is the case of *Drosophila virilis* where three species of the red group were found among twelve flies captured (Patterson, Stone, and Griffen, 1940 and 1941). *Drosophila virilis* represents a very sparse population where genetic changes would be expected to become homozygous and thus fixed in certain localities. As pointed out by Wright (1931), this would not be probable in large dense populations.

Of interest and significance is the fact that *mulleri* and *aldrichi* are closely related species living in the same locality. Volterra showed mathematically that two species competing for the same food supply and dependent on the supply could come to equilibrium only when one completely replaced the other (Chapman, 1931). This concept has been elaborated by Gause (1934). Thus two species competing for the same ecological niche would come to equilibrium only when one was completely replaced.

However, if some part of the utilization of the environment is different for the two species, there may be an equilibrium between the two where both may persist. *Drosophila mulleri* and *aldrichi* fit very nicely into this scheme. Although very similar morphologically and having arisen presumably from a common stem, they do have different feeding habits and thus occupy different ecological niches. Thus both are able to survive in the same environment because each uses a slightly different part of it.

The fact that the two species are so similar does not necessarily mean that they are of very recent origin, since the populations are dense enough to be evolving quite slowly and the environmental factors influencing the two groups are very nearly identical.

Drosophila mulleri and *aldrichi* are most dense in the southern parts of the State of Texas and apparently have not invaded the northern and eastern parts of the United States, but the population is known to extend into Mexico. Also the two western forms are found in warm climates. It was suggested (Patterson and Crow, 1940) that perhaps the mulleri group arose as two branches from a common ancestor somewhere to the south, perhaps in Central America, and that these branches migrated along the east and west sides of the Rocky Mountain system to their present locations. Further information appears to strengthen this hypothesis.

The discovery by Patterson that *buzzatii,* from Sicily and Argentina, is closely related to the North American *mulleri* group is very significant in this connection. It seems quite probable that there are forms related to *mulleri* between Mexico and Argentina that form a link between these two groups, and that in some regions of the Western Hemisphere there may be the more primitive forms from which both the North and South

American groups have come. If such forms exist, one would expect to find them in tropical localities. Here fly populations would be dense, large, and constant, and therefore under conditions favoring very slow evolution.

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SUMMARY

1. A series of tests with five species of the mulleri group show them to be related by some degree of cross fertility, although most of the species combinations do not cross reciprocally.

2. In most cases the hybrids produced show various abnormal characteristics such as unusual sex ratios, and sterility usually restricted to the males.

3. Nearly all the combinations of crosses show definite sexual isolation.

4. A strain of *aldrichi* carries an X-chromosomal gene that produces no noticeable effect within the species but acts as a dominant semi-lethal in hybrids with *mulleri*. The male hybrids from this cross show an unusually high incidence of an abnormal abdomen effect similar to the mutant *bobbed*.

5. The sum of the isolating factors greatly reduces the amount of cross breeding in the laboratory, and the very low percentage of *mulleri-aldrichi* hybrids found in nature indicates that these mechanisms are effective there.

6. Some of the species differ in gene arrangements, but two of the five are very similar. The hybrid chromosomes often show a tendency toward non-synapsis.

7. It is suggested that the Texas, West Coast, and South American members of the group arose as branches from a common stem and that more primitive forms related to these groups might be present in tropical Central or South America.

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TABLE 1

Hybrids Produced in Interspecific Crosses

Males / Females	Mulleri	Aldrichi	Mojavensis	Arizonensis	Buzzatii
Mulleri		Sterile ♂ Sterile ♀	Sterile ♂ Fertile ♀	Sterile ♂ No ♀	No ♂ Sterile ♀
Aldrichi	None		No ♂ Sterile ♀	No ♂ Sterile ♀	None
Mojavensis	None	None		Fertile ♂ Fertile ♀	None
Arizonensis	None	None	Sterile ♂ Fertile ♀		Larvae only
Buzzatii	None	None	None	None	

TABLE 2

Percentage of Females Fertilized in Pair Matings

Males / Females	Mulleri	Aldrichi	Mojavensis	Arizonensis
Mulleri	84	0	5	0
Aldrichi	0	88	17	0
Mojavensis	0	0	96	77
Arizonensis	0	0	33	90

V. RELATIONSHIPS IN THE MELANICA SPECIES GROUP

A. B. GRIFFEN

Among the North American members of the subgenus *Drosophila* the melanica group of species is conspicuous because of its wide geographical distribution and its relatively large population size. In these respects the *melanicas* are surpassed by only one other member of the subgenus, the repleta-hydei complex. These groups present excellent opportunities for the study of speciation within categories having large and widespread populations in nature. It is the purpose of the present paper to give a preliminary account of the relation between members of the melanica group. The data presented here were obtained from genetic and cytological studies of three cross-fertile members. *Drosophila micromelanica* has been omitted because of its complete sterility to other forms and *melanissima* because of its failure to survive in laboratory cultures.

MATERIALS AND METHODS

The following stocks have been used in the initial determination of the relationship and grouping of the members:

Drosophila melanica Sturtevant. Strains from Coffeeville, Kan., and the Ozark Mountains were the source of the test stocks for this form. These strains were kindly sent by Professor A. H. Sturtevant. Other *melanicas*, discussed in a subsequent section, were collected in the southern portion of the United States and in northern Mexico by members of the Texas laboratory.

Drosophila melanica paramelanica Patterson (Article I, this Publication). Five strains of this subspecies were used from collections at Madison, Wis.; Woodstock, Md.; Woodbury, Conn.; Zealand, and Wooster, Ohio. The first four were supplied by Professor Sturtevant and the fifth by Professor W. P. Spencer.

Drosophila nigromelanica Patterson and Wheeler. Three strains were obtained from collections made at Wood's Hole, Mass., and Wooster, Ohio, by Professor Spencer and at Cleveland, Texas, by Professor J. T. Patterson and the writer.

There are several sharp differences between *melanica* and *nigromelanica*, such as the darker body color and the red eyes of the latter as contrasted with the lighter body color and brownish eyes of *melanica*. Moreover, the males of *nigromelanica* have orange-colored testes whereas in *melanica* these organs are a dull yellow. The differences between *melanica* and *paramelanica* are not so marked, the general lighter coloration of *melanica* being the only outstanding quality. This lighter coloration is quite conspicuous in southwestern *melanicas*, most of which have a tan or very light brown coloration that easily permits identification.

The relationships which are presented here are based on cross-fertility between members and strains of the species and upon the gene arrangements as seen in salivary gland chromosomes of hybrids. All breeding tests were carried out on banana food which, though not entirely suitable for *nigromelanica,* has made possible comparisons of fertility under standard conditions; all cultures and matings were grown at 72° F. since this temperature was found to provide optimum conditions for mating, oviposition and development. The test stocks of the several strains were developed from inbred pairs.

Cytological Observations

The metaphase chromosomes of *melanica, paramelanica,* and *nigromelanica* all show the same configurations; all stocks thus far examined have one pair of large V's, one pair of small V's, two pairs of rods and a pair of microchromosomes which are generally small and rounded except in *nigromelanica,* where these bodies are somewhat larger and rod-shaped. It is probable that the large V-shaped element arose through the fusion of two rod-shaped chromosomes as has been demonstrated in the virilis complex (Patterson, Stone, and Griffen, 1940); the identity of this V is being determined through segregation tests. The small V is the result of a pericentric inversion which moved the centromere to a submedian position. This element is readily detectable in salivary gland cells and consists of two completely euchromatic arms. A similar case has been reported for *D. montana* (Stone, Griffen, and Patterson, 1941).

Cross Fertility and Gene Order

For the determination of cross-fertility in the melanica group, crosses of the test stocks were prepared as follows: All the possible combinations were set up as mass matings of ten pairs in each of ten vials. In cases showing no fertility the number of cultures was increased to a total of 150 ten-pair matings and the cultures were kept for eight weeks with several changes to new food. At the end of this period the crosses were counted fertile or sterile; cases of sterility have been indicated by the letter *S* in the table. Any crosses which produced offspring were then tested for degree of fertility through pair matings. Vials which had both members of the cross alive at the end of eight weeks, but which showed no indication of larvae, were counted as sterile. For each cross a minimum of one hundred living pairs was used, and in those cases where a cross-fertility of less than 1 per cent is indicated, a minimum of 150 pairs. The gene orders were determined in salivary gland cells of F_1 larvae.

On the basis of their cross-fertility the test stocks can be arranged as shown in the table. The *nigromelanicas, melanicas,* and *paramelanicas* form three distinct mating groups. There is considerable fertility within

the groups, but very low fertility between them; *melanica* and *nigromelanica* are slightly cross-fertile to *paramelanica,* but sterile to each other. All hybrids which have been obtained thus far are fertile, and the sexes appear in equal numbers.

As a preliminary step in the comparisons of gene orders each test stock was examined cytologically for the presence of rearrangements. In two cases, Madison and Wooster, of the *paramelanica* group, inversions were present in the longest autosome in approximately half of the preparations; other strains have shown no such heterozygosity thus far. Within the *paramelanicas,* aside from the inherent Madison and Wooster rearrangements, the following differences were noted in comparisons with Madison as a standard: In Woodstock a small proximal inversion in the longest autosome; no rearrangements in Zealand and Woodbury; in Wooster, three small inversions in the X, two small, proximal, overlapping inversions in the longest autosome and two small inversions in a third autosome. In the *melanicas* the two test strains showed identical arrangement; each in comparison with *paramelanica,* represented by Madison, shows a large central inversion in the X and in the longest autosome a proximal rearrangement which has not been sharply delimited at present, but which is apparently different from any of those mentioned above. From these observations it is apparent that, with the exception of Wooster, the *paramelanica* strains have common gene orders in all of the chromosomes except the longest autosomal element. Similarly the *paramelanicas* and the *melanicas* have the same order in all but the X and the long autosome. In the *nigromelanicas* Wood's Hole and Wooster show the same gene order, while Texas shows two conspicuous inversions in relation to either; the details of differences between this group and *paramelanica* are at present obscure because of the low degree of synapsis found in salivaries of the scarce hybrid larvae.

With the initial grouping on the basis of fertility and gene order as the basis for further study, survey tests have been begun upon strains derived from population samples collected by Dr. G. B. Mainland, Mr. M. R. Wheeler, and Mr. R. B. Wagner, and by Dr. J. T. Patterson and the writer. Thus far it has been found that all samples are highly fertile to the *melanicas* and slightly fertile, often in only one direction, to the *paramalenicas;* no fertility to the *nigromelanicas* has been observed. The gene order is predominantly that of *melanica* with several variations indicating the geographical extent of common genomes. For example, a group of Southwestern forms from Arizona, New Mexico, Utah, and northern Mexico (Sonora) seem to differ from Ozark and Coffeeville only in two small distal inversions in the long autosome. Strains from a group of populations extending northward through central Texas and into Oklahoma show a striking gene order difference in the form of a series of overlapping inversions in one of the autosomes; and finally a group of strains from Louisiana, Mississippi, and Florida show no gene order differences.

Discussion

Between the three members of the melanica group examined in this study there is a rather strong degree of isolation. In view of the fact that hybrids, when produced, are viable and fertile, the mechanism which can best be indicated is that of sexual isolation in P_1 crosses between the groups. Geographical isolation enters to an extent which can only be surmised; yet the distribution of the *paramelanicas* across the northern and the *melanicas* across the more southern regions gives evidence of a temperature barrier of some effectiveness. Ecological isolation can best be seen in *nigromelanica*. The Texas populations of this species are primarily forest dwellers (Patterson and Wheeler, 1942) and can be found feeding on fungi on the ground and in the cavities of stumps and logs. In Texas and other southern collecting points this species is found only in small numbers in traps; hence it may be classed as a fungus-feeder, although it is not completely dependent upon this type of food. This food preference and the marked sexual isolation of *nigromelanica* readily account for its identity as a species in spite of the geographical coincidence of its populations with those of both *melanica* and *paramelanica*. There is no apparent thermal barrier between this form and its relatives.

Within the species there is evidence of distinct geographical varieties. In the *melanicas* the division may be made on the basis of gene orders so that distinct southwest, central and southeastern races can be recognized, the Coffeeville and Ozark test-strains belonging, of course, to the southeastern division. There is within these varieties little or no restriction of particular gene orders to small local populations, and the geographical extent of each type is very broad. Incomplete tests of a strain from Beaumont, Texas, have revealed an intermediate condition between the central and southeastern types; Beaumont-Ozark hybrids show a portion of the series of overlapping inversions which, as previously mentioned, are a striking characteristic of hybrids between the two races. The zone of junction between these varieties probably extends northward through eastern Texas and Louisiana into the Ozark region.

In the *paramelanicas* there is as yet no comparable evidence of racial groups; such might be expected, however, in the form of populations north of the Great Lakes and in regions of the northwest. One detail which should receive comment here is the low fertility of the Wooster test stock. It has been demonstrated in the virilis group (Patterson, Stone, and Griffen, 1942) that continued inbreeding of progeny from original crosses between some geographical strains of the domestic type eventually resulted in a great reduction of fertility. The continued inbreeding which produced the Wooster test strain likewise has resulted in low fertility, which is expressed in both sexes as shown in out-crosses. The presence of different gene orders is indicative of a heterogeneous local population, which must also have been heterozygous for numerous factors; the continued inbreeding which produced the test strain has

allowed these factors to express themselves to a degree so marked as to indicate that the local population was the result of fairly recent hybridization between adjacent or overlapping groups, each of which had already developed numerous and different sterility factors. This explanation for the heterozygous condition of the Wooster stock is plausible since it is a member of the subspecies which can be called the genetic intermediate of the melanica group.

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FERTILITY AND AVERAGE NUMBER OF OFFSPRING PER TUBE

	♀♀ \ ♂♂	NIGROMELANICA			PARAMELANICA					MELANICA	
		Texas	Wood's Hole	Wooster	Madison	Woodstock	Zealand	Woodbury	Wooster	Ozark	Coffeyville
NIGROMELANICA	Texas	86% av. = 24.2	45.2% av. = 46.6	69.2% av. = 34	less than 1%	S	less than 1%	S	S	S	S
	Woods Hole	51.9% av. = 44.5	14.3% av. = 40.6	38.1% av. = 36.4	S	S	S	S	S	S	S
	Wooster	52.2% av. = 49.3	22.4% av. = 40.2	36% av. = 38	S	S	S	S	S	S	S
PARAMELANICA	Madison	S	S	S	82% av. = 43	1.5% av. = 9	4.1% av. = 30.2	22% av. = 35.7	8.0%	less than 1%	0.21%
	Woodstock	S	S	less than 1%	23.0% av. = 38	75.2% av. = 52	27.3% av. = 32	39.1% av. = 41	15.4% av. = 48.2	less than 1%	S
	Zealand	S	S	S	24.2%	32%	42.3% av. = 17	20% av. = 12	incomplete; approx. 10%	S	S
	Woodbury	S	S	S	13.3% av. = 32.9	3.6%	21.3% av. = 7	52% av. = 21	15% av. = 60.9	S	S
	Wooster	S	S	S	34.0% av. = 60	16% av. = 46.8	48% av. = 43	10.5% av. = 15.8	13.5% av. = 51.6	S	less than 1%
MELANICA	Ozark	S	S	S	0.95%	less than 1%	less than 1%	less than 1%	3.2%	67.0% av. = 17	47.7% av. = 23.8
	Coffeyville	S	S	S	S	less than 1%	less than 1%	less than 1%	less than 1%	5.8% av. = 19.2	70.0% av. = 35

VI. GENETIC RELATIONSHIPS IN THE *DROSOPHILA FUNEBRIS* GROUP

G. B. MAINLAND

In connection with the study of genetic relationships between different populations of *Drosophila* being conducted at The University of Texas, the *Drosophila funebris* group of species was selected for investigation. The distributions of the populations in this group and the intra- and interspecific hybrids between them differ from similar types of studies with other species groups of *Drosophila*. This report presents a preliminary account of the study.

GEOGRAPHIC DISTRIBUTION

The *Drosophila funebris* group is composed of three species, namely, *D. funebris* (Fabricius), *D. macrospina* Stalker and Spencer, and *D. subfunebris* Stalker and Spencer.

Drosophila funebris was described in 1787 by Fabricius as *Musca funebris* (Sturtevant, 1921). This species is cosmopolitan, having been reported from the temperate zone of every continent. In the northern part of the United States, it is fairly common in the woods and around the habitations of man, while in the southern United States, it is relatively rare. Among the 671,500 *Drosophilinae* which have been collected in Texas by this laboratory, only 30 specimens of *D. funebris* have been taken, of which the large majority were collected in wholesale produce houses. In all tests attempted between *D. funebris* and the other members of the group, no hybrid progenies have been produced; hence, little reference will be made to this species in this report.

In 1939 Stalker and Spencer described *Drosophila macrospina macrospina* from a stock established from three females taken on The University of Texas campus by Parker in 1935. Subsequently, there have been described two additional subspecies: *D. m. ohioensis* Spencer (1940b) and *D. m. limpiensis* Mainland (1941). The subspecies *macrospina* has been taken in wooded areas along streams in the central, eastern, and northeastern portions of Texas, in Oklahoma, Missouri, Arkansas, Louisiana, Tennessee, Mississippi, Alabama, and Florida. Northward in Ohio (and Michigan?), *macrospina* is replaced by the subspecies *ohioensis*. Since various combinations of the morphological characters which are used to distinguish between the subspecies *macrospina* and *ohioensis* are found among the flies collected in the areas between Ohio and Texas, the assignment of stocks from these areas to either subspecies is, to some degree, arbitrary. Inasmuch as both subspecies give similar results in intraspecific and interspecific crosses, they both will be considered as geographical strains of *macrospina* for the purposes of this report.

Drosophila macrospina limpiensis has been completely described recently by Patterson and Wheeler (1942). This subspecies was first found by

Patterson in the Limpia Canyon of the Davis Mountains of west Texas in July, 1939. Subsequently it has been taken at various points in New Mexico, southeastern Arizona, central Sonora, and at one point in Zion National Park, Utah. Many of the *limpiensis* collections have been made from the bracket fungus, *Polyporus farlowii* Lloyd, found growing on willows, *Salix* sp.

At present little is known concerning the distribution of *Drosophila subfunebris* Stalker and Spencer (1939). Spencer has collected this species at two points in California: within the city of Pasadena, and thirty miles east at Camp Rincon, San Gabriel Mountains. Sturtevant (private communication) has stated that *subfunebris* is one of the rarest species which have been taken in the vicinity of Pasadena.

Population Densities

Patterson's data (unpublished) for the collection of *Drosophilinae* at the Aldrich Farm, three miles east of Austin, Texas, indicate that the *D. m. macrospina* population has two maxima during the year in central Texas, the first being in April and May, and the second, in September. The collection records for *macrospina* at the Aldrich Farm from October, 1938, to June, 1940, are shown in Table 1. During this period, the number of traps, their position, the manner of collecting, and the type of bait used remained essentially the same. Although the number of *macrospina* per collection appear to be similar for the same seasons of different years, it is readily apparent that some of the other species were not subject to a similar variation. The average number of *macrospina* per collection in April, 1939, is very similar to that of April, 1940; but *macrospina* composed 7.30% of all the flies trapped during April, 1939, while in April, 1940, they represented 24.32%. Fluctuations in the number of the different species attracted to the traps may be due to various factors, e.g., actual population size, abundance of natural foods, temperature, humidity, wind, etc. At the present time there is no method of measuring accurately the absolute population sizes of different species even at a given place.

D. m. macrospina is almost strictly a woodland species being found usually in wooded areas along streams or in swampy areas. Of the 8,004 specimens taken in Texas by this laboratory, only one was taken in a wholesale produce house. Small numbers have been captured in or near wooded areas within cities. In those parts of Texas where *macrospina* is found, it forms from about 1% to 15% of the flies taken in the collections, with a mean about 10%. Eastward and northward, with the exception of Oklahoma, the records of this laboratory and those of Spencer (private communication) indicate that the subspecies *macrospina* and *ohioensis* are much less common than in Texas, composing from about 0.1% to less than 0.01% of the number of the flies collected. It is rather probable that the season of the year during which the out-of-state collections were made did not always coincide with the season of the maximum development of the *macrospina* populations.

Patterson (1941) has pointed out that various species of *Drosophila* may be differentially attracted to the baited traps. At several of the points where collections of *D. m. limpiensis* were made, there is rather good evidence that this is the case. At Magdalena, Sonora, Mexico, the ratio between *D. m. limpiensis* and *D. victoria* Sturtevant taken from the traps baited with fermenting bananas was 1:1, while in the immediate vicinity, the ratio between these two species collected on the fungus, *Polyporus farlowii* Lloyd, was 1.7:1. At San Bernardino, Arizona, similar, but more striking, results were obtained; the ratios were respectively 1:3.7 from the traps and 6.8:1 from the fungus.

Considering the fluctuations in population densities, the differential attraction to traps, the ecology of different regions, the different species supported thereon, and many other factors, it appears that quantitative comparisons of populations from different areas, as judged by material taken from traps, may indicate very little regarding actual population densities of a given species in different areas. Hence, at least in the case of *macrospina* comparisons of the frequency of occurrence of *macrospina* in different areas are of very little value.

Stocks Used in These Investigations

The source of the various stocks used in these investigations are listed below. To facilitate the keeping of the pedigrees in the various test crosses, a letter has been assigned to each stock.

Macrospina Stocks

M. (527.6a) Standard *macrospina* stock. Single female, collected three miles east of Austin, Texas, at the Aldrich Farm, Dec. 23, 1939.

A. (1281.10) Pair, collected at Petit Jean State Park, Arkansas, Sept. 17, 1941.

C. (1112.6d) Pair, collected near the Mississippi River on the northern outskirts of New Orleans, Louisiana, June 13, 1941.

K. (1148.8) Pair, collected at Lake McKethan north of Tampa, Florida, June 19–20, 1941.

O. (Sp. 1) Type stock for subspecies *ohioensis*. Two pairs, collected at Overton, Ohio, by W. P. Spencer, July, 1939.

Q. (854.4) Pair, collected near Columbus, Mississippi, by Dr. O. P. Breland, Aug. 8, 1940.

V. (874.9a) Single female, collected on the banks of the Rio Grande River near Del Rio, Texas, Nov. 13, 1940.

Limpiensis Stocks

L. (268.3i) Type stock for subspecies limpiensis. Single female, collected in Limpia Canyon of the Davis Mountains, west Texas, July 2, 1939.

B. (1248.1h) Pair, collected at San Bernardino, Arizona, Aug. 19–20, 1941.

G. (1256.2e) Pair, collected about 1 mile south of Magdalena, Sonora, Mexico, Aug. 23, 1941.

H. (1261.2g) Pair, collected on *Polyporous farlowii* at Hermosillo, Sonora, Mexico, Aug. 25, 1941.

J. (1253.2j) Pair, collected at Punta del Agua, Sonora, Mexico, Aug. 22, 1941.

N. (968.2) Pair, collected 17 miles west of Silver City, N.M., by Mr. A. B. Cutler, Supt. of CCC Camp SCS–20–N, Nov. 2–9, 1940.

R. (1241.5a) Pair, collected near Radium Springs, New Mex., Aug. 16, 1941.

U. (1263.3f) Pair, collected on *P. farlowii* near Patagonia, Arizona, Aug. 27, 1941.

Z. (1223.7a) Pair, collected in Zion National Park, Utah, Aug. 1–3, 1941.

Subfunebris Stock

S. (Sp. 4) Type stock for species subfunebris. Single female, collected at Pasadena, Calif., by Dr. W. P. Spencer, May 5, 1937.

The letters used in the following manner: The female parent is always written first, e.g., M x L = M ♀ x L ♂ ; a double series of letters indicates F_1 hybrids, e.g., *ML* x *ML* is a cross of F_1 ♀ x F_1 ♂ both of which were derived from the cross *M* ♀ x *L* ♂ ; a triple set of letters indicates progeny derived from a backcross, e.g., *(ML)L* indicates that such an individual was the result of backcrossing a hybrid *ML* ♀ to an *L* ♂, while *L(ML)* indicates progeny from the cross *L* ♀ x hybrid *ML* ♂. The progeny from more complex crosses are indicated in a similar manner. In a few cases exponents are used with a letter, e.g., $M^{10}(ML)$ is an individual derived from the tenth backcross of hybrid *ML* ♂ to *M* ♀ ♀.

Methods Employed

Relationships between the geographical races, subspecies, and species were tested by cross-matings in a number of ways. The first type of test was the determination of the willingness or the ability to cross between the several strains. In all of the matings reported in this paper, the standard banana-yeast-karo-agar medium of the Texas laboratory was employed.

Initially, with the exception of certain intraspecific crosses, small mass mating of about five pairs per vial were made. At two- or three-day intervals the parental flies were changed to new food, and the vials from which the parents had been removed were saved. This procedure was continued over a period of from 20 to 30 days. From time to time the vials were examined for progeny. A part of the data of Tables 1 and 6 regarding cross-fertility of parental crosses was obtained in this manner.

In the case of some interspecific matings which did not produce offspring within the limits of the small mass mating tests, certain of the crosses were repeated in large mass matings of 25 pairs per half-pint milk bottle.

At 10 days the mating bottles were examined for larvae, and the parental flies were transferred to new half-pint bottles. If no larvae were noted, the bottles were retained for a ten-day period at which time they were examined for progeny which may have escaped detection at the time of the first examination. Subsequently, this procedure was continued at five-day intervals up to 65 days, provided that the parental flies remained alive without producing progeny. The initial number of half-pint bottles used for these large mass matings was normally two or three, but in tests employing *funebris* as one of the parental species, the number varied from two to twenty-four. In a few specific cases where the parental species proved to be cross-sterile in the first large mass matings, second and third tests were run in the same manner. The data in Table 6 were obtained from these tests.

The second type of test was the determination of the fertility of the F_1 progenies by inbreeding. Series of four or five pairs per vial were made. At 10 days these vials were examined for larvae, and the F_1 adults were transferred into new vials which were also examined for larvae on the twentieth day. A part of the data in Table 2 and all of the data of Table 7 were obtained from such crosses. If no larvae were in evidence in either the first or second series on the twentieth day, the F_1 males and females were separated. A part of the F_1 males were backcrossed to females of one of the parental strains, and the other portion of the males, to females of the other parental strain. The F_1 females were similarly backcrossed to males of the parental strains. In all of these backcross tests, mass matings were employed, four or five hybrids being mated to eight or ten flies of the parental species. All backcross matings were examined for larvae on the 5th, 10th, 15th, and 20th days. Tables 3, 4, 8, and 9 list these data.

After the exploratory crosses outlined above, the experiments, the data for which are listed in Table 1, were carried out by making 120 pair matings in vials between flies that were at least three days of age. Ten days later all the vials were examined; if one or both members of the pair had died or if the vials were contaminated with mold, such vials were discarded. If fewer than 100 pairs remained, the number retained for the test is recorded within parentheses on the Table. On the twentieth day, the vials containing offspring were counted. Originally the number of young adult flies in each vial was counted, but it was soon apparent that there was a great variation in the number of adults which had emerged in the various vials by the twentieth day, the variation being from zero to 125. Afterwards the production of progeny was considered "normal" if the large majority of pairs of the mating had produced 30 or more progeny. When the average number was below 30, the average number of young per fertile pair is recorded. Table 1 and subsequent tables give both the percentage, to the nearest per cent, of fertile pairs in a given mating, and the average number of progeny produced per fertile pair.

In the intraspecific crosses, F_1 progenies were tested by three types of pair matings: inbreeding, and backcrossing to both parental strains. In

these tests 60 pairs were employed. If fewer than 50 pairs remained after the examination of 10 days, this is noted on the table. These data are listed in Tables 2, 3, and 4.

The interspecific hybrids were tested in pair matings only by means of the backcross; otherwise, the procedure was the same as for the intraspecific tests of F_1. The data for these interspecific backcrosses are listed in Tables 8 and 9.

The fertility of F_2 backcross flies was obtained by making pair matings to the *L* stock for intraspecific crosses and to both parental strains for the interspecific crosses. Intraspecific crosses were made in sufficient quantity to insure 100 living pairs at the end of ten days. In many of the interspecific crosses, it was not possible to obtain 100 F_2 progeny; the number of pairs tested in each interspecific test is recorded on the Table. The results of the intraspecific crosses are shown on Table 5; those for the interspecific crosses, in Tables 10 and 11.

In certain of the interspecific crosses observations were made regarding the courtship and mating behavior of the flies in attempt to learn some of the reasons for the failure to mate.

Another test employed was the dissection of the females to determine if motile sperm were present in the spermathecae of the females.

The cytological results were obtained by making acetocarmine smears of the larval ganglion for metaphase plates and the salivary glands for the giant salivary gland chromosomes.

Results of Intraspecific Crosses

The results listed in Table 1 indicate that all matings within the species *macrospina* are rather fertile. There is considerable variation in the degree of cross-fertility both within and between the several subspecies. In general *limpiensis* males appear to mate almost as readily with *macrospina* females as they do with *limpiensis* females since the percentage of both types of matings producing offspring are about equal. On the other hand, *macrospina* males appear to mate less readily with *limpiensis* females than with *macrospina* females because there is a smaller percentage of pairs of the former type of mating which produced progeny. These observations are substantiated by those made at the time of the ten-day check. The matings within both subspecies or between *limpiensis* males and *macrospina* females generally had larger, more mature larvae than those between *macrospina* males and *limpiensis* females. This appears to be due to earlier copulation having occurred in the former crossed than in the latter.

In obtaining the data for Table 1, all pairs which had produced larvae by the twentieth day were considered fertile regardless of the age of the offspring. With but few exceptions, fertile pairs in the various crosses has some offspring in the imagine stage by the twentieth day. Males and females of the Florida *macrospina* stock, K, were exceptional in that they had a much higher initial isolation to both *limpiensis* and *macrospina* stocks than did any other *macrospina* stock tested. With increasing lengths

of time during which the matings were maintained, the percentage of pairs producing progeny increased. In many *K* matings the oldest larvae were in only the first or second instar at the time of the fertility check. This type of isolation was more marked in the case of *K* males than *K* females. Similar results were obtained in the cross: Mississippi *macrospina, Q,* males to *ohioensis, O,* females.

In all of these intraspecific crosses, the number of hybrid males and females produced was approximately equal.

The data of Table 2 obtained from inbreeding the F_1 hybrids show that all progenies tested were fertile to a marked degree with the exception of those from the crosses of *limpiensis* females to *macrospina* males. All hybrids showed heterosis to some degree, the hybrids being larger and more active than individuals of the parental strains. With the exception of the males from the crosses noted above, the hybrids were also more fertile, bred somewhat more rapidly, and produced more progeny.

The hybrids from the cross: *limpiensis* ♀ x *macrospina* ♂, were vigorous flies, but their fertility was poor, varying from sterile to slightly fertile. The fertile hybrid pairs produced six or less offspring; hence, they are considered as being semi-sterile since control matings under the same conditions produce from thirty to over one hundred offspring. From Tables 3 and 4 it is evident that the sterility was due to an unbalance in the hybrid males since such males continued to show sterility or semi-sterility when backcrossed. On the other hand, the hybrid females were as fertile in the backcrosses as were the females from the reciprocal crosses. Dissections of the spermathecae of the females to which these hybrid males were known to have mated revealed sperm which were only slightly motile.

There is a general tendency of the F_1 males to be less fertile with increasing western origin of the *limpiensis* female parent. The F_1 males have a similar, but less striking, reduction in fertility with increasing eastern origin of the *macrospina* male parent. Exceptions were noted among the F_1 males when the source of the *limpiensis* female parent was from southeastern Arizona and Sonora, but these data are incomplete.

The data for the first backcross progenies are presented in Table 5. Obviously the offspring from any single cross varied considerably in their genetic constitution. It is to be noted that the first backcross females were generally quite fertile, their cross-fertility comparing favorably with that of the F_1 hybrid females when tested to males of the *L* stock. The percentage is similar to the 85% of cross-fertility in the cross, *O* x *L*, and 85% in the mating, *OL* x *L*.

The first backcross males showed a considerable variation in the percentage of pairs producing progeny by *L* females. Some of the ratios obtained are rather suggestive, but the data are insufficient to warrant conclusions in most cases.

When pairs of offspring from the cross, *L* x *M*[10]*(ML)**, were inbred, it was found that 48% of the pairs were fertile. Females derived from the same cross were tested to *M* males; all of these females were fertile. Hence, it is apparent that several factors are operative in the production of sterile males in the crosses between *limpiensis* and *macrospina*.

RESULTS OF INTERSPECIFIC CROSSES

The data for interspecific parental matings between the several species and subspecies of the *Drosophila macrospina* group are presented in Table 6. Although large mass matings were made of various *funebris* strains to *subfunebris* or to the different subspecies and races of *macrospina*, no progeny were produced in any of the experiments.

Crosses between *subfunebris* and the several subspecies and geographical races of *macrospina* result in an interesting series of cross-fertility relationships. With the exception of two *macrospina* strains from near the known western limits of this subspecies, no *macrospina* (or *ohioensis*) strains were cross fertile to *subfunebris*. These two exceptional strains, *M* and *V*, produced progeny only if used as the female parent. In contrast, all *limpiensis* strains, with the exception of the *J* strain from Punta del Agua, Sonora, Mexico, have produced progeny in at least one direction with *subfunebris*. The ease with which the various *limpiensis* strains crossed shows considerable variation.

In all cases where interspecific hybrids were obtained, the number of offspring was less than that produced by a similar number of pairs of either species. In all successful crosses, the interspecific hybrids consisted of about equal numbers of males and females. Neither sex in any of the progenies was considered as being abnormal morphologically.

The cross, *M* ♀ x *S* ♂, went poorly after a period of 20 to 40 days from the time the pairs were placed together. Recently this cross produced progeny after 12 days; in this case the age of the parental flies was 20 days or more. The reciprocal cross has never yielded offspring even though it has been attempted repeatedly for periods up to 65 days. *M* males have never been observed to court *S* females, although males court one another consistently even when in the immediate vicinity of the *S* females.

In one small mass mating *V* females produced a few hybrid progeny by *S* males after a period of approximately thirty days. Other small mass matings of this same series were cross-sterile as were all of the reciprocal crosses.

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*The males used in this cross were taken from the progeny of the tenth backcross generation of *M* females by *ML* males, i.e., such males were obtained by backcrossing the hybrid *ML* males and the males from each successive backcross generation to *M* females in turn for ten generations. Ten generations were used since there is a 99.5% chance of having replaced all *L* chromosomes, except the *Y*, by the homologous *M* chromosomes provided that the Y chromosome was not dependent upon certain autosomes in order for the males to be fertile. The development of the Table from which this determination of chance was taken is presented in the Appendix, p. 102.

Hybrids have been obtained from the cross, *S* ♀ x *L* ♂, but the majority of the attempts have been failures. In the few successful crosses, progeny were first noted about the twenty-fifth day or afterwards. The number of progeny produced was fair once females began laying fertilized eggs. *L* males were noted to court *S* females with much less vigor than they do while courting females from their own or from other strains of *macrospina* and *limpiensis*. If an *L* male lost his orientation with respect to the *S* female, he made little effort to regain it. The reciprocal cross, *L* ♀ x *S* ♂, has been attempted repeatedly without the production of progeny. *S* males actively courted *L* females, but without the same persistence as females of their own species. Initially the females were rather passive to the courtship behavior of the males until the mates attempted copulation. Thereupon the females became "violent," attempting to brush the males off by using both posterior pairs of legs, clipping their wings, shaking and running. Thereafter the females usually resisted further advances of the *S* males.

When the males of the following *limpiensis* stocks: *R, B, U,* and *H,* were paired with *S* females, larvae appeared in the cultures from 10 to 15 days afterwards. The reciprocal crosses were unsuccessful although continued for a period of 30 days and upward. However, all of these stocks were tested by means of small mass matings which do not always permit one to determine whether progeny might be produced in large mass matings.

The *N* stock produced offspring in reciprocal matings with *S*. When *N* was used as the female parent, larvae usually appeared in cultures by the tenth day. The reciprocal cross produced progeny less readily, the first larvae appearing about the twentieth day. In the latter cross fewer progeny were produced by the same number of pairs during the same length of a productive period than by the former. The *G* stock has reacted very similarly to the *N* stock in crosses to *subfunebris*.

A fairly large collection of *limpiensis* was made at one point in the floor of Zion Canyon, Zion National Park, Utah, during the summer of 1941. The vials in which these flies were brought back to the laboratory contained numerous larvae. The adults which developed from them were mated to *subfunebris* reciprocally. In both cases nine small mass matings were made. Both series showed four fertile sets at the end of ten days. The remainder of both sets had not produced offspring at the end of twenty days. Subsequently the *Z* stock, which originated from a single female taken at Zion, has been mated reciprocally to *S*. If *S* was used as the female parent, the cross resulted in progeny after a period of 15 days; data indicate that, if the reciprocal cross goes, it does so after a considerably longer time. Other stocks from Zion are being investigated to elucidate these contrasting results.

From the data presented in Tables 7, 8, and 9, it is clear that the interspecific F_1 female hybrids are markedly fertile while all F_1 hybrid males are sterile. F_1 males, in addition to those shown on Table 8, were

tested in large mass matings to both parental species. In no case were any progeny produced.

With two exceptions all F_1 females proved to be fertile. One-half of the *MS* females failed to produce offspring by *M* males in pair matings; however, when such females were subsequently mated to 5 *M* males or to a single *S* male, all proved to be fertile. Hence it appears that *M* males are reluctant to mate with the hybrid *MS* females. One female failed to produce progeny in each of the following crosses: *NS* x *S* and *SN* x *N*. These females were not mated to other males to determine whether or not they were fertile.

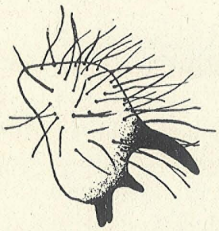
The F_1 interspecific hybrids were examined with regard to morphological differences and similarities to their parental strains. With the exception of *GS* males, the dark red eye color of *subfunebris* was dominant to the light red *macrospina* and *limpiensis*. The *GS* males had an eye color somewhat lighter than *subfunebris* but considerabl darker than *G;* hence, it is rather likely that the *G* stock carried a recessive sex-linked gene (or genes) which also affects the pigmentation of the eye in addition to the autosomal gene (or genes) noted above. The number of arista branches for *subfunebris* was also dominant in all crosses to *limpiensis* and *macrospina*. On the other hand the dark body color of *M* was dominant in both *MS* females and males. All *subfunebris-limpiensis* hybrids had divergent rows of median acrostichal hairs, a diagnostic character of *limpiensis*. Although difficult to determine, the puparia color of *macrospina* and *limpiensis* appeared to be dominant.

Although considerably larger, the hybrid females were intermediate to both parental species with respect to body build and shape. The color of the ovipositor plates of *subfunebris* are a clear yellowish-tan while those of both subspecies of *macrospina* are dark brown or black. The ovipositor plates of the hybrids were a light brown with a darkened central area. In addition the plates were intermediate in shape.

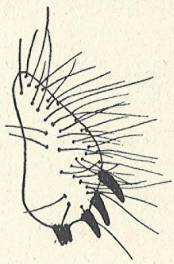
Generally the hybrid males resembled the males of their maternal stock more than those of their paternal. Although larger, males carrying a *subfunebris* X chromosome showed less sexual dimorphism than those carrying a *macrospina* (or *limpiensis*) X chromosome. It should be pointed out that *subfunebris* males have considerably less sexual dimorphism than those of the species *macrospina*. Hybrid males having a *subfunebris* mother had a *subfunebris*-like bristle pattern on their anal plates (vid. Plate I), and a *subfunebris*-like build and carriage. In all cases the shape of the anal plates was more nearly like that of *subfunebris*. On the other hand, hybrid males having a *macrospina* or *limpiensis* X chromosome displayed sexual dimorphism similar to that of *macrospina*, a *macrospina*-like pattern of bristles although the posterior two were more nearly of a size (vid. Plate I), and a *macrospina*-like build and body carriage.

Tables 10 and 11 present the data concerning the fertility of the first backcross progenies. From Table 10 it is evident that the first backcross females are not as fertile as the F_1 hybrid females. However, until more

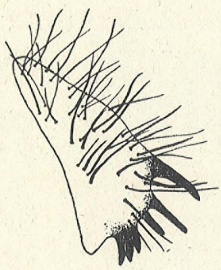
MALE ANAL PLATES



D. MACROSPINA LIMPIENSIS



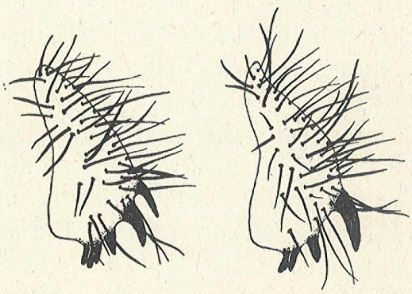
D. SUBFUNEBRIS



D. M. LIMPIENSIS ♂

X

D. SUBFUNEBRIS ♀



D. SUBFUNEBRIS ♂

X

D. M. MACROSPINA ♀

extensive data are available, it appears to be inadvisable to conclude much regarding the factor or factors operative in this reduction. It should be pointed out, though, that sexual isolation is apparent when the first backcross females are tested to the parental strain other than that used as their fathers. In Table 11 the data for the fertility of the first backcross males are given. In the tests so far conducted, only two of these males have produced progeny. Until further data are available regarding crossing-over, disjunction, segregation, and second backcross males, little can be decided regarding the sterility of the F_1 and first backcross males.

Among the first backcross progenies from *MS* x *M* and *SL* x *L*, it was observed that there was a 1:1 segregation for the dark eye color of *subfunebris* and the light of *macrospina*. The dark body color of *M* gave an approximate 1:1 ratio in the cross *MS* x *S*. However, among the "lighter" group there appeared varying degrees of lightness, indicating that several genes must affect body color.

From the cross, *(MS)S* x *S*, fertile females which produced progenies having *macrospina*-like genitalia were selected and backcrossed to *S* for several generations and then inbred. Thus it was possible to obtain a *subfunebris*-like stock with *macrospina* type genetalia.

Other morphological differences noted among the F_1 progenies either gave "complex" arrays of combinations not analyzable with the data at hand or were not noted.

Discussion

Numerous workers (review Dobzhansky, 1941, pp. 51–93) have shown that some of the differences existing between races, subspecies, and species are similar (or identical) to and follow the same Mendelian laws of segregation as gene differences within a single population. In the *Drosophila macrospina* group certain morphological differences have been noted and followed intra- and inter-specific crosses.

The dark body color of the *M* stock behaves as a simple dominant in both intra- and inter-specific crosses. That other genes, recessive and dominant, also cause darkening of the body color in these crosses does not mitigate against this conclusion. The divergence of the median acrostichal rows of hair immediately in front of the scutellum is a diagnostic character of *D. m. limpiensis*. In both intra- and inter-specific crosses, this character acts like a simple dominant gene. Apparently in some *limpiensis* stocks the expression of this character is modified through the action of other genes, but in the species and subspecies hybrids the full expression of the character is realized. Hence it appears likely that dominant alleles of such "modifying" genes are present in the other species and subspecies.

Stalker and Spencer (1939) stated the following in their introduction to the description of *D. macrospina* and *D. subfunebris:*

"In some of these (small sub-groups of *Drosophila*) one of the characters proves particularly valuable for taxonomic purposes, i.e., sex combs

in the *affinis* group (Sturtevant and Dobzhansky, 1936). In the *funebris* group the plate of the male and female genitalia differ markedly in the three species. It is extremely difficult to separate the specimens of *funebris* and *subfunebris* of either sex on other characters, but reference to the genital plates makes the classification easy."

This author has found that this statement has been true for all collections of members of this group which have been taken in different geographical localities. Consequently, it is very interesting to note that it has been possible, by repeated backcrossing and subsequent inbreeding, to establish a stock of *subfunebris*-like flies having *macrospina* type genitalia. From the evidence at hand, it appears that this "taxonomic characteristic" depends upon not more than a few sex-linked genes.

Within the *D. macrospina* group, every indication points to the fact that the morphological differences between the species and subspecies are very similar to known gene mutations and are inherited in the same manner as mutations within a species.

Despite the sterility encountered in both intra- and interspecific hybrids, the sex ratios in all of the numerous experiments were very close to normal. Therefore it appears that the genic balance in the determination of sex for the various strains of the species *macrospina* as well as that of *sub funebris* are sufficiently similar so that a chromosome from one strain, subspecies, or species may replace its homologue in another without producing any gross phenotypic changes such as found by Wharton (1942) in the *repleta* group.

With the assembling of material regarding the nature of species in the genus *Drosophila* (Patterson, 1942a, b), one principle has become especially evident, namely, isolating mechanisms (Dobzhansky, 1941, pp. 255–330; Patterson, 1942b) inhibit or prevent the spread of genes from one population to another. Depending upon the efficacy of the isolating mechanisms involved in the separation of races, subspecies, and species, the different categories are able to maintain their identity with varying success.

One very obvious type of isolation is geographic isolation. Unfortunately this term has been used to cover two types of isolation, namely, biogeographic isolation and isolation due to distance alone. Biogeographic isolation indicates that the populations are separated by geographic regions through which the biotic environment, e.g., soil, climate, hosts, is unsuitable. Hence, populations separated in such a manner connot exchange genes with one another except rarely when a waif is carried into their midst. In contrast, the isolation due to distance implies that the several populations are not truly isolated, but merely that the exchange of genes is reduced by the distances between populations of the continuous series. In this case the amount of gene exchange is largely determined by the effective range of the single individuals (Wright, 1940).

Isolation due to the distance seems to be the major type of isolation found within the subspecies *macrospina* and *ohioensis*. From collections

made by Patterson, Spencer, and this author (all unpublished data), it appears in general that *macrospina* and *ohioensis* populations are limited to regions along stream banks. Rarely in heavy forested, semi-swampy areas, these subspecies are apparently widely distributed over an area without regard to streams. Wright (1940) has shown that in populations which are essentially one-dimensional (shore-line, river, etc.) the differentiation increases much more rapidly with distance than in populations which are two-dimensional, i.e., a population distributed uniformly over a large area. Since *macrospina* and *ohioensis* populations appear to be more nearly like a one-dimensional population, it seems that an explanation is offered for the large amount of morphological and physiological differences noted in the various populations and the intergradations between them.

All present evidence points to the fact that the *limpiensis* populations are separated from one another biogeographically. Their distribution through the southwest appears to be discontinuous even along rivers which flow the year around. Most of such western rivers flow through regions unsuited to the growth of *Salix* sp., e.g., gorges. However, it should be pointed out that populations of *limpiensis* living along the same river system may not be completely isolated from one another at the present time. The bracket fungus, *Polyporus farlowii,* not only grows upon the rotting heart wood of living *Salix* sp. but also upon larger pieces of dead wood. It is highly possible that dead wood carrying *P. farlowii* infected with the larvae of *limpiensis* may be washed down the river during the time of floods. Hence, occasionally a undirectional transfer of individuals from one population of *limpiensis* to another may occur.

Field observations, although inconclusive, tend to indicate that *limpiensis* populations are also small. Wright (1932, 1937, 1940) has shown that small completely isolated populations tend to fixate a chance combination of genes. Such combinations may not be the most adaptive. The discontinuous nature of the morphological and physiological variation found among the different samples of *limpiensis* populations is not at variance with the probability that many of them are small isolated populations. On the other hand, all experimental evidence indicates that *limpiensis,* although heterogeneous, forms a natural group distinctly set off genetically from the other members of the *Drosophila macrospina* group.

Very little is known regarding the distribution of *subfunebris.* However, it seems likely that this species is separated geographically from *limpiensis* populations by a desert area unsuited biotically to either of the two.

Funebris is widespread through North America, but like *virilis* (Patterson, 1941) it seems to be a species recently introduced by man, preferring a "domestic habitat." Sturtevant (1921) has reported this species to be especially common around stables, and it has been observed around and breeding upon stale formalin preserved animal material. He stated, "It will breed upon fleshly fungi, but is rarely found about them in woods. It

is, in fact, seldom to be found in woods at all, though quite common about houses, barns, or grocery stores." The extensive collections of the Texas laboratory are in complete agreement with the observations of Sturtevant and also those of Stalker and Spencer (1939). Stalker and Spencer further stated, "In view of the almost constant association with the habitations of man and its rarity in the woods it would seem to be an introduced species in the United States." Although the geographic distribution of *funebris* overlaps that of *subfunebris* and the three subspecies of *macrospina,* it is, apparently, almost completely isolated ecologically from them since with the exception of *subfunebris,* about which we know little, the other members of the *macrospina* group are woods dwelling species.

Another type of mechanism engendering the separation of populations is sexual isolation. In many forms of higher animals there exists a behavior pattern preliminary to the act of copulation. These patterns may, and often do, differ in various populations. Variations of this preliminary behavior may weaken or fail to elicit the normal response on the part of either or both sexes. In other instances, such differences may evoke a negative response, e.g., females may resist the advances of or run away from males courting them. The lack of the proper behavior pattern is one of the causes contributing to sexual isolation. Regardless of the manner by which the sexual isolation is achieved, the net result is the same, namely, reproduction between such populations is reduced. Sexual isolation is the most efficient type of isolation which may exist between populations in contact since the reproductive effort is preserved and the potential competition of the hybrids with the parental forms is reduced or eliminated.

Within the genus *Drosophila* there are numerous known instances of sexual isolation. In mixed cultures of *D. melanogaster* and *D. simulans,* Sturtevant (1920, 1921) has shown that each species exhibited a preference for mating with representatives of its own species. Similar results were obtained by Lancefield (1929) in the matings of *D. pseudoobscura* species *A* and *B.* Boche (Dobzhansky, 1941, p. 264) has extended these observations to show that this preference exists between geographical strains of the same species of *pseudoobscura,* i.e., race *A* and race *B.* Dobzhansky and Koller (1938) have demonstrated that sexual isolation exists also between *D. miranda* and both species of *D. pseudoobscura.* Patterson, Stone, and Griffen (1940), Spencer (1940b), Stalker (1941, 1942), Griffen (1941), and Crow (1942) have obtained similar results within other species groups. Wharton (1942) has found that profound sexual isolation is apparently the only mechanism which prevents the interbreeding of strains of *D. repleta.* Patterson (1942b) has reviewed much of this material in his recent paper concerning isolating mechanisms. Spett (1931) and Diedrich (1941) reported that even mutant types of *D. melanogaster* have preferential mating behavior.

In the *Drosophila macrospina* group, various degrees of sexual isolation exist. Different strains of the same subspecies cross with divers

degrees of ease. In no case does this isolation seem to be very great except in the case of the Florida stock, *K*. In this case the initial isolation is broken down gradually as the pairs remain together over a period of time.

A greater amount of sexual isolation is found in crosses between subspecies. Again the degree depends largely upon the strains employed in the tests. In several specific instances, e.g., *M* ♀ x *L* ♂, there apparently exists a negative isolation in that a greater percentage of the females produce progeny by *L* males than by *M* males. In the former cross, *M* ♀ x *L* ♂, 86% to 99% of the pairs produce progeny while in the cross of *M* ♀ x *M* ♂ only 73% give rise to offspring. That this is not due to sterility on the part of the *M* males is shown by the cross of *ML* ♀ x *M* ♂, in which 94% of the pairs are fertile. The factors involved are completely unknown. On the whole males of the subspecies *macrospina* show the most sexual isolation in intraspecific crosses, while males of *limpiensis* have the least.

It is very interesting to note that the isolation is reduced or absent in backcrosses of the F_1 intraspecific hybrids to their parental strains. Hence, the factors causing sexual isolation between the parental stocks are recessive.

A much greater degree of sexual isolation exists between the several species of the *D. macrospina* group.

The large majority of the strains of the subspecies *macrospina* are completely isolated sexually from *D. subfunebris*. Strains of *macrospina* originating east and north of central Texas have never produced progenies within the limits of any of the tests. The two strains of *macrospina* which are cross-fertile to *subfunebris* are from the western known limits of the distributional area of this subspecies. It is interesting to note that in these cases hybrids were produced usually after a period of 20 days only if *macrospina* was used as the female parent. Thus it is apparent that both the *macrospina* males and females carry factors which engender sexual isolation. If the same factors are responsible for the isolation in both sexes, they have a sex-limited action. But there is no proof that factors producing the physiological preference of mating are the same in both sexes.

With the exception of the *J* strain from Punta del Agua, Sonora, all *limpiensis* stocks have produced progenies by *subfunebris* in at least one direction. The majority of the strains are cross-fertile only if *limpiensis* is used as the male parent; all of such strains are from the southern distributional area of the subspecies. On the other hand both of the *limpiensis* stocks, the parents of which were taken from localities situated on tributaries of the Colorado River, are cross-fertile to *subfunebris* reciprocally. In at least one direction, these strains also produce progeny by *subfunebris* in a shorter length of time than the other *limpiensis* strains with the exception of the *G* stock from Magdalena, Sonora. Whether these results are a coincidence or are indicative of a closer relationship of the "Colorado

River" stocks to *subfunebris* cannot be decided from available data. However, this author is inclined to the latter view.

There is an interesting series of cross-fertility relationships between *subfunebris* and the *G, J,* and *U* stocks of *limpiensis.* The Magdalena, Sonora, stock, *G,* is reciprocally cross-fertile, but it is more readily fertile if used as the female parent in the cross. Thirty miles northward on the same river system the parents of the *J* strains were taken at Punta del Agua, Sonora. The *J* strain is cross-sterile to *subfunebris.* Thirty miles northward from the latter point near Patagonia, Arizona, is the type locality of the *U* stock. The *U* males are cross-fertile to *subfunebris,* while the females are cross-sterile. Although the last two points are located on different watersheds, the headwaters of both lay in fairly well forested rolling hills which may well support a *limpiensis* population. Conceivably the cross-sterility of the *J* may have originated through the combining of the isolating factors carried in *G* and *U* stocks.

As in the case of intraspecific crosses, the factors causing sexual isolation in this series of interspecific crosses are recessive in the hybrid females. A majority of the females from the first backcross again showed sexual isolation when tested to males of the species not used as the male parent in the first backcross. The results also support the certainty that the major factors engendering sexual isolation are recessive.

It was pointed out that *M* males continue to show a rather high degree of sexual isolation to hybrid *MS* females; with respect to the other tests these results were exceptional.

In crosses between the subspecies of *D. macrospina* and *D. subfunebris,* it was pointed out that generally those strains of *macrospina* originating from areas closer to that inhabited by *subfunebris* were inclined to show less sexual isolation to *subfunebris* than those coming from more remote localities. One particular exception to this geographical rule was noted in that strain of *limpiensis* which originated at Punta del Agua, Sonora. In contrast to these results Dobzhansky and Koller (1938) found the opposite to be true in crosses between *D. miranda* and both races of *D. pseudoobscura.* Strains of either race of *pseudoobscura* coming from localities in or near that of *miranda* had a greater degree of isolation to *miranda* than those coming from greater distances. They too noted one particular exception to their geographical rule; the strain from Oaxaca, Mexico, displayed an unexpectedly high degree of isolation.

Upon consideration, these contrasting results may not be as conflicting as they apparently seem. In the case of *pseudoobscura-miranda,* the distributional areas of the two species overlap. If there exists a frequent opportunity for interspecific matings, a high selective advantage accrues to those *pseudoobscura* strains coming from in or near the distributional areas of *miranda* provided such strains have a high degree of sexual isolation. On the other hand, *macrospina* and *subfunebris* distributional areas do not come in contact as far as known. Hence, there would be no selective advantage accruing to *macrospina* strains having high degrees of

sexual isolation. Rather to this author, the results indicate a closer phylogenetic relationship between *subfunebris* and those strains of *macrospina* having less sexual isolation to it.

The degree of sexual isolation between two stocks is difficult to measure in a quantitative manner. In the cross *M* x *L*, a variation of 13% was found in two successive runs. However, usually there was a considerably closer similarity in the numerical results obtained. A variation of more than 18 days was encountered in the appearance of the first larvae in different crosses between *M* females and *subfunebris* males. In this instance one of the conditions causing the difference was the length of time during which the adults were aged before being mated. Adults aged for longer periods before mating produced offspring sooner after being placed together.

Drosophila funebris proved to be cross-sterile to both *D. subfunebris* and the several subspecies of *D. macrospina* in all tests attempted. As yet all factors involved in these cross-sterility relationships are unknown. However, observations indicate that one of the more important factors is sexual isolation. It appears probable that gametic and zygotic mortality are causal factors also since *funebris* males have twice been observed in copulation with *macrospina* females. The spermathecae of these females were not dissected for sperm since it was hoped that they might produce progeny. The eggs laid by these females did not hatch.

Still another isolating mechanism encountered in the *Drosophila macrospina* group is hybrid sterility. Sterile hybrids have been reported in nearly every species group studied in the genus *Drosophila*. In some cases sterility is limited to second and subsequent generations, e.g., the *virilis* group (Patterson, Stone, and Griffen, 1940). In other cases sterility occurs in F_1 males from a cross in a specific direction while the reciprocal cross produces fertile F_1 males, e.g., the *macrospina* group (Mainland, 1941, 1942); *micromelanica* group (Sturtevant and Novistki, 1941a); *mulleri* group (Patterson, 1942b; Crow, 1942); and the *virilis* group (Patterson, 1941, 1942b). Among interspecific progenies frequently sterile F_1 males are obtained in both of reciprocal crosses, e.g., *pseudoobscura A* and *B* (Lancefield, 1929); *pseudoobscura-miranda* (MacKnight, 1939); and *macrospina-subfunebris* (Mainland, 1942). In still other cases both the F_1 males and females are sterile, e.g., *melanogaster-simulans* (Sturtevant, 1920, 1921); *pseudoobscura-miranda* (Dobzhansky, 1935a; MacKnight, 1939); *mulleri* group (Patterson and Crow, 1940; Crow, 1941, 1942; Patterson, 1942b); *affinis* group (Sturtevant and Dobzhansky, 1936; Miller, 1939, 1941). There are several series of progenies among which it has been possible to determine the fertile-sterile relationships of the hybrids from a cross in one direction only as a result of sexual isolation preventing a reciprocal cross, e.g., the *mulleri* group (Patterson and Crow, 1940, etc.); and the *affinis* group (Miller, 1939, 1941). Sterile hybrids have been obtained from both intra- and interspecific matings within the *macrospina* group.

In crosses between *macrospina* and *limpiensis*, it was pointed out that F_1 males from the cross, *limpiensis* ♀ x *macrospina* ♂, were sterile or semi-sterile depending upon the strain of each employed. Very close to 50% of the first backcross males from the cross of hybrid females to *macrospina* males have produced progenies when tested to *L* females. Since these data are very similar to that obtained in the case of sex-linked genes, this author (Mainland, 1941) stated:

"Apparently, a limiting factor (or factors) of a complimentary nature in the *limpiensis* Y is necessary for the fertility of males which carry a *limpiensis* X."

In testing males of the constitution $L[M^{10}\ (ML)]$ to *L* females (vid. results and footnote on p. 81), it was found that about 50% of the males were fertile although such males, of necessity, carried both a *limpiensis* X and Y chromosome. Hence it is apparent that the initial explanation of these data is incorrect.

Such data as that obtained from the cross mentioned in the preceding paragraph can result from the following genic relationships: (1) In order to be fertile, males with a *limpiensis* Y chromosome must have this Y chromosome complemented by a dominant factor (or factors) carried in at least one specific *limpiensis* autosome. (2) In order to be fertile, those males carrying a *limpiensis* X chromosome must have this X chromosome complemented by a recessive factor (or factors carried homozygously in the same chromosome or one of the same chromosomes) which complements the *limpiensis* Y.

The *macrospina* X chromosome is not limited in a manner similar to the *limpiensis* X since *ML* males are completely fertile, e.g., 100% of *L* x *ML* pairs were procreant. Sufficient data are not at hand to determine whether the *macrospina* Y chromosome is complemented by either *macrospina* autosomes or the *macrospina* X.

The present known complementary factors, though, are sufficient to explain all of the sterility which has been encountered so far in the F_1, F_2, and first backcross *limpiensis-macrospina* males. These complementary autosomal factors apparently have no effect in *limpiensis-macrospina* hybrid females. In the tests of the F_1 hybrid females and the females from the first backcross (four types tested), the fertility of the females was uniformly high, varying from 85% to 100% among the F_1's and from 88% to 97% among the first backcrosses.

Patterson, Stone, and Griffen (1940) have found Y-autosome complementary factors in both *americana* and *texana* which are similar to those found in *limpiensis*. In their case, the Y chromosome was complemented by chromosomes 2 and 5. In both groups, i.e., *macrospina* and *virilis*, the autosomal complementary factors are dominant; consequently, male sterility occurs only in certain second generation combinations. Sturtevant and Novitski (1941a) demonstrated an analogous situation in the Texas strain of *micromelanica*. However, in this case, the factor complementing the Y chromosome was located in the X. Muller and Pontecorvo

(1940a, b, 1942) have shown that males having a *simulans* Y in place of a *melanogaster* Y in an otherwise *melanogaster* male genotype are sterile. The *simulans* Y in a *melanogaster* female apparently has no effect.

In the interspecific hybrids between *subfunebris* and two of the subspecies of *macrospina,* all F_1 males have proved to be sterile while the F_1 females are completely fertile. Fertility tests of the first backcross progenies from the divers backcrosses showed that the fertility of the first backcross females varied from about 50% to completely fertile while that of the males was 1% or less.

In those cases where 50% of the first backcross females are fertile, two pairs of chromosomes (or factors) must complement one another in the production of female fertility. Since it is known that when these chromosomes (or factors) are either both homozygous or both heterozygous, the females are fertile, it follows that, when either pair is homozygous and the other heterozygous, sterility results. In cases where 75% of the first backcross females are fertile, it is again indicative that two chromosomes (or factors) are involved in the production of fertile females. Here, however, fertility is impaired only when one specific homozygous-heterozygous combination segregates.

From the data obtained by testing the fertility of the first backcross males, it is evident that many factors, a part of which is probably located on every chromosome, are involved in the production of fertile males. Also it would seem that males, in order to be fertile, must have a genotype very similar to that of one of the parental species.

Until marked chromosome stocks are available or sufficient cytological markers are known, it will not be possible to carry the analyses of the factors causing sterility in both intra- and interspecific hybrids.

Dobzhansky (1936) has concluded that male sterility in hybrids between *pseudoobscura A* and *B* is due to a series of multiple factors carried on all chromosomes except the Y and the small 5th. However, the data of Dobzhansky concerning the Y's not carrying factors concerned with male sterility are inconclusive. The results obtained among the first backcross males and *pseudoobscura* are sufficiently similar to those obtained in *macrospina-subfunebris* to suggest that probably a series of multiple factors, carried on most if not all chromosomes, conditions the male sterility in the latter case also. Muller and Pontecorvo (1940a, b, 1942) have demonstrated that *"melanogaster"* males homozygous for the *simulans* 4th chromosome are sterile although when heterozygous they are fertile. These investigators also determined that certain combinations of *melanogaster* and *simulans* chromosomes in "pseudo-hybrids" caused sterility or death in both males and females. Among interspecific hybrids between more distantly related forms, it is apparent that each genotype has its own peculiar balance. If this balance is changed, various morphological and physiological abnormalities result in the reaction system. In the genus *Drosophila* the fertility of the males is usually the first reaction system which is unbalanced.

Haldane (1922) stated: "When in the F_1 offspring of two different animal races one sex is absent, rare, or sterile, that sex is the heterozygous sex." All the data obtained in this work are in complete agreement with this statement.

RELATIONSHIPS IN THE DROSOPHILA FUNEBRIS GROUP

By means of the various tests employed, the members of the *Drosophila funebris* group may be divided into two subgroups. First, there is *D. funebris* consisting of strains from many areas in North America and Europe. Until the relationship of *funebris* can be determined by crosses to another member of the group, it will remain as a satelite to the group as a whole. Next is the endemic *Drosophila macrospina* subgroup consisting of the species *macrospina* and *subfunebris*. The species *macrospina* can be further subdivided on the basis of genetic tests into the two following parts: the subspecies *macrospina* (including *ohioensis*), and the subspecies *limpiensis*. These general divisions do not imply that these groups are homogeneous.

Evidence from geographical distribution, ecology, phenotypes, and genetic and cytological relationships are to be considered in the determination of the phylogenetic relationships.

Drosophila funebris is world-wide in distribution (Sturtevant, 1921; Kikkawa and Peng, 1938). At least in North America it is usually found close to the habitat of man (Sturtevant, 1921; Stalker and Spencer, 1939). Morphologically the three species of the group are rather similar. Grossly *subfunebris* and *funebris* resemble each other more closely than either resembles *macrospina;* however, the morphologies of the male and female genital plates of *funebris* are distinctly different from those of the other two species, while both the male and female genital plates of *macrospina* are quite similar to those of *subfunebris*. In addition *funebris* is cross-sterile to other members of the group, while *subfunebris* is cross-fertile to certain of the members of *macrospina*. From these facts, especially distributional and ecological, it does not seem amiss to consider *funebris* as an exotic species recently introduced into North America.

The *Drosophila macrospina* subgroup is a unit the parts of which are connected by differing degrees of cross-sterility, hybrid fertility, and chromosomal rearrangements. Geographically the several members of the subgroup replace one another across the southwestern, southern, and eastern portions of the continent. Ecologically, the members are woods-dwelling forms not commonly found about the habitat of man; hence, from these considerations, it appears that the *Drosophila macrospina* subgroup is very probably endemic. Phenotypically the several members resemble one another. The genital plates (vid. Plate I) of both *macrospina* and *subfunebris* are more similar than either of them are to those of *funebris*. From the little cytological data available it appears that with decreasing genetic relationships, there are increasing changes in gene

order. In all cases studied synapsis appears to be quite normal. Furthermore it should be noted that at least a part of all hybrid progenies were fertile. Taking these facts into consideration, it appears evident that there is a closer relationship between the several members of this subgroup than between *funebris* and them.

It has been pointed out previously that the subspecies of *macrospina* replace one another across the continent and that certain genetic reaction systems are localized to specific geographical areas. This is true of subspecies in other groups of animals (Dice, 1940a, b; Mayr, 1940). Morphologically all strains of the species *macrospina* are quite similar. In crosses between the subspecies, less hybrid sterility and, generally, less sexual isolation is encountered than between *subfunebris* and any strain of the species *macrospina*. Hence, it appears evident that the various subspecies of *macrospina* are more closely related than any of them are to *subfunebris*.

In crosses between the strains of the subspecies *macrospina*, usually little sexual isolation is found, and no hybrid sterility has been encountered among such progenies. Similar results were obtained in crosses between strains of *limpiensis*. Regardless of the strains employed in crosses between *macrospina* and *limpiensis*, the type of hybrid sterility obtained was basically the same. Hence, these tests indicate that each strain has a considerable part of its genic system in common with the other strains of the same subspecies.

A geographical trend was noted in the case of semi-sterility in the case of males from the cross, *limpiensis* ♀ x *macrospina* ♂. Another geographical trend was noted in the degree of sexual isolation which the several strains of the species *macrospina* were observed to have with respect to *subfunebris*. Assuming that these trends are indicative of phylogenetic relationships, it might be stated that in general the closer the locality of the origin of the several strains in the *macrospina* subgroup, the closer their relationship, except that members of the same subspecies are more closely related, regardless of geographic origin.

From these divers considerations, the following scheme is proposed to show the relationships of the members of the *Drosophila funebris* group.

Subfunebris—*Limpiensis*—western *Macrospina*—| *Ohioensis* / *Florida strain*

Funebris

Any one of the above members of the endemic group may have been the original one. The relationship of *funebris* to the other members is unknown at the present time. This designation of relationships is tentative. It fits the available data; however, when the cytological analysis is complete and further collections are made in western Arizona and southern California, a more detailed and better picture of the relationships should be possible.

The work of Antevs (1938, 1939, 1940, 1941) offers a possible explanation regarding the origin and distribution of the present members of the endemic *macrospina* subgroup. During the time of the last North American Glacial age, there occurred a Pluvial Age in the southwestern portions of the United States and northern Mexico. During this time the temperatures were lower and the rainfall much greater than present now throughout this region. During the Pluvial Age hickory (*Carya* sp.) and *Populus* sp. were known to occur in the southeastern part of Arizona (Antevs, 1941, p. 33) which is now semiarid. It is quite possible that during the Pluvial age, which lasted for 10,000 years, the range of the progenitors of the *macrospina* subgroup may have extended from coast to coast.

About 10,000 years ago, the climax of the Pluvial age was reached in the southwest. Subsequently the temperature rose and the rainfall decreased. Antevs (1938) states, "When the changing climate has become about as it is today, the Post-pluvial is understood to have commenced." If that was the case, then the populations of the *macrospina* subgroup would have then been separated roughly into three divisions, namely, the mountains of southern California, the highland of the Southwest, and the eastern portion of Texas. Probably the southern California population would have been the first to be separated from the main body since the region between the first and second groups is the driest region in the southwest today.

The post-pluvial is divided into the Early, the Middle, and the Late Post-pluvial stages. The Middle stage embraced the warm age of 5500–2000 B.C. This stage was characterized by extreme dryness in the southwest. Hence it would appear that the ancestors of *subfunebris* and *limpiensis* may have been reduced to very small populations residing in the higher mountains during this time.

During the Late Post-pluvial stage, i.e., the last 4000 years, the rainfall has increased in the southwest. The first half of this stage was more moist than the later half. Hence it appears that the various members of the group may have extended their range considerably during the period of 2000 B.C. to the beginning of the Christian era. Subsequently, during the past 2000 years, it appears that a portion of these populations were able to maintain themselves where they are now found while other intermediate populations may have disappeared. This correlation, although admittedly rough, is in fair agreement with the established genetic relationships and distributional data.

The evolutionary pattern of the *Drosophila macrospina* group is somewhat different from those of other groups which have been studied. This group forms a chain of strains across the North American continent, with a general east-west relationship between the strains. Among animal groups which form chains, this is the first group which has been subjected to a genetic analysis.

TAXONOMY BASED ON A GENETIC SCALE

Spencer (1940) has stated well the difficulty of evaluating the taxonomic status of two organisms which have evolved from a common source. Various taxonomists and geneticists (Standfuss, 1896; Shull, 1923; Kinsey, 1930, 1937; Lotsy, 1931; Dobzhansky, 1935; Emerson, 1935; Thorpe, 1940; Mayr, 1940; Timofeeff-Ressovsky, 1940; Sturtevant, 1942, p. 32; and others) have set forth criteria in an attempt to facilitate this evaluation. However, in view of the material presented in this paper and recent work in allied fields, it is well to review certain of the criteria.

Customarily external morphology is used as a means of evaluating relationships. Usually accompanying changes in morphology, one finds that there have been established some positive isolating factor or factors, namely, ecological isolation, sexual isolation, mechanical isolation, gametic mortality, zygotic mortality, and hybrid sterility. (See Patterson 1942b.) But only when morphological and isolating factors accompany one another, are structural changes reliable for the differentiation and the identification of the various taxonomic categories. Within limits, which may be broad indeed, morphological changes are incapable of separating a species into two non-interbreeding components, e.g., the various mutants of *D. melanogaster*. From our present knowlege of mutations, it appears that physiological mutations which effect positive isolation do not condition the organisms to an increase of the morphological mutant types, nor do morphological mutations condition the organisms to physiological mutations. These two types of gene replacement are independent phenomena. Morphologically, *D. mulleri* and *D. aldrichi* may be separated only with difficulty, but positive isolating factors completely prevent gene interchange between them. On the other hand, *D. mojavensis* and *D. arizonensis* are easily differentiated morphologically, but Crow's results (1942) show that the exchange of genes between them is quite possible. Numerous cases of physiological species have been reported in the literature (see review Thorpe, 1940; Dobzhansky, 1941, pp. 371–378). In many of these cases, very small morphological differences were determined. In some cases, e.g., *D. pseudoobscura* species *A* and species *B*, no reliable morphological differences have been found between "so-called" physiological species. Hence from these considerations, it is apparent that morphology *per se* may not prove to be a good measure of divergence of two forms from a common ancestor in all cases.

Regardless of morphological changes, when any single one of the previously mentioned positive isolating factors is completely operative between two populations, gene interchange cannot take place. In such cases, two populations must be considered as separate species since each is entirely free to evolve independently.

Taxonomic categories which can be subjected to a complete genetic analysis, of necessity, cannot have any one of the positive isolating factors absolute in its effect. Certain authors, previously cited, have suggested that in those cases where hybrids may be obtained, the final determination

of the taxonomic rank is dependent upon the fertility of the hybrids, i.e., if all hybrids are sterile, the parental forms are of specific rank. Does this mean that an animal geneticest can never study interspecific hybridization beyond the F_1 generation? Because one may obtain fertile F_1 hybrids in some cases under laboratory conditions, it does not appear to this author that the parental forms are necessarily below the rank of a species. In many cases yielding fertile F_1 progeny, the parental forms rarely mated even when given no choice of mates. Thus in such cases, it is highly improbable that gene interchange would occur under natural conditions. Griffen's tests (1942) within the *melanica* group indicate the almost complete separation of the three forms, *melanica, paramelanica,* and *nigromelanica.* The latter species broadly overlaps the former two geographically, but as yet no indication of natural hybridization has been found although a few fertile hybrids may be produced in the laboratory. Dobzhansky (1941) has reported a similar case for *pseudoobscura A* and *B,* both of which he considers good species. In almost all cases hybrids between *pseudoobscura* and *miranda* are sterile (Dobzhansky, 1937), but MacKnight (1939) reported that Dobzhansky found the fertility of the hybrid females varies from slight to nil, depending upon the geographic races employed as parents. When the potentiality of gene interchange between two populations is reduced by positive isolating factors to a point less than their innate tendency to diverge, then in the author's opinion, one is dealing with two populations at the species level, at least in the case of animals. Admittedly such a point is almost impossible to determine; hence the evaluation of a population as a species near this point is dependent upon the considerations of the investigator.

The passive factor of biogeographic isolation can also prevent the exchange of genes between two populations derived from a single population. Subsequent changes in this passive factor may permit two such groups to come again into contact, but unless positive isolating factors had been established previously or are established shortly afterwards, the differences which may have accumulated between the two populations during the separation would be swamped out and a "hybrid swarm" formed (Dobzhansky, 1941, pp. 280–288). In many cases, it is not possible to determine the actual taxonomic status of biogeographically isolated races without recourse to experimental techniques.

Tentatively this author proposes to define a species as follows:

> A species is an actually or potentially interbreeding array of forms whose net mutation rate is greater than the actual or potential gene interchange with other arrays of forms.

Such an evolutionary stage as that defined above can only be reached as a result of the action of positive isolating factors in preventing a "swamping out" of physiological and morphological differences which may arise between populations.

SUMMARY

1. The *Drosophila funebris* group is composed of three species: (1) *Drosophila funebris* (Fabricius) which is general in its distribution through the United States and Canada; (2) *D. subfunebris* Stalker and Spencer known only from a region near Pasadena, California; and (3) *Drosophila macrospina* of which three subspecies have been described: *Drosophila macrospina macrospina* Stalker and Spencer which is found in central Texas, Oklahoma, Arkansas, Missouri, Tennessee, Louisiana, Mississippi, Alabama, and Florida; *D. m. ohioensis* Spencer from Ohio and Michigan: and *D. m. limpiensis* Mainland which is distributed through west Texas, New Mexico, Arizona, southern Utah, and Sonora, Mexico.

2. Crosses between races of the same subspecies show variations in the degree of cross-fertility, but no cases of hybrid sterility have been found among the progenies from such crosses.

3. Crosses between *D. m. macrospina* and *D. m. limpiensis* generally go less readily than crosses between strains of the same subspecies. When *D. limpiensis* is used as the female parent, the F_1 hybrid males from such crosses are sterile or semi-sterile depending upon the strains of each employed. In general there is a decrease in the amount of male sterility with decreasing geographical separation between the points of origin of the parental strains. A part of the hybrid sterility of *limpiensis-macrospina* males was found to be due to a *limpiensis* X-autosome complementary factor (or factors) and to a *limpiensis* Y-autosome complementary factor (or factors).

4. Interspecific crosses between *D. subfunebris* and *D. m. macrospina* are cross-sterile with the exception of strains from the western limits of the distribution of *D. m. macrospina.* These exceptions are cross-fertile only when the latter subspecies is used as the female parent. *D. subfunebris* is cross-fertile to all but one strain of *D. m. limpiensis* when the latter subspecies is used as the male parent. Three strains of *D. m. limpiensis* are cross-fertile reciprocally to *D. subfunebris.* In general there is increasing sexual isolation between *D. subfunebris* and strains of both *D. m. limpiensis* and *D. m. macrospina* with increasing geographical separation between the points of origin of the parental strains of the two species.

In interspecific F_1 progenies from crosses between *D. subfunebris* and either subspecies of *D. macrospina,* all F_1 males are sterile while all F_1 females are fertile. Among the first backcross progenies, 1% or less of the males are fertile, and from 50% to 100% of the females are fertile. It is postulated that the male sterility is due to numerous factors located on all, or almost all, chromosomes, and that the female sterility is due to a few complementary factors.

5. No interspecific hybrids have been obtained between *D. funebris* and either *D. subfunebris* or *D. macrospina.*

6. It is postulated that *D. funebris* is an exotic species recently introduced into North America and that *D. subfunebris* and *D. macrospina* are

endemic. It is further postulated that the latter two species had a common ancestor which spread from coast to coast during the Pluvial Age and that during the Post-pluvial Age the populations of this common ancestor were broken into three populations which underwent differentiation, giving rise to the three major groups now found, namely, *D. subfunebris*, *D. m. limpiensis*, and *D. m. macrospina*.

7. Several of the critera used by the systematists in differentiating taxonomic categories are discussed with relation to genetic isolating mechanisms.

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APPENDIX

Since marked chromosome stocks of *D. macrospina* were not available, the following calculations were made in order to ascertain the number of backcross generations which were necessary in order to be practically certain of transferring a Y chromosome from one stock into another without carrying along any of the paternal autosomes, provided, of course, that a paternal autosome or autosomes did not carry a complementary factor or factors which were necessary for the fertility of the males carrying the Y chromosome in question.

A hybrid male from a cross between two *D. macrospina* strains will carry the maternal X and the paternal Y chromosome. The five pairs of autosomes of such a male will be heterozygous with respect to their origin; hence, there will be 6 possible combinations of autosomes in either the X bearing gamete or the Y bearing gamete. When such a male is backcrossed to females of the maternal strain, some of the progeny will be homozygous for none of the maternal autosomes, others for 1, 2, 3, 4, or 5. The proportion of each type of offspring may be determined by the expansion of the binomial theorem to the 5th power, i.e.:

$$(a + b)^5 = a^5 + 5a^4b + 10a^3b^2 + 10a^2b^3 + 5ab^4 + b^5$$

where a^5 represents the proportion of the offspring homozygous for all maternal autosomes, $5a^4b$, the proportion homozygous for 4 maternal autosomes, $10a^3b^2$, the proportion homozygous for 3 maternal autosomes, etc.

In random selection of first backcross males to be used as parents of a second backcross generation, the different types would be in the proportion as given above. Those males which were heterozygous for all of the maternal autosomes would give a segregation ratio the same as their father; those heterozygous for 4 autosomes, a segregation ratio according to the expansion of $(a + b)^4$; those heterozygous for 3, according to expansion of $(a + b)^3$; etc. Each of these various segregation ratios should be weighted according to its proportion of the first backcross progeny. Hence, one is able to determine the proportion of each class theoretically expected among the second backcross progenies.

Using the same random selection of males in subsequent backcross generations and the same method of calculation, one is able to determine the expected frequency of each class among subsequent backcross generations.

In Table 15 are given the expected frequencies of the various classes of progenies among the first through tenth backcross generations.

TABLE 1.

The Occurrence of *D. m. macrospina* in Collections of *Drosophilinae* at the Aldrich Farm by Months

Month and Year	Number macrospina collected	Average number per collection	Percentage of total flies collected
October, 1938	163	12.54	3.10%
November, 1938	103	10.30	3.12
December, 1938	4	4.00	1.89
January, 1939	0	0	0
February, 1939	---	---	---
March, 1939	25	4.17	4.66
April, 1939	802	100.25	7.30
May, 1939	491	28.88	2.80
June, 1939	304	27.64	3.03
July, 1939	215	21.50	5.34
August, 1939	324	19.06	2.57
September, 1939	1,243	103.58	7.17
October, 1939	232	17.85	1.48
November, 1939	21	1.62	.61
December, 1939	27	2.08	.88
January, 1940	1	.14	.36
February, 1940	10	.83	2.13
March, 1940	97	6.47	4.70
April, 1940	1,134	94.50	24.32
May, 1940	1,553	119.46	11.33
June, 1940	29	29.00	1.49

TABLE 2.

Fertility of Intraspecific *macrospina* Crosses

macrospina x macrospina			limpiensis x limpiensis		
Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair	Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pairs
M X M	73–73	normal	L X L	80–89	Normal
K X M		fertile	L X G		fertile
O X M	69	normal	L X N	86	normal
Q X M		fertile	L X Z		fertile
V X M		fertile	B X B		fertile
A X A		fertile	B X Z		fertile
K X A	95	fair	G X L		fertile
C X C		fertile	G X G		fertile
K X C		fertile	G X Z		fertile
M X K		fertile	H X H		fertile
A X K	96	normal	H X Z		fertile
C X K		fertile	J X J		fertile
K X K		fertile	J X Z		fertile
M X O	74	normal	N X L	91	normal
O X O	58–58	normal	N X N	100	normal
Q X O		fertile	N X Z		fertile
V X O		fertile	R X R		fertile
M X Q	89	normal	R X Z		fertile
O X Q		fertile	U X U		fertile
Q X Q		fertile	U X Z		fertile
V X Q		fertile	Z X L		fertile
M X V	93	normal	Z X B		fertile
O X V		fertile	Z X G		fertile
Q X V		fertile	Z X H		fertile
V x V		fertile	Z X J		fertile
			Z X N		fertile
			Z X R		fertile
			Z X U		fertile
			Z x Z		fertile

TABLE 2—(Continued)

Fertility of Intraspecific *macrospina* Crosses

limpensis x macrospina			macrospina x limpiensis		
Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair	Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair
L X M	53–60	normal	M X L	86–99	normal
L X K		fertile	M X G		fertile
L X O	53	normal	M X N	92	normal
L X Q		fertile	M X Z		fertile
L X V		fertile	A X Z		fertile
B X K	67	normal	C X Z		fertile
G X M		fertile	K X L		fertile
G X K	69	fair	K X B	70	normal
H X K	74	normal	K X G	87	normal
J X K		fertile	K X H	96	normal
N X M	60	normal	K X J		fertile
N X K		fertile	K X N		fertile
N X O		fertile	K X R	100	normal
N X Q		fertile	K X U	98	normal
N X V		fertile	O X L	85	normal
R X K		fertile	O X N		fertile
U X K	75	normal	Q X L	97	normal
Z X M		fertile	Q X N		fertile
Z X A		fertile	V X L	93	normal
Z X C		fertile	V x N		fertile
Z x K		fertile			

TABLE 3.

Fertility of Intraspecific F_1 Hybrids Tested by Inbreeding

macrospina x macrospina			limpiensis x limpiensis		
Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair	Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair
MK X MK	fertile	normal	LG X LG	fertile	normal
MO X MO	71	normal	LN X LN	83	normal
MQ X MQ	fertile	normal	BZ X BZ	fertile	normal
AK X AK	100	normal	GL X GL	fertile	normal
KM X KM	fertile	normal	GZ X GZ	fertile	normal
KA X KA	98	normal	HZ X HZ	fertile	normal
KC X KC	fertile	normal	JZ X JZ	fertile	normal
OM X OM	87	normal	NL X NL	89	normal
OQ X OQ	fertile	normal	UZ X UZ	fertile	normal
QM X QM	fertile	normal	ZB X ZB	fertile	normal
QO X QO	fertile	normal	ZG X ZG	fertile	normal
CK X CK	fertile	normal	ZH X ZH	fertile	normal
			ZJ X ZJ	fertile	normal
			ZR X ZR	94	normal
			ZU X ZU	fertile	normal

TABLE 3—(Continued)

Fertility of Intraspecific F_1 Hybrids Tested by Inbreeding

limpiensis x macrospina			macrospina x limpiensis		
Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair	Cross ♀ ♂	Per cent pairs producing progeny	Average progeny per fertile pair
LM X LM	33	2.0	ML X ML	98	normal
LK X LK	sterile	0	MG X MG	fertile	normal
LO X LO	22	2.0	MN X MN	fertile	normal
LQ X LQ	semi-sterile	low	MR X MR	91	normal
BK X BK	38		AZ X AZ	fertile	normal
GM X GM	semi-sterile	low	CZ X CZ	fertile	normal
GK X GK	24	ca. 10	KL X KL	fertile	normal
HK X HK	39		KB X KB	97	normal
JK X JK	17		KG X KG	98	normal
NM X NM	0	0	KH X KH	98	normal
NK X NK	semi-sterile		KJ X KJ	95	normal
NO X NO	0	0	KR X KR	96	normal
RM X RM	12	.75	KU X KU	93	normal
RK X RK	semi-sterile		OL X OL	98	normal
UK X UK	27		ON X ON	fertile	normal
ZA X ZA	semi-sterile		QL X QL	fertile	normal
ZC X ZC	semi-sterile				
ZK X ZK	2				

semi-sterile indicates that the pairs produced few progeny.
normal indicates normal fecundity.
fertile indicates fertility.
sterile indicates sterility.
low indicates that a small number of progeny were produced.

TABLE 4.

Fertility of Intraspecific F_1 Male Tested by Backcrossing.

Mating	Per cent fertile pairs	No. progeny per fert. pr.	Mating	Per cent fertile pairs	No. progeny per fert. pr.
M x MO	59%	normal	M x OM	79%	normal
O x MO	52	normal	O x OM	67	normal
L x LM	34	2.7	L x ML	96	normal
M x LM	35	3.3	M x ML	98	
N x NM	2	2.0	N x MN	fertile[1]	normal[1]
M x NM	2	1.0	M x MN	fertile[1]	normal[1]
L x NM	0	----			
Z x ZM	slightly* fertile	1.0*	Z x MZ	fertile*	
M x MZ	0*	----	M x MZ	fertile*	
Z x ZK	0	----			
L x LV	3	4.0			
L x LO	23	2.0	L x OL	95	normal
O x LO	11	1.8	O x OL	86	normal
N x NO	0	----			
L x NO	4	4.0			
L x LN	72	normal	L x NL	86	normal
N x LN	83	normal	N x NL	99	normal
Z x LZ	fertile*		Z x ZL	fertile*	
L x LZ	fertile*		L x ZL	fertile*	
Z x NZ	fertile*		Z x ZN	fertile*	
N x NZ	fertile*		N x ZN	fertile*	

*Data from small mass matings.

[1]Matings were contaminated with mold so that per cent of pairs was unreliable; however, the number of progeny in fertile mold-free cultures was normal.

TABLE 5.

Fertility of Intraspecific F_1 Female Hybrids Tested by Backcrosses.

Mating	Per cent fertile pairs	Mating	Per cent fertile pairs
MO x M	73%	OM x M	67
MO x O	66	OM x O	69
LM x L	100	ML x L	97
LM x M	94	ML x M	88
ZM x Z	fertile*	MZ x Z	fertile*
ZM x M	fertile*	MZ x M	fertile*
LO x L	94	OL x L	85
LO x O	68	OL x O	63
NO x N	96	MN x N	79
NO x L	93	MN x M	67
NM x L	93		
LN x L	80	NL x L	81
LN x N	85	NL x N	85
LZ x L	fertile*	ZL x L	fertile*
LZ x Z	fertile*	ZL x Z	fertile*
NZ x N	fertile*	ZN x N	fertile*
NZ x N	fertile*	ZN x Z	fertile*

*Data from small mass matings.

TABLE 6.

Fertility of First Backcross Progenies.

Mating	Per cent fertile pairs	Per cent semi-fertile pairs	Per cent sterile pairs	Mating	Per cent fertile pairs
L x (OM)O	68	....	32	(OM)O x L	94
L x (LO)L	82	....	18	(LO)L x L	97
L x (LO)O	48	2	50	(LO)O x L	88
L x (LM)L	62	10	28	(LM)L x L	97
L x (LM)M	49	3	48	(LM)M x L	93
M x M(LM)					

TABLE 7.

Cross-fertility in Interpecific Matings.

Mating	Fertility	Mating	Fertility
S x *funebris*[1]	cross-sterile*	*funerbris*[1] x S	cross-sterile*
macrospina[2] x *funebris*[1]	cross-sterile*	*funebris*[1] x *macrospina*[2]	cross-sterile*
S x O	cross-sterile*	O x S	cross-sterile*
S x Q	cross-sterile*	Q x S	cross-sterile*
S x C	cross-sterile	C x S	cross-sterile
S x A	cross-sterile	A x S	cross-sterile
S x E[3]	cross-sterile*	E[3] x S	cross-sterile*
S x M	cross-sterile*	M x S	fertile*
S x V	cross-sterile	V x S	fertile
S x L	fertile*	L x S	cross-sterile*
S x R	fertile	R x S	cross-sterile
S x N	fertile*	N x S	fertile*
S x B	fertile	B x S	cross-sterile
S x U	fertile	U x S	cross-sterile
S x J	cross-sterile	J x S	cross-sterile
S x G	fertile	G x S	fertile
S x H	fertile	H x S	cross-sterile
S x Z	fertile	Z x S	fertile

*Results from large mass matings; others from small mass matings.
[1]Eleven geographical strains from North America and Europe.
[2]*Macrospina* stocks: *E*[3] *L*, *M*, *N*, *Q*, and *V*.
[3]Several stocks of *macrospina* from east Texas; each tested individually.

TABLE 8.

Fertility Interspecific F_1 Hybrids Tested by Inbreeding

Mating	Fertility	Mating	Fertility
		MS x MS	sterile
SL x SL	sterile		
SN x SN	sterile	NS x NS	sterile
SB x SB	sterile		
SU x SU	sterile		
SG x SG	sterile	GS x GS	sterile
SH x SH	sterile		
SZ x SZ	sterile	ZS x ZS	sterile

TABLE 9.

Fertility of Interspecific F_1 Hybrid Males Tested by Backcrossing

Mating	Per cent fertile	No. tested individually	Mating	Per cent fertility	No. tested individually
S x SL	0	11	L x LS	0	11
S x MS	0	29	M x MS	0	30
S x SN	0	45	N x SN	0	45
S x NS	0	43	N x NS	0	43
S x SZ	0	small mass	Z x SZ	0	small mass
S x ZS	0	small mass	Z x ZS	0	small mass

TABLE 10.

Fertility of Interspecific F_1 Hybrid Females Tested by Backcrossing.

Mating	Per cent fertile	No. tested individually	Mating	Per cent fertile	No. tested individually
MS x S	100%	18	MS x M	50%	28
VS x S	fertile	small mass	VS x V	fertile	small mass
SL x S	100%	8	SL x L	100%	8
SR x S	not	tested	SR x R	fertile	small masses
SN x S	100%	17	SN x N	89%	18
NS x S	92%	48	NS x S	100%	53
SB x S	not	tested	SB x B	fertile	small mass
SU x S	not	tested	SU x U	fertile	small mass
SZ x S	not	tested	SZ x Z	not	tested
ZS x S	fertile	small mass	ZS x Z	fertile	small mass

TABLE 11

Fertility of First Backcross Female Progenies of Interspecific Hybrids

Tested to same parental species as used in first backcross			Tested to parental species other than that used in first backcross		
Mating	Per cent fertile	No. tested individually	Mating	Per cent fertile	No tested individually
(SL)L x L	52%	52	(SL)L x S	12%	52
(SL)S x S	69%	54	(SL)S x L	41%	49
(MS)M x M	100%	7	(MS)M x S	not	tested
(MS)S x S	85%	20	(MS)S x M	not	tested
(NS)N x N	81%	107	(NS)N x S	43%	93
(NS)S x S	74%	100	(NS)S x N	37%	41

TABLE 12

Fertility of First Backcross Male Progenies of Interspecific Hybrids

Cross	Per cent fertile	No. tested	Cross	Per cent fertile	No. tested
L x (SL)L	1%	58	S x (SL)L	0%	37
S x (SL)S	0%	53	L x (SL)S	0%	33
M x (NS)M	0%	8	S x (MS)M	not	tested
S x (MS)S	0%	11	M x (MS)S	not	tested
N x (NS)N	0%	61	S x (NS)N	4%	28
S x (NS)S	0%	16	N x (NS)S	0%	39

TABLE 13

Probability of Various Heterozygous and Homozygous Chromosome Combinations *vs.* Number of Backcross Generations

No. of Gen. Back-crossed	Homo-zygous	Hetero. for 1 Auto.	Hetero. for 2 Auto.	Hetero. for 3 Auto.	Hetero. for 4 Auto	Hetero. for 5 Auto.
1	3.13%	15.63%	31.25%	31.25%	15.63%	3.13%
2	23.73	39.55	26.37	8.79	1.46	.10
3	51.29	36.64	10.47	1.50	.11	.0031
4	72.42	24.14	3.22	.21	.01	----
5	85.32	13.76	.89	.03	----	----
6	92.43	7.34	.23	----	----	----
7	96.16	3.79	.06	----	----	----
8	98.06	1.92	.01	----	----	----
9	99.03	.97	----	----	----	----
10	99.51	.49	----	----	----	----

VII. A STUDY OF INTERSEXES PRODUCED BY A DOMINANT MUTATION IN DROSOPHILA VIRILIS, BLANCO STOCK

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University of Utah

PART I. THE MORPHOLOGY OF THE INTERSEXES

A dominant mutation, *Ix*^{B}, was discovered by Elwood Briles in the Blanco stock of *Drosophila virilis*, established in the Genetics laboratory of the University of Texas. Dr. Wilson S. Stone has kindly permitted me to use material from this stock and has carried out the breeding experiments by which the specimens for the study were obtained. He has offered helpful suggestions and our discussions on the problem have been invaluable.

1. External Sexual Characteristics of Normal Flies

(a) Female (Figs. 1a-1c).

Eight tergites are present. Numbers 1–6 are unspecialized and have a spiracle present below each ventral edge. The seventh tergite is narrow dorsally and presents, in a lateral view, an almost triangular appearance. The spiracle on each side of this tergite is near its anterior border. The eighth tergite lies just anterior to the anal valves. It is smaller than the others and its mid-lateral region is narrower than its dorsal and ventral portions. It extends ventrally almost to the vaginal plates. There are no spiracles associated with it.

Six sternites are present and each is in the same segment as the tergite of the next higher number; i.e., sternite 1 lies below tergite 2, etc. The sixth sternite is bifurcated and the two lateral parts extend posteriorly on each side of the anterior portion of the vaginal plates (Fig. 1b).

The anal valves (Fig. 1c) are transverse in position and the posterior edge of each is partly divided by a median notch. The vaginal plates (Figs. 1a-1c) have somewhat the shape of plow-shares with the points directed posteriorly.

(b) Male (Figs. 2a-2c).

Six complete tergites are present. The sixth has been described as being a fused sixth and seventh in *melanogaster* (Dobzhansky and Bridges, 1928 p. 426, and Strasburger, 1935, p. 47). These writers illustrate two spiracles associated with each ventral edge. Behind the sixth tergite of

*The writer has been a guest of the Department of Zoology and Physiology of The University of Texas during the year 1941–42. He wishes to express his gratitude for the facilities and materials generously placed at his disposal and for the help and encouragement freely given him by Drs. J. T. Patterson, Wilson S. Stone, and other members of the staff of the department. This paper is a report upon the research done during the year.

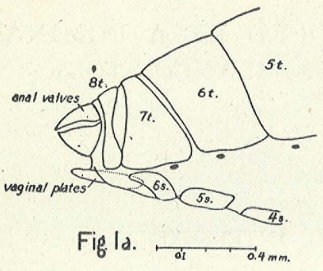


Fig 1a.

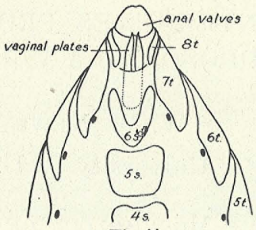


Fig. 1b.

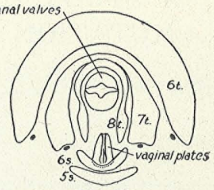


Fig. 1c.

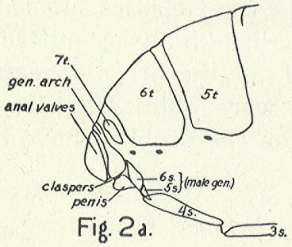


Fig. 2a.

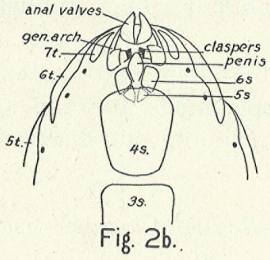


Fig. 2b.

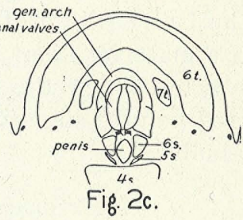


Fig. 2c.

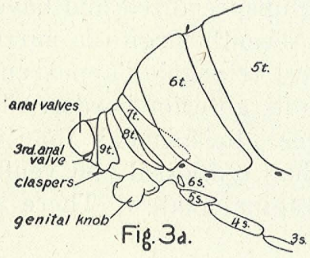


Fig. 3a.

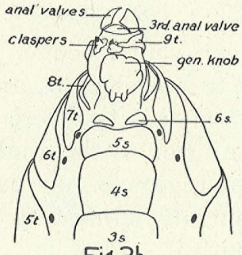


Fig.3b.

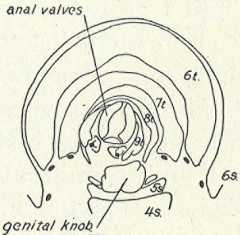


Fig.3c.

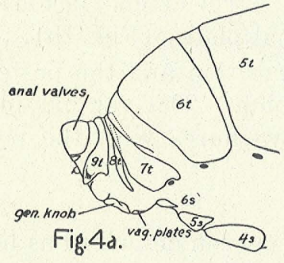


Fig.4a.

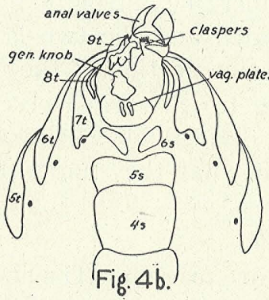


Fig. 4b.

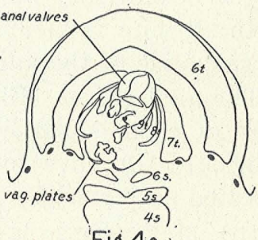


Fig.4c.

NOTE: The scale of magnification for Figs. 1–4 and Fig. 8 is the same as that given with Fig. 1a. The abbreviations 3s–6s refer to the 3d to 6th sternites and 6t–9t refer to the 6th to 9th tergites.

Figures 1a-c. Lateral, ventral and posterior views, respectively, of the posterior part of the abdomen of a female fly.

Figures 2a-c. Lateral, ventral and posterior views, respectively, of the posterior part of the abdomen of a male fly.

Figures 3a-c. Lateral, ventral and posterior views, respectively, of the posterior part of the abdomen of an intersex fly with a well developed genital knob.

Figures 4a-c. Views like those of Figs. 3a-c of a specimen with a poorly developed genital knob.

virilis (Blanco stock) is a pair of small, oval plates, dorso-lateral in position, and a spiracle lies on each side, ventral to them (Figs. 2a and 2c). It seems evident that in this species these plates correspond to the fused seventh tergite of *melanogaster*.

The genital arch is formed by the fused eighth and ninth tergites in *melanogaster* (Dobzhansky and Bridges, 1928, p. 427; Strasburger, 1935, p. 47). In *virilis* (Blanco stock) it probably has the same origin. The arch (Figs. 2b and 2c) is fairly narrow dorsally but ventrally, where it is associated with the claspers, it is enlarged and bulbous.

There are four complete sternites. Numbers one to three are opposite tergites two to four respectively, but sternite number four is opposite tergites five and six.

The anal valves (Fig. 2c) are vertical in position and their posterior edges do not have the notch characteristic of the valves of the female.

The copulatory apparatus (Fig. 2b) consists of a pair of claspers, and the penis which is laterally supported by two pairs of plates. Each clasper consists of a comb-like row of 6 straight, sharp spines at the end of a short, stout stalk. The base of the latter is partly hidden beneath the ventral border of the genital arch. The exposed part of the penis consists of a smooth, lightly chitinized, hollow process. This part is supported laterally by two plates each of which bears a single bristle. The rest of the penial apparatus is tucked inward dorsal to the fourth sternite for a variable distance. The invaginated part of the penis is also hollow and is supported ventrally by a second pair of small, triangular plates. The two pairs of plates which support the penis probably represent two additional sternites. The male thus has the same number of sternites (6) as the female.

2. *External Sexual Characteristics of Intersex Flies*

A total of nine tergites are present (Figs. 3a, 4a). Numbers one to six are large and are similar to those of normal flies. Tergite seven is well developed, but is smaller than number seven of the normal female, and has a pair of spiracles associated with it. Tergite eight is a narrow band of variable width but is always present and is completely separated from tergites seven and nine. Tergite nine is a small band just anterior to the anal valves. In many specimens this tergite bears a notch of variable depth on each ventral edge.

Five fully formed sternites are present (Figs. 3b, 4b). The fifth is, in many specimens, partly divided into right and left halves. Posterior to sternite five is a pair of small plates, which bear a few bristles and represent the sixth sternite.

The anal valves (Figs. 3c and 4c) of the intersexes are vertical in position and never possess the notch characteristic of the plates of the normal female. A small, third anal valve is present at the ventral side

of the anus, between the bases of the two primary valves. This valve is not found in normal males.

A pair of claspers (Figs. 3b and 4b) are present below the anal valves. The stalks of the claspers are somewhat shorter than those of the normal male and irregular chitinous plates, not found in the normal male, support their bases. The spines of the claspers, but four or five in number, are about half the length of those of the normal male. Many specimens have a very large "genital knob" (see below) and on some of these the claspers are borne at the sides of this structure. The claspers often have an asymmetrical arrangement. The plates between and at the bases of the claspers or that part of the genital knob lying between the bases of the claspers probably represents the median part of the penis. This statement is based upon developmental studies.

The entire gential region, including the eighth and ninth tergites is symmetrical in many specimens. In some, however, this region, particularly the anal portion, as seen from the posterior, is rotated clockwise (Figs. 3c and 4c). The direction of the rotation is constant. The degree of rotation varies but never exceeds about 20°.

On the ventral side of the body, between the anus and the sternites, is an enlargement which I have called the "genital knob" (Figs. 3a-3c). This is the most variable of the external structures found in the intersexes. It is symmetrical in most specimens but may be involved in the rotation of the genital region. In specimens in which it shows its greatest development, the genital knob is a large, irregularly rounded, heavily pigmented and chitinized mass. In other specimens it shows various degrees of reduction in size, pigment or chitinization until in some it is virtually absent, being represented by a small plate ventrally and by the plates at the bases of the claspers dorsally (Figs. 4a-4c). In one random sample of 29 flies the genital knob was well formed in 18, poorly formed in 8 and was absent in 3 specimens.

In specimens in which the genital knob is poorly formed or absent, rudimentary vaginal plates are present (Figs. 4a-4c). These lie just posterior to the sternites and show considerable variation in development. In other specimens they are either absent or form an indistinguishable part of the genital knob.

Serial sections show that the genital knob, in most specimens, is filled with undifferentiated tissue. However, in some specimens parts of the vagina or some of the coils of the ventral receptacle are present in the cavity formed by the knob. The ventral portion of the knob, therefore, represents the posterior part of the female genitalia. This has been further shown by developmental studies.

It should be noted that all of the described structures are not readily visable. In about one-fifth of the specimens I have examined, the entire genital area, including the eighth and nineth tergites and the sixth sternites, are deeply invaginated. This seems to be particularly true of

recently emerged flies. However, cleared specimens and serial sections show that all of the structures described are present in these individuals.

Summary. These intersexes of *virilis* (Blanco stock), which are zygotic females, are very uniform in regard to the external anatomy of their genital region. The presence of nine tergites and six sternites is a larval character retained by the adults. The vertical anal valves and the presence of claspers are distinctly male characteristics. The small ventral valve represents the ventral valve of the normal female. The two vertical, lateral valves represent two dorsal valves of the immature female which have failed to fuse. This statement has been verified by means of developmental studies and is, in part, in harmony with the condition described by Lebedeff (1939, p. 560) and Dobzhansky and Bridges (1928, p. 426). It is further verified by a specimen to be described below.

3. Internal Sexual Characteristics of Normal Flies

The internal genitalia of *virilis* are to be described by J. T. Patterson in a future Bulletin and need be only briefly considered here.

(a) Female

Inward from the genital opening is a short, somewhat enlarged *vagina* (termed uterus in some descriptions). From the inner, anterior end of the vagina open the pair of *spermathecae*, the pair of *parovaria* and the single, highly coiled *ventral receptacle*. Extending forward from the vagina is the *azygous oviduct* which, in turn, gives rise to the *paired oviducts* joining the *ovaries*.

(b) Male*

Inward from the penis is the short *posterior ejaculatory duct* (ejaculatory duct) which leads from the fairly large, bilobed *ejaculatory bulb* (ejaculatory pump or sperm pump). Extending forward from the ejaculatory bulb is the *anterior ejaculatory duct* (vas deferens). The posterior ejaculatory duct, the ejaculatory bulb and the anterior ejaculatory duct together make one complete, counterclockwise loop around the posterior part of the gut. The anterior end of the ejaculatory duct is somewhat enlarged. From this enlargement lead a pair of elongated thin walled sacs, the *accessory glands* (paragonia) and into it open a pair of *vasa deferentia* (vasa efferentia) leading from the coiled, yellow-colored testes.

4. Internal Sexual Characteristics of Intersex Flies

A sample of 26, randomly selected, intersex flies was taken. Serial sections were made of these and the internal genitalia were studied by means of graphic reconstructions (Fig. 5).

*Miller (1941) has revised the terminology used to designate the various parts of the male internal reproductive organs to conform with that of general insect morphology. His terminology is used in this paper but synonymous terms are given in parenthesis.

Table 1 summarizes the results of the study. The data obtained were arranged to demonstrate the types of intersexes obtained. Specimens numbered 1–3 were almost wholly female. Numbers 4–15 were hermaphroditic and possessed both male and female structures. Numbers 16–21 possessed only one female structure while numbers 22–26 were wholly, but incompletely, male. Only one structure, the ejaculatory bulb, was found in all specimens. The associated anterior ejaculatory duct was found in over three-fourths of the specimens. Another male structure, the accessory gland, was not found in any specimen. It is interesting to note that the loop of the genitalia about the gut, which is characteristic of the normal male, was not found in these intersexes.

All except two of the genital structures of the intersexes showed great variability in development. They could all be readily recognized by either their structure or morphological relationships but were more or less incompletely formed in many individuals. The spermathecae and the ventral receptacle were the exceptions; these were well developed in all specimens in which they were present.

An additional study was made of 12 late pupal intersexes. Data from these, when added to those of Table 1, do not greatly change the percentages given in the table or the relative number of each of the types of intersexes.

5. Gonads of Intersex Flies

The gonads were either rounded or, in some cases, lobed (Fig. 5) and bore no resemblance in shape to either the normal ovary or testis. The tissues surrounding the gonads had, in all cases, a yellow color similar to that of the tissue of the testes.

Histologically the gonads were, with one exception, ovary-like in character but varied from those markedly ovarian to an intermediate type with some male characteristics. Accurate classification was impossible but each type was about equally represented. In the ovary-like gonad groups of large cells were surrounded by a cellular follicle and closely resembled the upper part of the normal ovary (Compare Figs. 6 and 7a).

In the intermediate type gonad (Fig. 7b) a few large, egg-like cells, some with and some without a follicle, were present, but groups of smaller cells were surrounded by a connective tissue sheath. These groups resemble, fairly closely, the cysts of spermatogonia found near the upper end of the normal testis. In several, but not all, of the specimens with the intermediate type gonad, one or the other gonad was attached to the hypodermis (Fig. 7b).

One specimen only (No. 24, Table 1, p. 137) possessed gonads with no large egg-like cells. In this individual the cells were of medium to small size, groups of the latter in all cases being enclosed within a connective tissue

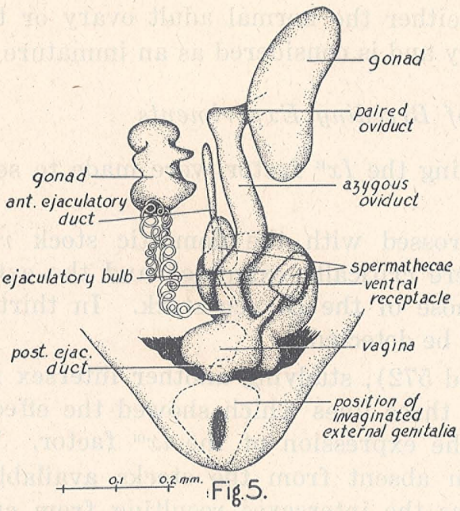


Fig. 5.

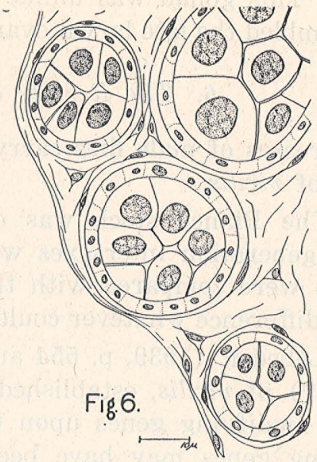


Fig. 6.

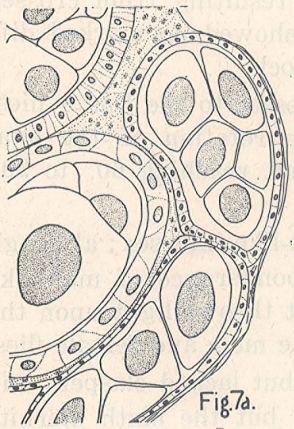


Fig. 7a.

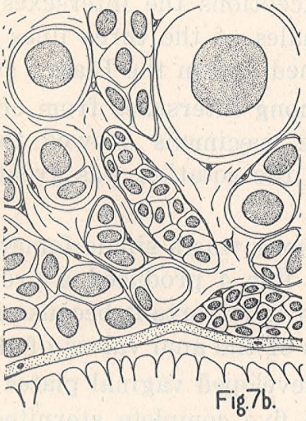


Fig. 7b.

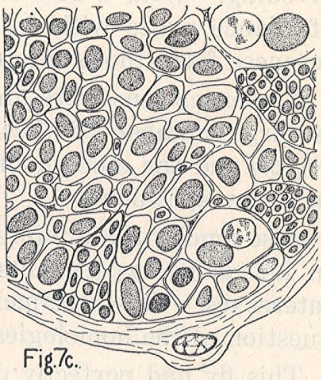


Fig. 7c.

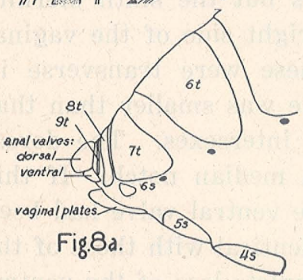


Fig. 8a.

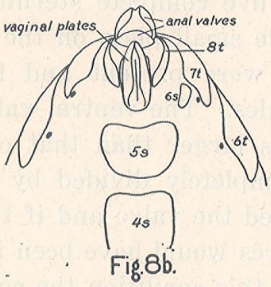


Fig. 8b.

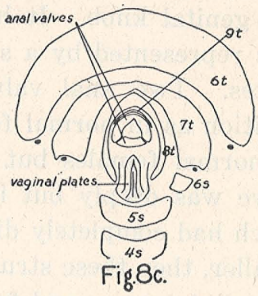


Fig. 8c.

Figure 5. Ventral view of the internal genitalia of an intersex fly (Specimen 5, Table 1). In this specimen the external genitalia were invaginated.

Figure 6. A camera-lucida drawing of a section of the upper part of the ovary of an adult female fly. The scale of magnification is given and also applies to Figs. 7a-c.

Figure 7a. A drawing of a section of a female-like gonad of an intersex fly (Specimen 8, Table 1).

Figure 7b. A drawing of a section of an intermediate type gonad. This specimen like several others of this type was attached to the hypodermis (Specimen 4, Table 1).

Figure 7c. A drawing of a section of an immature type gonad (Specimen 24, Table 1).

Figures 8a-c. Lateral, ventral and posterior views, respectively, of the posterior part of the abdomen of an exceptional female-type intersex resulting from a cross of a Blanco stock male and a female of Line 4 of a stock established by Lebedeff (1939).

sheath. This gonad was unlike either the normal adult ovary or testis, but resembled the late larval ovary and is considered as an immature type.

6. The Results of Breeding Experiments

Out-crosses of male flies carrying the Ix^B factor were made to several strains of *virilis*.

(a) The Blanco stock was crossed with the domestic stock *virilis*. Fourth generation intersexes were critically examined and the external features were compared with those of the Blanco stock. In thirty-five flies no difference whatever could be detected.

(b) Lebedeff (1939, p. 554 and 572), studying another intersex mutation (ix^m) of *virilis*, established three lines which showed the effects of various modifying genes upon the expression of the ix^m factor. These modifying genes may have been absent from the stocks available for breeding. With a few exceptions the intersexes resulting from crosses of Blanco males with females of the three lines showed no marked differences from those obtained within the Blanco stock.

The exceptions were among intersexes from crosses to the line 4 flies. A large number of these specimens showed the rotation of the anal region and the amount of the rotation was greater, reaching 60° to 90° in a few specimens.

Also among these intersexes was a single female-like intersex; although in Lebedeff's experiments line 4 produced a preponderance of male-like intersexes. This specimen is important because it throws light upon the question of the homologies of the anal valves of the male and female flies.

This fly had perfectly developed vaginal plates but lacked claspers and the genital knob. It had five complete sternites but the sixth sternite was represented by a single small plate on the right side of the vaginal plates. Two anal valves were present and these were transverse in position as in normal females. The ventral valve was smaller than that of normal females but was larger than that of intersexes. The dorsal valve was deeply but incompletely divided by a median notch. If this notch had completely divided the valve and if the ventral valve had been smaller, then these structures would have been identical with those of the other intersexes, and from this condition the complete loss of the ventral valve would produce the normal male valves. If, on the other hand, the dorsal valve had been undivided and the ventral valve had been somewhat larger the normal female condition would be obtained. This single specimen, therefore, indicates that the valves of the normal male are homologous to only the dorsal valve of the female; the ventral valve being absent. In the intersexes the ventral valve is present but is greatly reduced in size. Developmental studies further verify this idea of the relationship between the anal valves.

7. Summary

Zygotic female flies of the Blanco stock of *virilis* develop into intersexes under the influence of the dominant factor Ix^B. Externally, these intersexes have unspecialized segmentation; nine tergites and six sternites being present. They have male-like anal valves and claspers but these are incompletely formed. They have very rudimentary female genitalia, the vaginal plates, but many of them have a large, chitinious genital knob associated with the female internal genitalia. Internally, the intersexes are less uniform. All specimens examined had one or more male-like structures but over half of them had some female-like structures in addition. The gonads were usually either ovary-like, or had in addition, groups of cells resembling, to some extent, the cells of the upper part of the testis.

When Blanco stock flies were outcrossed to a domestic stock of *virilis* the expression of the Ix^B factor was essentially unchanged.

Certain lines of *virilis* have factors which are known to have modified the expression of another intersex producing factor, ix^m. Crosses of Blanco stock flies with the Ix^B factor were made to these lines. If the modifying factors were present in the stocks used for breeding they had but little influence upon the expression of the Ix^B factor.

PART II. THE DEVELOPMENT OF THE INTERSEXES

Numerous studies have been made by various authors upon certain aspects of development of the reproductive system of several species of *Drosophila*. Some of these studies will be referred to later. However, a complete, stage-by-stage study of the development of an intersex and a comparison to comparable stages in normal development seem not to have been made. Furthermore, the development of the reproductive system of *virilis* is evidently unknown. The writer has attempted to trace the development of this intersex character in *virilis* from the earliest stage in the development of the reproductive system to the adult condition and to compare each stage with the corresponding stage of normal male and female flies.

1. Methods

Male flies were tested by breeding for the presence of the intersex factor, Ix^B. Those possessing the factor were mated, the pairs being allowed to remain in the culture vial for one day, after which they were transferred to another vial. The age of the specimens taken from the cultures was thus known to within one day. When certain critical periods in the development were established a second set of similar matings were made, in which the pairs remained together in each culture for four hours. When the age of a specimen is given in this report it means the approximate elapsed time after the laying of the egg. The tests and matings were all made by Dr. Wilson Stone.

The larvae and pupae were taken at regular intervals and were killed in hot (100° C.) Bouin's fluid, after the heads had been punctured to permit rapid penetration of the fixative. Studies were made of serial sections prepared from the specimens.

2. Development of the Reproductive System During the Larval Period

The differentiation of the gonads begins during the embryonic and early larval periods. By the third day the testes are oval bodies about 40μ x 80μ in size, with cells about 10μ in diameter. The ovaries are nearly spherical bodies 25μ–30μ in diameter, with very small cells.

The imaginal disk which gives rise to the internal reproductive system begins its development shortly before the third day. Its history during the larval period is essentially the same in the two sexes and the intersex. Early in the third day the disk appears as a plate of cells about 15μ long and 50μ broad (Fig. 9). It appears to have arisen, in part, by delamination of a single disk of cells from the ectoderm in the midventral line, a short distance anterior to the anus. None of my preparations show any evidence of a double invagination of this ectoderm such as has been described for some other insects (e.g., *Hydroporus,* a water beetle, Heberdey, 1931, p. 421).

Between the imaginal disk and the anus are a pair of muscle bands. These are united in the mid-line and join the body wall just below the most posterior part of the gut. They extend dorsally and anteriorly on each side of the gut and insert on the body wall. A narrow, cellular fiber extends from either side of the imaginal disk and attaches to the muscular band. The cells of the fibers are intimately associated with those of the lateral part of the disk (Fig. 9). Their nature and that of many of the disk cells suggest that these two groups of cells arise from embryonic connective tissue, presumably of mesodermal origin. Other cells of the disk are evidently formed by a differentiation of the hypodermis. My studies lead me to conclude that the disk has a double origin. The epithelial lining of the reproductive tract arises from the ectoderm but the associated muscle and connective tissues are of mesodermal origin and develop with the ectodermal parts from the earliest stage.

During the third and early part of the fourth day the imaginal disk rapidly increases in size. Most of this growth is due to the addition of cells from the hypodermis. By the middle of the fourth day the disk is 150μ–200μ broad and about 30μ long (Fig. 10).

There are several questions concerning the nature of the conversion of hypodermal cells into disk cells which cannot be satisfactorily answered by means of sections. It appears, however, that the hypodermal cells enter the disk primarily from the sides. As they reach the stalk joining the disk they undergo a marked reduction in size and become greatly flattened. Their nuclei also shrink and become very dense. The center of the stalk of all specimens of the fourth and fifth days consists of some fibrous substance which I can only interpret as being discarded material from the metamorphosing cells.

The cells of the dorsal surface of the disk become arranged as an epithelial plate during the last half of the third and early part of the fourth day. By the middle of the fourth day they become separated from the underlying cells and a flattened cavity develops along the zone of separation. This cavity becomes the lumen of the reproductive tract but it does not open to the outside for several days.

The disk retains its lateral connection with the muscle band through the fourth and fifth days by means of the cellular fibers. These show little change in size but become more fibrous and less cellular with increased age.

The testis and ovary may be readily distinguished by the middle of the fourth day. The former reaches an approximate size of 60μ x 100μ x 150μ while the latter is but 40μ x 40μ x 60μ. The markedly larger size of the testis cells helps to distinguish this organ from the ovary.

During the fifth day considerable growth occurs in the imaginal disk. The relation of the stalk to the hypodermis makes it seem likely that additions from this tissue continue during the day. The disk cells are too small and the tissue is too dense to make mitotic figures very evident and but few appear in my preparations. However, the increase in the number of cells is so great that it seems probable that a large part of the growth is due to the multiplication of the disk cells.

In earlier periods intersex individuals cannot be distinguished, but by the fifth day they may be identified by the size and character of the gonads. The testis is large (140μ x 180μ x 230μ), the cells are quite uniform in size (14μ–18μ) and extend inward in columns from the outside of the organ. The ovary is smaller than the testis (70μ x 80μ x 135μ), and its small cells (8μ–10μ) are without definite arrangement. The intersex gonad is ovary-like but is larger (80μ x 100μ x 135μ) and in it are many small cells like those of the four-day ovary. Interspersed between these are larger cells like those of the six-day ovary. By the end of the fifth day the intersex gonad is much larger than the ovary and the difference in size between the small cells, now arranged in groups, and the larger ones, is much more evident. Little change in structure but considerable increase in size occurs in the intersex gonad after the sixth day.

At the beginning of the sixth day the imaginal disk (Fig. 11) is 250μ broad and 75μ long. There are no essential differences between the disks of the male, female and intersex larvae. The lateral borders of the disk remain attached to the muscle bands, but the connecting fibers have lost their nuclear elements.

The lumen of the disk is larger than in younger specimens. During its expansion the lateral edges divide into dorsal and ventral chambers separated by a prism of epithelial cells. The cells bordering the lumen become arranged as a stratified columnar epithelium. Dorsal to the epithelial layer a compact layer of irregularly arranged cells is formed. A layer of cells also forms below the ventral lining of the cavity (Fig. 11). These have an epithelial character at first, but later become loosely arranged. Both these groups of cells first appear at the lateral borders of the disk, near the attachment of the fibers connecting the disk and muscles. This

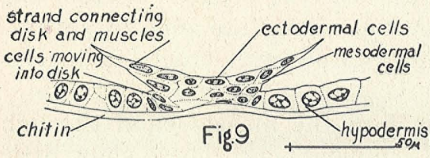


Fig.9

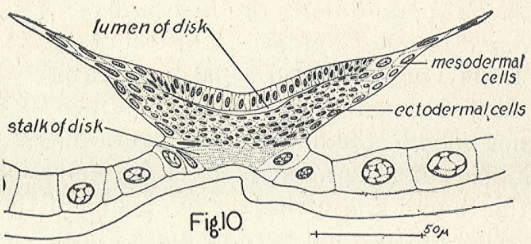


Fig.10

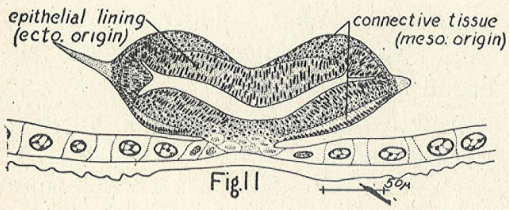


Fig.11

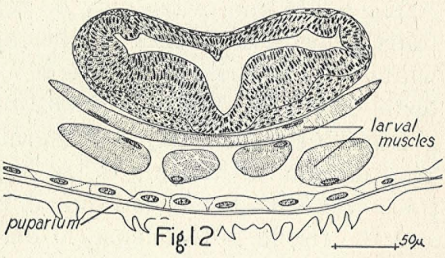


Fig.12

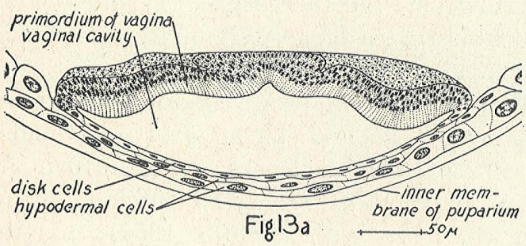


Fig.13a

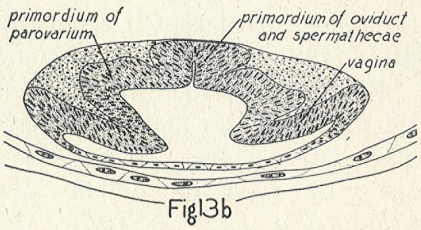


Fig.13b

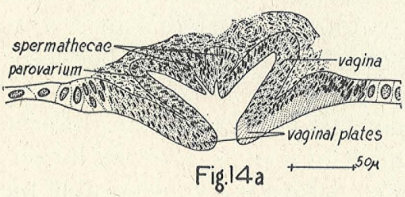


Fig.14a

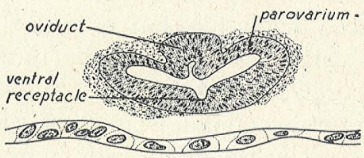
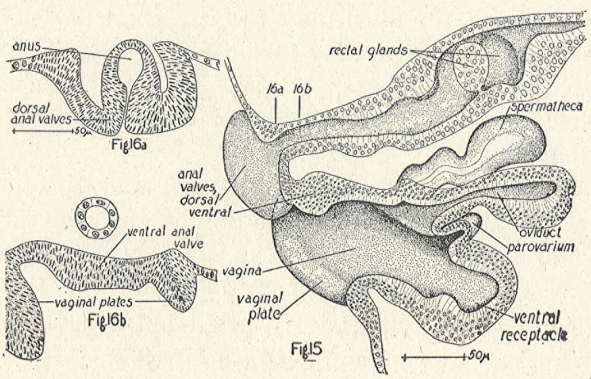


Fig.14b



fact suggests that they probably have the same embryonic origin, the mesoderm, as the fibers.

The disk, at the sixth day, is still attached to the hypodermis by a stalk which is narrower than in previous stages. The cells at the stalk do not show the transition from the hypodermal to disk type but are small and have poorly defined nuclei. They appear to be degenerating and it would seem that the contributions of the hypodermis to the imaginal disk have about ceased by the sixth day.

3. Development of the Reproductive System During the Pupal Period

Pupation occurs in this strain of *virilis* (under the laboratory conditions), toward the end of the sixth day. It is at this time also that the differentiation of the male, female and intersex reproductive systems begins. The imaginal disk in both sexes and the intersex remains attached to the hypodermis at the posterior end but there is little evidence that the hypodermis continues to contribute cells to the disk. The fibers suspending the disk from the muscles break up and disappear during the sixth day. Externally, the shape of the disks of the three types of individuals is about the same; internally they differ.

(a) Development of the female system.

Late in the sixth day the imaginal disk of the female becomes much thicker due to the enlargement of its lumen (Fig. 12). The double chambered character of the lateral part of the cavity largely disappears and a deep groove, which extends the full length of the cavity, develops on its floor. The epithelium of the roof of the cavity becomes thinner and that of the floor becomes thicker. A basement membrane separates the epithelium from the surrounding connective tissue.

Early in the seventh day the cavity of the imaginal disk, near its posterior end, opens to the outside to form the genital pore. The opening does not develop through the stalk of the disk but occurs immediately

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Figure 9. A transverse section of the genital imaginal disk of a 3-day larva.
Figure 10. A transverse section of the genital imaginal disk of a 4-day larva.
Figure 11. A transverse section of the genital imaginal disk of an early 6-day larva.
Figure 12. A transverse section of the genital imaginal disk of a late 6-day, female pupa.
Figure 13a. A transverse section of the genital imaginal disk of an early 7-day, female pupa. The section is through the posterior part of the disk, in the region of the opening to the outside.
Figure 13b. A more anterior section of the same specimen as that of Fig. 13a.
Figure 14a. A transverse section of the genital imaginal disk of a late 7-day, female pupa. The section is through the posterior part of the disk in the region of the vaginal plate primordia. The left side of the section is about 20μ anterior of the right.
Figure 14b. A more anterior section of the same specimen as that of Fig. 14a.
Figure 15. A stereogram drawn from serial sections of an early 8-day, female pupa. The drawing is made to represent the left side of the posterior part of the body, of a specimen dissected along the median plane.
Figures 16a and 16b. Vertical sections through the anal region at the positions shown in Fig. 15.

anterior to it. The epithelium of the disk, in the region of the stalk, becomes very thick. The hypodermis on each side of the stalk is also thick and consists of large, tall cells. From these two regions of the hypodermis a fold of flat cells grows mediad beneath the stalk and extends as a single plate under the hypodermis immediately anterior to it.

In the meantime, the cavity of the disk expands rapidly laterad, largely at the expense of the epithelium of its floor (Figs. 13a and 13b). This layer becomes very thin and there is evidently a migration of cells to the roof of the disk because this epithelium becomes thickened. The thin floor of the disk comes in contact with the hypodermis just behind the stalk and with that extending beneath the stalk (Fig. 13a). In the area of contact the two layers of cells disintegrate and the cavity of the disk thus opens to the outside.

The large cavity of the disk, in the region of the opening, becomes the vagina (Fig. 13a). Forward from the vagina the roof of the disk expands dorsally to form a deep, broad, inverted groove (Fig. 13b). This chamber, in turn, develops a pair of lateral outpouchings and a deep dorsal groove. Each lateral outpouching is the primordium of one of the pair of parovaria while the oviduct and the spermathecae arise from the dorsal groove.

During the seventh day the ovaries begin to assume the adult structure; the oögonia and oöcytes with their accompanying follicle cells may be readily recognized.

Toward the end of the seventh day the primordia of all the female reproductive organs, both external and internal, and the anal valves are formed.

The valves first appear as plates of thickened hypodermis. A pair of these plates form posterior and lateral to the anus and a single plate forms anterior to it. When the anus shifts to a terminal position the plates have, respectively a dorsolateral and a ventral position.

The genital pore is large by the end of the seventh day and a fold of tissue develops on each side of it (Fig. 14a). The median surface of these folds may, in part, be derived from the imaginal disk but most of the tissue is derived from the large hypodermal cells which were previously located on either side of the stalk of the disk. The two folds of tissue are the primordia of the vaginal plates.

The cavity above the genital pore is that of the vagina. The parovaria still appear as lateral, groove-like, outpouchings from the imaginal disk. The deep dorsal groove, described above, develops three narrow, tubular outpouchings. A pair form near the posterior end and these become the spermathecae. A single one forms at the anterior end and this becomes the oviduct (Figs. 14a and 14b). A groove also forms on the floor of the disk at the anterior end. This becomes the ventral receptacle. All of these structures form from the epithelium of the disk.

During earlier stages the connective tissue of the disk was held in close association with the epithelial lining by a membrane. During the seventh day this membrane largely disappears. The connective tissue becomes more loosely arranged and parts of it begin to differentiate into muscle tissue.

The anal valves form as folds of the hypodermis early in the eighth day and have the positions of the plates from which they arose; i.e., a pair form postero-lateral and a single valve forms anterior (ventral) to the anus (Figs. 16a and 16b).

On either side of the genital pore the vaginal plates now have the form of thick, well formed folds of tissue (Fig. 15). The vagina forms a distinct portion of the reproductive tract anterior to the plates. The spermathecae are elongated, tubular structures and each is somewhat enlarged at the anterior end. The oviduct is a single, median tube extending anteriorly from the dorsal part of the vagina and the ventral receptacle is a short tube ventral to it. The parovaria still have the form of lateral outpouchings from the imaginal disk. Although the anterior part of each pocket is somewhat deeper than in previous stages, they are otherwise unchanged (Fig. 15).

During the ninth day the development of the female reproductive system is about completed (Fig. 17). The openings of the digestive and reproductive tracts have been shifted to a terminal position. A fold of the body wall on each side of these openings partly encloses the plates guarding them but this cavity is temporary and is largely lost after the emergence of the adult. A portion of these folds, however, enclose the basal, anterior part of the vaginal plates. This part of the folds remains to form a sheath for the vaginal plates.

The two postero-lateral anal valves, now dorso-lateral in position, fuse in the mid-line above the anus to form a single, dorsal valve. The single, anterior valve, now ventral in position, increases in size.

The vaginal plates fuse in the mid-line, on the dorsal side to a limited extent, and on the ventral side for about half their length. It is the latter part which is enclosed within the sheath. The vagina is increased in length by this joining of the plates but it remains a simple, undifferentiated tube. From the anterior end of the vagina, in the dorsal region, the oviduct continues forward. Early in the ninth day the oviduct bifurcates and each branch joins the corresponding ovary.

The ventral receptacle opens from the vagina immediately below the oviduct. This organ now has the form of a long coiled tube. The tube itself is of epithelial (ectodermal) origin and has a narrow lumen. It is surrounded, however, by a matrix of connective tissue. During subsequent development it grows out of the matrix but retains a covering of the tissue.

Each spermatheca develops a large vesicle at the outer end. This vesicle, in turn, grows backward to surround the distal part of the duct which thus comes to enter the vesicle through a stalk. The chitinous lining of the vesicle becomes very evident at about this time and it subsequently continues its development to form the chitinous vesicle of the adult spermatheca.

During the early and middle parts of the eighth day the parovaria were outpouchings from the anterior part of the lateral walls of the vagina (Fig. 15). Early in the ninth day each pouch closes off from the central cavity, beginning at the anterior end, and forms a tube along each side.

The parovaria thus come to open from the central cavity immediately posterior to the spermathecae; the position of the posterior end of the original pouch (Fig. 17).

(b) Development of the Male System.

Toward the end of the sixth day two grooves of columnar tissue form by delamination from the dorso-lateral part of the male imaginal disk (Figs. 18a and 18b). These grooves soon develop into tubes which are closed at each end. Each tube ultimately becomes one of the vasa deferentia and accessory glands. A number of connective tissue cells become enclosed within the developing tube but these form a transitory tissue. These primordia form earlier than do those of any part of the female tract. The remainder of the imaginal disk is much the same as that of the female of the same age.

By the middle of the seventh day the two grooves become tubular and increase in both length and diameter. Their increased diameter causes them to push into the lumen of the disk from which they are separated by the epithelial lining (Fig. 19, left side). The posterior ends of the tubes similarly extend into the lumen of the disk (Fig. 19, right side) while their anterior ends are surrounded by connective tissue and extend a short distance beyond the disk.

The primordia of the anal valves form during the first part of the seventh day. Their time of formation and their position and shape is the same as those of the female.

At the close of the seventh and opening of the eighth days the lumen of the imaginal disk opens to the outside to form the genital pore. The manner in which this occurs is the same as the opening of the female disk but the time is some 12 to 18 hours later.

The portion of the disk just anterior to the genital pore forms from that part of the seventh-day disk which had lain behind the paired tubes. At this time, early eighth day, this part consists of a fairly large, smooth walled chamber. There are, however, a pair of swellings on each lateral wall which become the penis. The anterior part of the disk, on the other hand, now forms an elongated, narrow, median duct which lies between the previously formed lateral tubes (Fig. 20). These latter lose their core of connective tissue; probably by the disintegration and absorption of the cells. The median duct and lateral tubes are surrounded by connective tissue but the membrane outside of this now begins to break up (Fig. 20).

Throughout the eighth day both development and growth occur in all parts of the imaginal disk and by its end the primordia of all adult structures are established. A comparable developmental stage occurs almost 24 hours earlier in the female.

The anal valves of the male have, at the end of the eighth day, the form of two folds of tissue lateral to the anus and a single thickened plate below it (Fig. 21). The latter develops no further and soon loses its identity.

From the lateral surface of each anal valve and from the body wall beside it a second pair of folds develop. These are the primordia of the claspers. Ventral to the median anal plate are two pairs of blunt, finger-like folds (Fig. 21). These are formed from the swellings described above and are the primordia of the penis.

Lateral folds of the body wall, like those described for the nine-day female, partly enclosed the anal and genital regions. Most of the cavity thus formed is lost at the time of emergence but in the region of the penis a portion of it remains as the penial sheath.

In the female, the vaginal plates were seen to be formed almost exclusively from hypodermal tissue which lay lateral to the genital pore. In the male the picture of development is somewhat different. The claspers have almost the same position in the male as the vaginal plates of the female have when they are first formed. The penis, however, arises from two pairs of swellings, which first appear on the sides of the lumen of the disk shortly after the genital opening is formed. As these four swellings increase in size to become the primordia of the penis, the disk becomes constricted immediately anterior to them. Thus the penis forms from disk tissue but secondarily assumes a position posterior to the reproductive tract.

The median part of the disk forward from the penis becomes the ejaculatory duct. The constricted portion becomes the posterior ejaculatory duct, forward from this an enlarged part with developing muscles around it is the ejaculatory bulb. The rest of the disk lies between the lateral tubes and becomes the anterior ejaculatory duct (Fig. 21).

The two lateral tubes, which were formed so early, are as yet not joined to the median duct but the duct and tubes are still surrounded by dense connective tissue. The posterior ends of the tubes are now somewhat enlarged and become the accessory glands. The anterior ends have grown forward and turned laterad toward the testes. They become the vasa deferentia (Fig. 21).

Beginning late in the eighth day and continuing through the first half of the ninth day the extreme posterior part of the abdomen of the male pupa describes a clockwise rotation of 360° (Figs. 21, 22a, and 22b). The region envolved is that within the concavity formed by the folds of the body wall and includes the anal and genital structures (Figs. 22a and 22b). The surrounding region which goes to make up the seventh to ninth tergites and sixth sternite is envolved very little if at all.

At the beginning of the rotation the entire genital tract is ventral to the rectum (Fig. 21). As the rotation goes on the posterior part of the tract is free to move. The anterior part of the tract cannot turn with the posterior part because the vasa deferentia join the testes at about the time the rotation starts. The median duct, however, grows rapidly at this time and is thus long enough to be literally pulled as a loop around the posterior part of the rectum (Fig. 23).

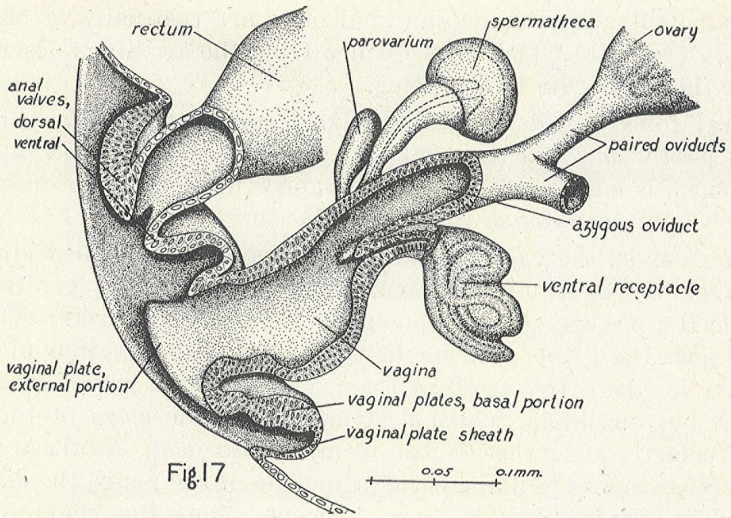

Fig.17

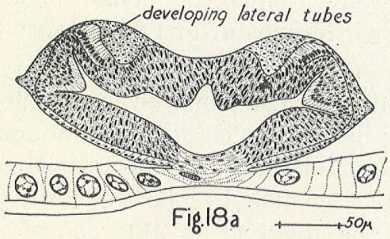

Fig.18a

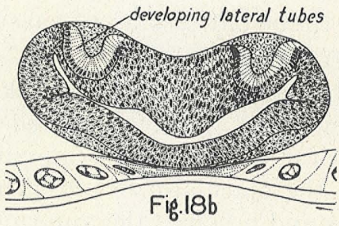

Fig.18b

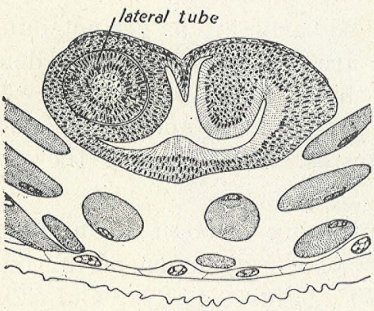

Fig.19

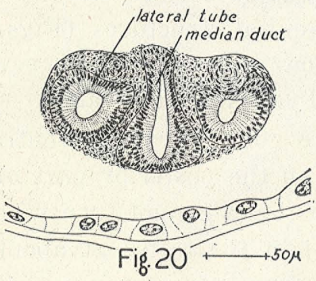

Fig.20

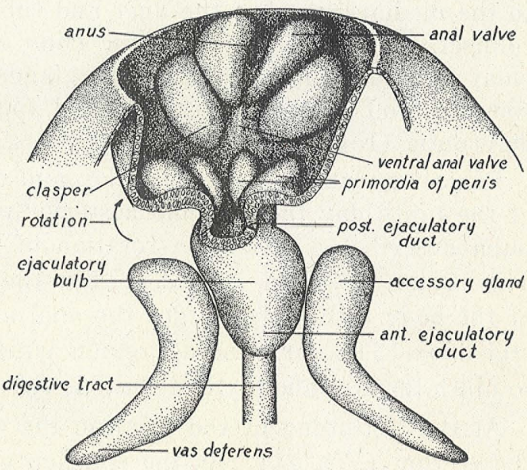

Fig.21

The rotation of the posterior region is a complete circle and the penis, genital pore and anal valves thus return to their original positions. This posterior ejaculatory duct and the ejaculatory bulb, being formed from a lineal portion of the original median duct, are part of the loop formed by the duct, and their rotation, particularly that of the bulb, is somewhat less than a circle.

The rotation of the posterior part of the body has been described in other Diptera by several workers. The most complete account for *Drosophila* is that of Gleichauf (1936) for *melanogaster*.

At the beginning of the tenth day the primordia of all parts of the male reproductive system are well formed but, of course, considerable development is yet to take place (Fig. 23). The rotation of the terminal region is completed and the external genitalia are again in the ventral position. The musculature of the ejaculatory bulb is forming (Fig. 23), but the chitinous plate, which gives the bulb its pumping action, has not yet begun to develop. The anterior ejaculatory duct extends forward to the level of the testes and terminates in an enlargement. The lateral tubes, previously described, are differentiated into anterior portions which join the testes; the vasa deferentia, and posterior, sac-like portions, the accessory glands. The lumens of these two pairs of structures have not yet joined that of the ejaculatory duct but will do so within the following day.

Stern (1942) has shown by experimental methods that the coiling of the testes is dependent upon the attachment to them of the vasa deferentia. My observations would confirm this because coiled testes were observed only in specimens which had the vasa deferentia and testes joined.

(c) Development of the Intersex.

The imaginal disk of the intersex begins its differentiation at about the same time the disks of sexual individuals begin theirs; i.e., late in the sixth day. The intersex disk of this age resembles that of the male more closely than it does that of the female (compare Figs. 24, 12, and 18a).

Figure 17. A stereogram like that of Fig. 15 made from serial sections of a 9-day, female pupa.

Figure 18a. A transverse section of the genital imaginal disk of a late 6-day, male pupa. The section is through the posterior part of the disk.

Figure 18b. A more anterior section of the same specimen as that of Fig. 18a.

Figure 19. A nearly transverse section of the genital imaginal disk of a 7-day male pupa. The left side of the section is about 20μ anterior to the right. The left side passes through the lateral tube and the right passes through the posterior end of the tube and its surrounding epithelium.

Figure 20. A transverse section of the anterior part of the genital imaginal disk of an early 8-day, male pupa. It shows the relation of the median duct and the lateral tubes.

Figure 21. A stereogram made from serial sections of a late 8-day, male pupa. The drawing is made to represent a ventral dissection of the posterior part of the body. The folds of the body wall which partly enclose the external genital and anal structures are represented as if removed from the ventral side. The asymmetrical position of the genitalia is due to the rotation of the posterior part of the body described in the text.

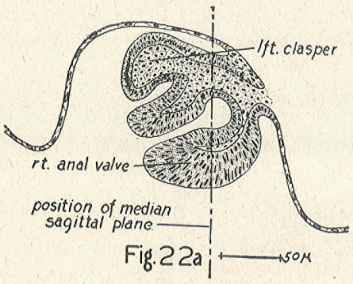

Fig. 22a 50μ

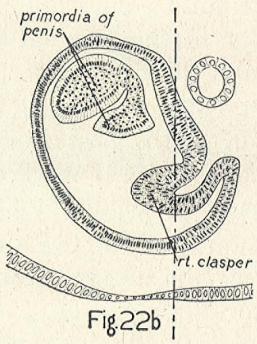

Fig. 22b

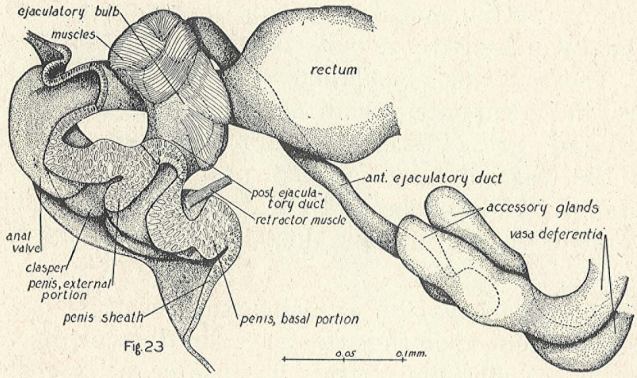

Fig. 23

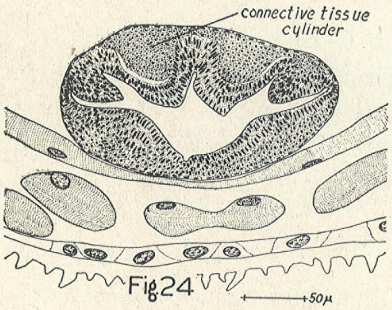

Fig. 24 50μ

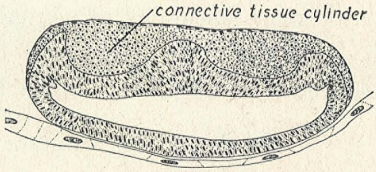

Fig. 25 50μ

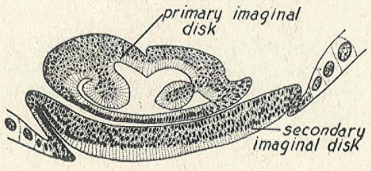

Fig. 26a 50μ

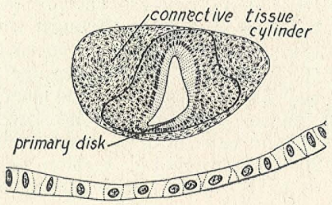

Fig. 26b

There is this difference however; the longitudinal grooves which form on the dorsal surface of the male disk do not form in the intersex although the connective tissue in this region has a similar arrangement.

The cavity of the intersex disk opens to the outside early in the seventh day. This occurs at about the same time as it does in the female and is not delayed as it is in the male. The cavity of the disk is fairly smooth and regular and the connective tissue of the dorsal surface forms two short cylinders (Fig. 25).

At the end of the seventh day the intersex disk is less well developed than is that of either sex. The connective tissue cylinders described above have almost exactly the position of the lateral tubes of a slightly younger male (compare Figs. 26a, 26b, 19, and 20). They protrude into the lumen of the disk posteriorly and extend anteriorly on each side of the epithelial portion of the disk (Figs. 26a and 26b).

Late in the seventh day the hypodermis, ventral and anterior to the opening of the disk, becomes thickened and the tissue takes on the histological characteristics of an imaginal disk. The lateral corners of this plate of cells form folds which extend mediad beneath it to establish a secondary imaginal disk (Fig. 26a). In most specimens the secondary disk does not completely close but grows anteriorly as a pocket (Figs. 27 and 28).

Thus the intersex, at the beginning of the eighth day, possesses two imaginal disks. The primary disk formed at the same time and in the same manner as the disks of sexual individuals. Its development is similar, in some respects, to that of the male disk, but is slower. The secondary disk forms by invagination, rather than by delamination, about five days after the primary. Recalling that the intersexes are zygotic females one might expect that the primary disk would become the female part of the reproductive "system." Such is not the case; the primary disk becomes the male part and the secondary disk becomes the female part.

I had six intersex specimens eight to eight and a half days old and these all possessed secondary disks. Another group of five specimens could be identified as intersexes by their gonads. Two of them had only thickened

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Figure 22a. A transverse section of the anal region of an early 9-day, male pupa. The position of the median sagital plane is shown. The distorted position of the anal valves and claspers is due to the clockwise rotation in this region which is described in the text.

Figure 22b. A more anterior section of the same specimen as that of Fig. 22a.

Figure 23. A sterogram drawn from serial sections of the reproductive tract of a 10-day, male pupa. The drawing is made to represent a lateral view of the system; the posterior part of which had been dissected along the median plane.

Figure 24. A transverse section through the genital imaginal disk of a late 6-day, intersex pupa.

Figure 25. A transverse section through the genital imaginal disk of a 7-day, intersex pupa.

Figure 26a. A transverse section through the posterior part of the primary imaginal disk and through the young secondary imaginal disk of a late 7-day, intersex pupa.

Figure 26b. A more anterior section of the same specimen as that of Fig. 26a.

plates of hypodermis in the position of the secondary disk and three had no secondary disks at all. These five specimens were eleven days old but their normal sibs had the structural characteristics of nine-day specimens. The primary disks were like those of other intersexes nine days old. From these facts it would seem that the secondary disks were particularaly sensitive to the environmental factors retarding general development. All of the specimens used for the study and descriptions possessed secondary disks and these showed greater variation than did the primary disks or the disks of normal sibs of the same age.

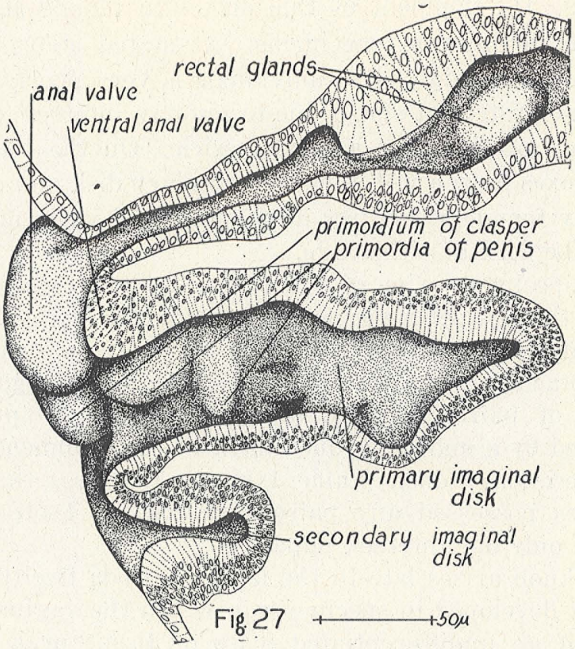
Very little change takes place in the primary disk during the eighth day. The secondary disk grows and in some specimens it becomes as large as the primary. It is still a simple tube (Fig. 27) partly surrounded by connective tissue which, I believe, is derived from that of the primary disk.

With one exception, all of the developmental features shown by the male during the sixth to ninth days are shown by the intersex during the ninth and tenth days. The exception is the formation of accessory glands and vasa deferentia, the primordia of which never develop. The tendency for lateral folds of the body wall to partly enclose the posterior region is expressed in an exaggerated manner and the entire anal and genital regions become enclosed in what might be called a "pseudo-cloaca." The size of this chamber is increased by what seems to be an actual drawing inward of the entire posterior region (Fig. 28).

The anal valves remain in the primitive state. The lateral primordia become valve-like but do not fuse dorsally as in the female. However, the ventral valve does not completely degenerate as in the male.

Swellings at the sides of the valves become claspers like those of the male (Figs. 27 and 28). The valves also rotate clockwise. The amount of rotation is never great and involves the genital region to only a very limited extent. As early as the eighth day swellings are present on the sides of the lumen of the primary disk. They vary in size and shape but during the ninth day they assume the form of two pairs of enlargements. In some specimens they remain as simple enlargements but in many they may be readily identified as being the primordia of the penis. I have never observed them to develop much further but chitinous plates form in this region in older pupae.

Anterior to the penis the primary disk of a typical specimen takes on the characteristics of the median duct of the male system. A posterior ejaculatory duct, an ejaculatory bulb and an anterior ejaculatory duct all form. In some specimens the disk forms only the ejaculatory bulb or the bulb plus one or the other of the ducts. The bulb may be identified by its thick muscular wall but its shape varies widely and its chitinous internal structure is undeveloped. The anterior ejaculatory duct is often quite long and a lateral extension may make contact with one of the gonads. This part of the duct is not a true vas deferens although it was so considered in Table 1. (Specimen No. 15, 22–24.)



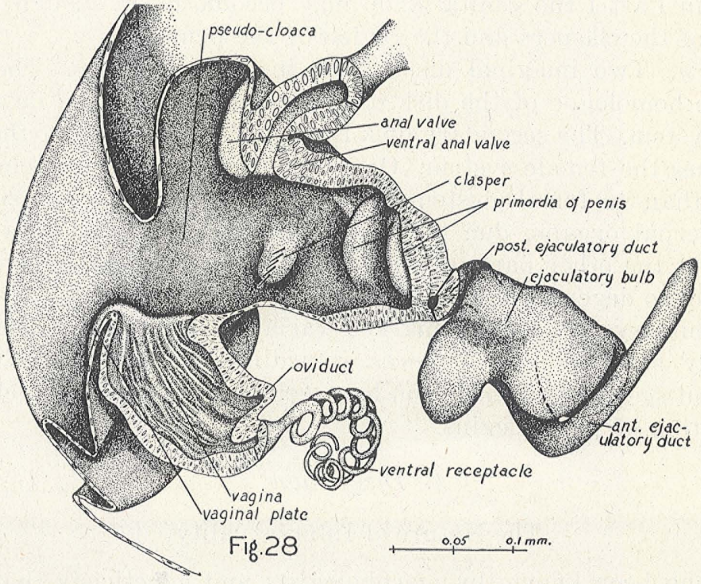


Figure 27. A stereogram of a middle 8-day, intersex pupa. The drawing was made to represent a specimen which had been dissected along the median plane.

Figure 28. A stereogram of an intersex pupa at the end of the tenth day. The drawing was made to represent a specimen which had been partly dissected along the median plane.

Development of the secondary disk is also largely limited to the ninth and tenth days. Development of this disk into female structures shows great variation in different specimens. As stated above, the secondary disk of some seven- to eight-day individuals is very poorly formed. Similarly, the female system of some nine-to ten-day intersexes consists of an amorphous mass of tissue or a small vesicle. On the other hand, some younger intersexes have a well formed secondary disk and older specimens also show many female structures developing. All variations between the extremes may be seen at either age.

In a typical specimen, primordia of vaginal plates form on either side of the opening of the disk; these seldom develop much further. The disk itself becomes vesicular to form a vagina and anterior tubular outgrowths form a ventral receptacle and oviduct. The latter may reach a gonad.

The failure of paired structures to develop in the primary disk is partly paralleled by a marked reduction in the development of such structures in the secondary disk. Table 1 shows that less than 25% of the adult intersexes possessed any paired organs and I observed a lateral outpouching in only one intersex pupa.

The genital knob arises late in the larval period; twelfth to thirteenth days. It is best developed in specimens in which the vaginal region of the disk consists of an undifferentiated mass of tissue or a simple vesicle. This becomes covered with the thick chitinous layer forming the knob. As noted in Part I the genital knob may become large enough to include the bases of the claspers and the region of the penis.

Summary. Two imaginal disks form in the intersexes. The primary disk is the homologue of the disk of sexual individuals and develops into the male system. The secondary disk is formed five days after the primary and becomes the female system. On the whole, the male system is better developed than the female system but is characterized by a complete lack of paired internal organs, due to the failure of their primordia to form. Variation in the other parts of the system is due to developmental variation and not to degeneration.

The secondary disk shows marked variation in size and seems to be particularly sensitive to influences retarding development. Subsequent development seems to depend upon how well formed the disk itself is when differentiation of parts begins.

4. Discussion

The Nature of Intersexuality

It has long been known by morphologists and geneticists that animals occasionally appear which show curious admixtures of male and female characteristics. That a study of such individuals might throw light upon the whole problem of sex determination and the related one of sex differentiation has also been recognized. The work of Dobzhansky and Bridges (1928), Lebedeff (1939), and that of Goldschmidt (1934) may be cited as examples of numerous studies of sex-intergrades among insects.

These studies have been characterized by a neglect of the details of the development of the intersexes. The authors have, to a large extent, attempted to reconstruct the course of intersexual development in the light of their findings made by an examination of the adult, and they have not given sufficient study to the development directly.

TABLE I

specimen no.	ejaculatory duct, post.	ejaculatory bulb	ejaculatory duct, ant.	vas deferens	accessory gland	vagina	parovaria	spermathecae	ventral receptacle	azygous oviduct	paired oviduct	gonads: female-like	gonads: intermediate	gonads: immature
1		small					1	2	X	X		X		
2		small				X			X	X	left	X		
3		X				X			X				X	
4	X	X	X			X	1	1	X	X	left		X	
5	X	X	X			X		2	X	X	left		X	
6	X	X				X		1	X	X		X		
7	X	X	X			X		1	X	X		X		
8	X	X	X			X		1	X	X		X		
9	X	X	X			X			X	X			X	
10	X	X	X			X			X	X			X	
11	X	X	X			X			X	X			X	
12	X	X	X			X			X	X		X		
13	X	X	X			X	1		X			X		
14		X	X			X			X	X	right		X	
15		X	X	left		X			X	X	right		X	
16		X	X							X			X	
17		X	X						X			X		
18		X							X			X		
19		small							X				X	
20		X	X			X						X		
21		X	X			X						X		
22		X	X	right								X		
23		X	X	right									X	
24		X	X	right										X
25		X	X										X	
26		X	X										X	
total	10	26	20	4	0	16	3	6	18	14	5	12	13	1
percent	39	100	77	15	0	62	12	23	70	54	19	46	50	4

Table 1. A table showing the structures present in different intersex specimens. The total number of individuals possessing each structure and the approximate per cent of such individuals is given at the bottom. The five structures to the left of the heavy line are male and the six to the right are female.

I have been able to obtain a very clear and complete picture of the development of the intersex of the Blanco stock of *virilis* by means of observations on a long series of specimens. This picture differs in almost every detail from those hypothecated by Dobzhansky and Bridges (1928) for *melanogaster* and by Lebedeff (1939) for *virilis*. The reason the method of development postulated for these intersexes differs from the actual development lies in the fact that these authors, and others, have based their reasoning largely upon the thesis of Goldschmidt (1934) that intersexes are characterized by development in the direction of one sex and a change at a turning toward the other. The present study shows that the "turning point" is not characteristic of *Drosophila* intersexes. Its actual existence elsewhere awaits experimental or observational verification.

The author has been fortunate in his studies in several regards. (1) In the Blanco intersex the condition is produced by a single dominant mutation and intersex individuals may be readily obtained. (2) The mutant gene has a highly specific effect and few, if any, modifying genes are present in the stocks. Thus, the intersexes are relatively uniform and the complication of variation among the specimens studied is reduced to the minimum.

Sex-intergrades have been named and classified in various ways. The terms hermaphrodite and gynandromorph have been used but these terms now have a very restricted meaning (Sturtevant and Beadle, 1939, pp. 250–253). The term intersex was suggested by Goldschmidt in 1915 (1934, p. 52) as a name for some sex-intergrades found among hybrids in the moth *Lymantria*. He defines it (p. 11) thus: "An intersex is an individual which starts development with its original, chromosomic, gametic sex, but changes sex during development. This change takes place at a certain point, the turning point, and development is finished with the other sex, though no change in the chromosomes has occurred." In a later paper, Goldschmidt (1938, p. 11) attempts to answer several criticisms of his work. Speaking of certain work on *Drosophila* he says: "It is a question whether the term intersexes ought to be used for such cases. . . . If these different types do not fit the laws of intersexuality (viz., development with a turning point), authors who call these types intersexes are prone to draw conclusions upon the real intersexes, which are bound to be erroneous."

Goldschmidt's views have been widely attacked from several quarters. However, his writings have been so voluminous and his ideas on sexuality have so thoroughly permeated the literature that it seems advisable to again call attention to the inadequacy of these definitions as they apply to *Lymantria*, as well as their inadequacy as applied to *Drosophila*.

In the 1934 paper, speaking of *Lymantria* intersexes, Goldschmidt (p. 95) says: "Or some organs might be unable to change after the turning point, their once finished determination not being reversible. An example is furnished by the derivatives of Herolds organ in intersexual males, which even after the turning point finish their once begun differentiation, whereas female equivalents may appear as a supernumerary struc-

ture." This is exactly what happens in the case of the internal genitalia of the Blanco intersexes—intersexuality expresses itself as a response to the developmental pressure of both sexes, not as development in one direction followed by a change.

This is but one of the several structures whose development in the intersex may be explained by an hypothesis other than that of the turning point.

(1) *The heredity of an intersex.* Goldschmidt's statements, given above, that an intersex starts life as one sex and then changes sex present certain serious difficulties. In the first place an individual starts development as a zygote and a zygote has no sex; it has only the capacity to develop sex, the same as it has the capacity to develop any of its other characteristics. In the second place there is no criterion by which the sexual potentialities of an individual may be determined at the zygote or early development stages. The sex chromosomes in a zygote may be known but Dobzhansky and Bridges' work (1928) has shown that the sex chromosomes alone are not the determiners of sex.

All of the chromosomes in a zygote may be like those of one or the other sex and the individual will still not develop into that sex. The intersexes of Lebedeff (1939) and the present Blanco intersex are examples of this fact. In the latter case the intersex zygote contains all of the chromosomes of a normal female but one normal gene (or possibly genes—see Stone, this Bulletin) is replaced by a dominant allel, Ix^B. The result of this replacement is that the zygote does not become a female because it has a genotype other than that of a female.

(2) *Development of an intersex.* From the statements concerning the characteristics of an intersex which are quoted above, Goldschmidt seems to ask that we apply the term "intersex" to only those individuals which meet the turning point requirement of this particular definition. If we agree to his request, we must also accept his thesis that sex-intergrades which develop without a turning point are, in some fundamental way, different from those which have one.

I do not agree that we should limit the use of the term intersex in this way. It is questionable whether we are yet in a position to define an intersex. Extensive studies of intersexes have only been made on those found in two genera of insects and additional studies have been made on but a few other animals. It would seem to be better that we await additional facts before we define an intersex and that we not attempt to reclassify sex-intergrades in order to eliminate those which do not fit any particular definition.

Neither do I agree that an intersex developing without a turning point differs, in any fundamental way, from one which does. My reasons for this stand are given here.

(a) Development of the gonad. During the late second, third, and fourth days the ovaries and testes alone can be distinguished. Not until the start of the fifth day can the intersex gonad be positively distinguished from the ovary in microscopic sections. Therefore, this organ might be

said to follow the "time-law" or turning point requirement for an intersex. These facts, however, must be considered: If I had dissected the specimens and had made only a gross examination of the gonads I could not have distinguished ovaries and intersex gonads until much later in development. If, on the other hand, I had used greater care in the preparations of my sections, had tried other fixative or stains, had made more careful measurements of average cell size; in other words, if I had used better technique, I have no doubt but that I could have distinguished ovaries and intersex gonads much earlier than I did. If, as is true, the time of the turning point depends entirely upon the technique used in its determination its significance as a "law" becomes greatly reduced and its actual existence is subject to doubt.

(b) Development of the primary imaginal disk. Until the onset of pupation the imaginal disks of all individuals of the same age are indistinguishable. The three types of disks could be recognized at the same time and the intersex disk did not follow, even briefly, the developmental pattern of the female. The cells forming the primary disk of the intersex have a different genotype than do those of the disks of either the male or female. Under these conditions, as should be expected, the disk of the intersex develops differently than does that of the male or the female. One fact is perfectly clear; the male reproductive organs of the intersex are formed directly from the primary disk and are not female organs which have passed through a turning point.

(c) Development of the secondary imaginal disk. This disk has no homologue in either sex but it is a "new" structure, developed under the influence of the factors producing this case of intersexuality. It gives rise to the female reproductive organs. Its history shows beyond doubt that these are not organs which failed to change at a turning point. These organs are a belated expression of female producing factors, acting upon tissue which would not normally have produced any part of the reproductive tract. This situation probably accounts, in part, for the wide variations in the structures developed from this disk. It is interesting to note that Lebedeff (1939, p. 564) suggests that a secondary imaginal disk forms in the intersexes he studied.

(d) Development of the secondary sexual characters. The tergites and sternites show no evidence of a turning point. They completely fail to become either male-like or female-like, but remain in an undifferentiated state. The anal valves, when they first form in either sex, are male-like. In the intersex they only retain, and in no sense assume, this male-like form. Associated with the valves are two male-like characters, the claspers and the terminal rotation. Both of these characters partly develop, without passing through a turning point.

(e) Accessory glands and vasa deferentia. The primordia from which these organs develop are never formed in the primary disk. This would indicate that the action of some factors in the genotype results in a deficiency which is just as definite and specific as are any of the structures produced.

From the above facts it may be seen that in the Blanco intersex we find five different expressions of the same set of factors. One of these, if uncritically examined, might be said to illustrate development through a turning point. The others show other types of development but none have a turning point. It therefore follows, that when the various parts of one organism develop in so many different ways no one way of development can be used as the single criterion against which they all must be evaluated. I must, therefore, conclude that the statement "development with a turning point" and the one "starts its development with its original, chromosomic, gametic sex" do not characterize an intersex and should not be used in its definition.

As has been noted above, the intersexes of *Drosophila* have been investigated and because my study was on this genus I feel that I am justified in being critical of some of this work. I will consider first the triploid intersexes of Bridges (Dobzhansky and Bridges, 1928 and Dobzhansky, 1930).

The study of these intersexes was, in many ways, much more difficult than the study of the Blanco intersexes because they varied so much. The authors, therefore, had the double problem of explaining intersexuality and also explaining the variation observed. I see nothing wrong with the generalized genetic explanation of intersexuality, ". . . the development of an individual into a female, an intersex or a male depends upon the variation of the balance between female-determining genes (localized chiefly in the X-chromosome) and male-determining genes (located chiefly in the autosomes). . . . The difference between the sexes is quantitative in nature." (Dobzhansky, 1930, p. 268). Neither do I find any objections to the explanation for the variation found among the intersexes. "The difference between the male-type and the female-type lines of triploids is probably due to a cumulative effect of many modifying factors localizd in the different chromosomes. . . ." (Dobzhansky, 1930, p. 268).

What I do object to is their acceptance of the turning point theory without a critical test of it in *melanogaster*. It may be argued that they did test the theory when they found that a correlation exists beween the time of development of an organ and its relative stability in the intersexes. That is, the late-developing male traits are unstable and are, therefore, good indices of maleness, whereas the early-development female traits are unstable and are good indices of femaleness. "Each intersex may be supposed to develop up to a certain point as a male individual and thereafter as a female. The intersex becomes more female-like if the moment of reversal comes early in development and more male-like if the reversal occurs late." (Dobzhansky and Bridges, 1928, p. 433).

The times at which the various characters appear in the normal flies was determined by dissection. I have indicated above that the timing of a developmental event depends entirely upon the technique used. For example: In the Blanco males the primordia of the accessory glands and

vasa deferentia form in the sixth day but they could not have been recognized in dissected material before the ninth day. Many other examples might be cited. It follows that correlations obtained by observations on dissected material have very little significance because this technique does not reveal when an organ develops. It only reveals an organ after it has developed.

The case of the rotation of the terminal region of the male is an illustration of the false ideas obtained by the use of the turning point "law." Dobzhansky and Bridges (1928, p. 429) describe the male and male-like intersex as having a 360° *counterclockwise* rotation of the terminal region. And they state: "This rotation is progressively undone in the passage to the female-type intersex." The turning point "law" requires that any rotation in the female-type intersex be an undoing of a previous male-type rotation. Thus, if the undoing is clockwise the doing must have been counterclockwise, and hence the error. I have verified Gleichauf's (1936) observation that the rotation in the male is clockwise and involves both anal and genital structures. I have also seen that the rotation in the Blanco intersexes is clockwise, partial, variable and involves primarily the anal valves. If, for the triploid intersexes, we assume that the rotation is clockwise, variable, partial, but involves primarily the genital structures, we may adequately explain all the observed facts without recourse to the theory of counter rotation.

Dobzhansky (1930, p. 270–271) reported upon the influence of temperature on intersexes and noted that higher temperatures result in an increase in the number of female-type intersexes and that lower temperatures result in an increase in the number of the male-type. He says (p. 271): ". . . , the moment of the reversal of the development occurs relatively late at lower temperatures, and relatively early at high temperatures." This statement is, of course, true only if a turning point exists. The theory that intersexes vary in the relative proportion of male and female characters but that both are co-existant will explain the temperature facts more satisfactorily than does the turning point theory. That is, low temperatures inhibit female development (or stimulate the male) and high temperatures stimulate female development (or inhibit the male).

In a recent paper, Dobzhansky (1941) reports on some intersexes observed in *pseudoöbscura* (race A). These were like the Blanco intersexes, in that their internal genitalia consisted of a male and a female system, both relatively complete. One statement is made regarding the female system which I believe is subject to question. It is said (p. 558) that, ". . . , the anterior portions of the female ducts may display grotesque modifications in the male direction." Specimens and text figures are then cited in which accessory glands (paragonia) are present at the anterior end of the azygous oviduct. These structures have the form of long, tubular vesicles but they are only found in specimens, or on that side of a single specimen, in which the paired oviduct does not join a gonad. That

is, these structures have the vesicular appearance of the accessory gland but have the structural relationship of a paired oviduct. Because oviducts and accessory glands are not homologous, I am confident that the morphological relationship of these structures is a better index of their character than is their vesicular appearance.

Dobzhansky also attempts to homologize the parts of the male and female systems. He concludes that only the median, unpaired parts are homologous, but my studies lead me to doubt the homology of even these parts.

In his discussion, Dobzhansky (1941, p. 560) contrasts the diploid intersexes of *Drosophila* and the triploid *melanogaster* and diploid *Lymantria* intersexes. The former are characterized by a double, male and female genitalia, whereas the latter ". . . , with rare exceptions, have a single set of ducts and of genitalia intermediate in structure between male and female." He cites his 1930(b) paper for data on the *melanogaster*, triploid intersexes. From this paper it is evident that specimens do appear with two sets of internal genitalia (Fig. 31, p. 111). Tables of coefficients of correlation are also given for various male and female characters. In one Table the lowest coefficient was –0.06, the highest was –0.69, and the average was –0.40. In another the lowest was –0.22, the highest was –0.97 and the average was –0.57. This would indicate that in a fairly large number of specimens male and female structures are coexistant. Until similar Tables are compiled for diploid intersexes and these are shown to be significantly different than those for the triploid, or until the development of the triploid intersexes can be shown to be different than that of the diploid, the question of the fundamental similarity or difference between the diploid and triploid intersexes must remain open.

Lebedeff (1939) studied an intersex of *virilis* which is much like the Blanco intersex. It is caused by a mutant, recessive, autosomal gene, and when homozygous makes intersexes of chromosomal females. Lebedeff made the same type of errors as Dobzhansky and Bridges and for the same reason. He, like they, assumed without verification, the existence of a turning point. Most of the facts available to Dobzhansky, however, tended to support the turning point hypothesis, whereas very few of the observations of Lebedeff support this hypothesis and many of them are in contradiction to it.

I wish to cite one outstanding example. He says (Lebedeff, 1939, p. 563) : ". . . all sexual organs, with the exceptions of the secondary ones, which have not completed their development at the time of reversal, do so after the occurrence of the turning point." Such a type of development is difficult to visualize, for if a turning point exists, an organ cannot change sex at the turning point and still continue development on the lines of the initial sex. Lebedeff recognizes this difficulty but still insists on the existence of a turning point. He says (1939, p. 564) : "The turning point does not interfere with the development of most of the sexual organs of the initial sex . . . once the imaginal discs of these organs have been

laid down . . . However, the occurrence of the reversal reaction stimulates the development of corresponding sexual organs of the other sex. The male organs develop from fresh outpushings (or else from new imaginal discs). As a result, the two systems, . . . , develop side by side in the same individual, . . ." It seems perfectly clear that if the organs of the "assumed" sex develop from a new disk those of the original disk had neither changed sex nor been subjected to a "reversal reaction." Lebedeff's facts do not fit the hypothesis of a turning point and he should have seriously questioned, rather than accepted, its existence.

CONCLUSIONS

The most important generalization which has come from the intersex studies of *Lymantria* and *Drosophila* is, that the expression of sex is the resultant of the action of two sets of hereditary factors. The one directs the developing organism toward maleness, the other toward femaleness. In normal sexes one or the other of these sets of genes "captures" the organism and succeeds in directing its development despite the continued influence of the other. In the intersex the influence of the set of genes which would otherwise have controlled development, is either weakened or that of the opposing set is strengthened. The weakening or strengthening may be due, in any one case, to one, or possibly a combination, of the following: (a) Mutations, such as those of the Blanco intersex and the *virilis* intersex of Lebedeff, (b) Genic unbalance produced by polyploidy, such as the triploid intersexes of Bridges, (c) Genic unbalance due to hybridization, such as that resulting from the *melanopalpa* female X *repleta* (Guatemala) male cross, described by Wharton in this Bulletin, (d) Gene differences and cytoplasmic factors (?), described by Goldschmidt for *Lymantria*, (e) Environmental factors, such as those described by Baltzer (1937) for *Bonellia*. (f) Additional study may reveal other factors which produce a similar effect. As a result of this action the two sets of factors controlling development are rendered more nearly equal in their influence and the sex of the developing organism is correspondingly modified. The manner in which the sex of an individual is modified is also variable and depends upon the modifying agent as well as upon the specific organ or structure being influenced.

In all probability an intersex, during early developmental stages, would be morphologically indistinguishable from either a male or a female of the same age, because the two sexes themselves cannot be differentiated when very young. However, work in experimental embryology has indicated that morphological differentiation is preceded by what has been called, chemical, physiological, or non-morphological differentiation. It would thus seem that a very real, although directly indetectable, difference exists between the sexes during the period proceeding morphological differentiation.

Now the question arises: does this non-morphological differentiation of the primary sexes also apply to the intersexes? This study would

indicate that the answer is in the affirmative. The reproductive organs of the intersexes exhibit their structural differentiation at the same time in development that the sexes exhibit theirs. We therefore have the same type of evidence for an early stage, non-morphological differentiation of the intersexes, as we have for the primary sexes. We have no basis for hypothecating a developmental period in the intersex when it has a non-morphological differentiation toward either sex. Unless such a period can be demonstrated by experimental means, we must conclude that the non-morphological differentiation of the intersex is intersexual and is not either male or female in character.

The intersex factors, acting with the normal factors for sex, then produce visable effects. In the Blanco intersex they cause the primary imaginal disk to develop into an incomplete male system. They cause a secondary disk to form and from it produce some of the parts of the female system. They cause the gonads to have the characteristics of both the immature ovary and testis. And they cause the secondary sexual characters to develop with very little or without any sexual differentiation.

The result of these various types of development is an organism which contains a mixture of male, female and undeveloped sexual characteristics in various proportions.

We may call such an organism an intersex.

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VIII. THE *Ix*^{B} FACTOR AND SEX DETERMINATION

WILSON S. STONE

A dominant intersex factor, *Ix*^{B}, was found by Mr. Elwood Briles in a stock of *Drosophila virilis* from Blanco, Texas. This stock came from a pair of flies captured in a grocery store on July 27, 1939, and is carried in the laboratory as 290.3. The case was not detected until the spring of 1941 when Mr. Briles discovered some intersexes in a series of pair matings. It is not possible to decide whether it was present in the Blanco population at the time the pair was collected.

The *Ix*^{B} factor is carried in stock through the male by breeding each generation from pairs that produce intersexes.

Professor A. B. Griffen has examined the *Ix*^{B} strain cytologically both in salivary gland and ganglion cells. No chromosome rearrangements—deletion, duplication, inversion, translocation, or fusion—were found to be present.

Linkage of Ix^{B}

Ix^{B} is a dominant autosomal factor (or factors) in chromosome 2, which causes 2 X + 2 A (chromosome female) to be intersex if present heterozygous. It has not yet been possible to obtain it homozygous, as all intersexes are sterile. Proof that the factor is in chromosome 2 and causes intersexuality of 2 X + 2 A individuals may be inferred from the following data. In each of the following tests individual males were used and the cultures that produced intersexes were counted. The numbers in parentheses indicate the chromosome.

(1) $R_{(2)}/R_{(2)}$ ♀ x $Ix^{B}/+$ ♂ ; F_1 ♂ $Ix^{B}/R_{(2)}$ x + ♀ ; F_2 gave 56 + ♂, 42 $R_{(2)}$ ♂, 61 $R_{(2)}$ ♀, and 56 intersexes which did not have *R*.

(2) P_1 *y w* $mt_{(X)}$ / *y w* $mt_{(X)}$ ♀ x Ix^{B} $R_{(2)}$ ♂ ; F_h 42 $R_{(2)}$ ♀, 71 *y w mt* ♂, and 38 (not $R_{(2)}$) intersex. Part of the males showed $R_{(2)}$, 19 *y w* $mt_{(X)}$ +, and 14 *y w* $mt_{(X)}$ $R_{(2)}$ in one count.

(3) P_1 *cn* $sv_{(3)}$ *cn* $sv_{(3)}$ ♀ x Ix^{B} + ♂
F_1 Ix^{B} / *cn* $sv_{(3)}$ ♂ x *cn* $sv_{(3)}$ / *cn* $sv_{(3)}$ ♀
F_2 29 + ♂, 26 *cn* $sv_{(3)}$ ♂, 11 + ♀, 9 *cn* $sv_{(3)}$ ♀, 19 + intersex, 13 *cn* $sv_{(3)}$ intersex.

(4) P_1 $px_{(4)}$ $pe_{(5)}$ / $px_{(4)}$ $pe_{(5)}$ ♀ *x* Ix^{B} / + ♂
F_1 Ix^{B} / $px_{(4)}$ $pe_{(5)}$ ♂ x $px_{(4)}$ $pe_{(5)}$ / $px_{(4)}$ $pe_{(5)}$ ♀

F_2	+	$px_{(4)}$ $pe_{(5)}$	$px_{(4)}$	$pe_{(5)}$
♂	16	12	20	25
♀	11	5	4	6
intersex	10	13	17	12

(5) P_1 $gl_{(6)}$ / $gl_{(6)}$ ♀ x Ix^B / + ♂
F_1 Ix^B / $gl_{(6)}$ ♂ x $gl_{(6)}$ / $gl_{(6)}$ ♀
F_2 55 + ♂, 39 $gl_{(6)}$ ♂, 17 + ♀, 21 $gl_{(6)}$ ♀, 25 + *intersex*, 32 $gl_{(6)}$ intersex.

In these tests there was no appreciable modification of the appearance of the intersexes on outcrossing, even with the replacement of any of the other autosomes, 3, 4, 5, or 6. As test (2) showed, Ix^B is linked in chromosome 2 and causes 2X: 2A individuals, which are heterozygous for Ix^B, to be intersexes. It does not affect the viability or fertility of the males. As there is ordinarily no crossingover in the males, it is not possible to prove that Ix^B is a single gene. Its lack of variability in these several crosses shows that it must be one, or less probably, several dominant genes in chromosome 2. It is therefore not an allele of the *ix* found by Lebedeff (1934) in chromosome 3.

Discussion

There are two somewhat different problems and genetic mechanisms connected with sexual reproduction. One is sexual differentiation. This is a problem of differentiation comparable to that of any other organ system. It is equally important in hermaphroditic and bisexual forms. The second problem is superimposed on the first. It concerns the alternative differentiation in the members of a species of only the male or the female system, rather than both.

The genetic system may be of such a nature that differentiation of a cell or tissue is inflexible and solely under the control of the genotype, or it may be labile and capable of responding to stimuli, chemical, and perhaps physical, from other cells or from the external environment.

Coe (1940), among others, has reviewed and discussed many examples of the several types of sex differentiation and sex determination. It is therefore unnecessary to consider many of them here. The advantages in evolution of cross fertilization and recombination are conferred on both hermaphroditic and bisexual forms so that they should be considered together.

There are two types of genetic control of these processes. In the first, one genotype produces the several types of differentiation. This may be an hermaphroditic form like the earthworm. It may have an inflexible genotype with a normal developmental cycle in which the male is the immature intermediate phase and the female the mature phase such as *Crepidula*. In others, such as the oyster, a more flexible genotype allows an alternation and reversal of the two sexual phases in response to external stimuli.

In the second type with genetic control there are alternative genotypes which determine the two sexes. This may consist of simple "+" and "—" factors such as determine crossing in forms like the Fungi. More often this is controlled at meiosis by incompletely homologous chromosomes,

the X and Y. In certain of these, a gene, or several linked genes, determine the sex of the organism. For example, in *Melandrium dioicum,* Warmke and Blakeslee (1940) have shown that the Y chromosome, or rather a part of it, determines maleness. If a Y is present the plant is male; if absent, a female, regardless of the frequency of the X or autosomes. Hermaphroditic plants occurred in a few combinations, but even here, always when a Y was present.

In other forms such as *Drosophila,* the *alternate* sexes are determined by differences in genic balance effected by changes in the relative frequencies of the same genes.

In mammals and birds it is not certain which of these two mechanisms, specialized genes or genic balance, is responsible for sex determination, although it is usually assumed to be determined as in *Drosophila.* In these higher vertebrates the genotype determines the sex of the organism, but the final sex differentiation is indirectly controlled by the genotype through hormone action. The genotype determines the hormone system which in turn controls the type of differentiation.

The genetic mechanism involved in the production of intersexes has been investigated only in insects. In these cases evidence of indirect control of sex differentiation of cells or tissues is exceedingly fragmentary. In *Habrobracon* the males are haploid (unfertilized eggs) while the females are diploid (fertilized eggs) and heterozygous for different genes which sum in activity to control female sex determination (Whiting, 1933). A few mosaic males in which the line of separation between the two genetically different haploid tissues pass through the genital region show a certain local feminization of the tissues at the place of juncture. Here there is local transmission of chemicals between the two tissues but certainly no general sex hormone control through the blood system. No other case suggesting sex hormones in insects where genetic investigations have been made has been satisfactorily demonstrated. Gynandromorphs occur in *Drosophila, Lymantria* and even in *Habrobracon.* In these cases no influence of sex hormones is detectable.

The intersexes studied by Newby (1942) therefore must be considered as occurring in a group (insects) where sex differentiation is entirely (or nearly so) under the control of the genotype in each cell. This is true even of tissue developing from the same imaginal disc, as shown in gynandromorphs.

Newby has pointed out the fallacy of a "time law" and "turning point" as postulated by Goldschmidt (1934) to explain the development of these intersexes. A turning point could exist if the development of organs was under sex hormone control, but this is not the case here. If each tissue should differentiate part way in the direction of one sex and then stop and develop in the direction of the other sex, there would be a turning point under the control of the genotype of the cells themselves. Newby has proven that partial differentiation in one direction with reversal is not the case in *Drosophila virilis* with Ix^B. The tissues do differentiate

according to their genotype. Until adequate studies similar to Newby's on *virilis* have been made, and actual development in the female direction followed by a reversal has been demonstrated in other insect material, an explanation similar to the one given by Newby for *virilis* must be considered much more probable for the other cases as well, on the scientific premise that explanation based upon experimental evidence must always be accepted in place of those based upon surmise from indirect evidence.

One of the pertinent features of the bisexual forms is that all genes necessary for the differentiation of the organ systems of both sexes are present but that only one organ system develops. This is true in the male of *Melandrium,* especially the polyploid forms, although the female lacks the male determiner genes in the Y. It is true in *Drosophila melanogaster* and *Drosophila virilis* in both sexes as the change in sex is accomplished by a change in the relative frequency of certain of the genes. It is true for *Habrobracon* with certain reservations. The haploid males have the same genes as the diploid females but cannot have different alleles heterozygous. In the case of the Ix^B factor, the *ix* gene studied by Lebedeff, triploid intersexes, and even *Lymantria,* part, and sometimes almost all of both organ systems, can coexist in the same organism. In the case of Ix^B this development was consistent, even in crosses between different strains. Sex determination demands the stimulation of one set of genes to produce one organ system with lack of stimulation or even inactivation of the other set of genes which determine the formation of the other type of reproductive system. It is not clear whether the two systems require stimulation to act, or require inactivation to prevent their acting. Regardless of this point, the gene or genes which by their presence *(Melandrium)* or by their change in frequency *(Drosophila)* control this selective activation of one type of differentiation are the sex determining genes, while the actual differentiation of the reproductive system is controlled by other genes just as any other developmental process.

In the determination of the sex of males and females in *Drosophila,* the change in frequency of the genes in the X chromosome relative to those in the autosomes changes the concentration of the products of incompletely dominant genes which are cumulative in their effect. This change in relative concentration of the products of gene action in these cases controls an all or none reaction. The reaction thus established stimulates one type of differentiation to occur, and inhibits the other. Dobzhansky and Spassky (1941) have made several suggestions concerning the mutations which have produced intersexes in *Drosophila pseudoobscura, Drosophila simulans* (Sturtevant 1921), and *Drosophila virilis* (Lebedeff 1934, 1938, 1939). They state (p. 561) concerning these mutations "the fact that a mutant allele of a gene causes a modification of the development in the direction of maleness does not prove that the normal allele of the same gene is also a gene for maleness, *or in fact that it has anything to do with sex determination*" (italics mine). With the last part of this statement I am in decided disagreement. In the first place neomorphic mutations,

that is, those with effects unrelated to their normal alleles, are rare. There are now at least four mutations which cause intersexuality, two of them in *virilis*. Furthermore Dobzhansky himself is very decidedly of the opinion that many genes are concerned in female sex determination in *melanogaster*. (Dobzhansky and Schultz 1934.) If we grant this and still assume that these mutations which cause intersexuality are in fact neomorphs, then we must be prepared to go back and reinvestigate the type of allelic relations that exist between almost all mutations and their normal alleles, before we can decide anything about the function of the normal gene. In almost all known cases there is a relation between the action of the normal gene and its mutant allele. Therefore Dobzhansky and Spassky seem unduly conservative in their reluctance to make inferences concerning these normal genes from their mutant alleles.

The interpretation of the effect of these mutations which cause intersexuality in *Drosophila* will depend on the type of gene activity which determines sex. We may assume that the genes which control the differentiation of the two types of reproductive systems would receive the necessary stimulus for the initiation of development but that the sex determining genetic reactions act to inhibit one or the other. On this assumption the normal allele of these intersex factors would act to inhibit the initiation of the development of the male system in the 2X:2A frequency of gene balance. It would, in effect, be a female sex determiner, and would be a limiting factor in the reaction. The mutant alleles fail to carry out the inhibition of the development of the male system and therefore both sex systems appear. This would place in the autosomes one or two (in *virilis*) female determiner genes whose action depended on the quantitative X-autosome gene action relation. Another gene (or genes) would be necessary to inhibit the development of the female system in the alternative X-autosome frequency. The data of Dobzhansky and Schultz (1934) and Pipkin (1940) show that there are several genes in the X which are additive and sum to produce the quantitative X-autosome balance, but certainly do not rule out the presence, elsewhere in the gene system, of genes necessary for the production of a female. We may make the alternative assumption that a stimulation is necessary to initiate the development of each of the two reproductive systems. In this case the intersex producing mutations stimulate the process of male differentiation in addition to the stimulation by the 2X:2A condition on the female system. Here the mutations would be more active than their normal alleles in male sex determination. It is possible, and seems even probable, that both stimulation and inhibition are involved in selection between the possible reproductive systems. In this case we might be inclined to interpret the intersex factors in different ways. As Wright (1934) has suggested, it seems much more probable that a gene carrying out a positive reaction will be dominant over an allele which does not. This seems to be the usual case despite the fact that a deficiency sometimes has a pseudodominant effect (e.g., *Notch*). On the other hand, a certain suppressor

of *vermilion* which seemed to be an allele of the *white* series was recessive in its action. If we assume increased dominance with increased activity, we might expect that the recessive genes causing intersexuality fall into one category while the dominants fall into another. On this basis the normal alleles of the recessive factors in *simulans* and especially in *virilis* may be female determiner genes, while *ix* failed to carry out the normal function. On the other hand, the normal alleles of the dominant mutations in *pseudoobscura* and *virilis* would be male determiner genes, and their alleles would be regarded as acting in the same direction but more effectively. In fact if Ix^B were strong enough to stimulate the development of both male and female gonads from the primary germ cells, individuals carrying this factor would be almost complete hermaphroditic forms.

In the evolutionary history of the origin of bisexual forms with an X-Y mechanism, such as in *Drosophila,* the male factor must have been located in what is now the Y and limited to the individuals that carried it. At the present time the function of that original factor has been transferred to a gene or genes elsewhere; certainly some male genes are in the autosomes. The genetic degeneration of the Y chromosome must have been conditioned by the loss of the ability to undergo recombination, a type of change similar to the degeneration of the Y-linked third chromosome of *miranda* (MacKnight, 1939). The X chromosome was gradually selected to be essentially haploid in the male, but diploid in the female, and the differentiation of the two systems must have been gradually coördinated with this change in genic balance. *Melandrium* seems to be a somewhat intermediate stage in such a system, as the Y has a limited region that carries the male determining factor or factors and which does not cross-over with the X. The occurrence of such a strong male factor as that in *Melandrium,* if it carried sufficient selective advantage in any way, would seem a probable intermediate step between an hermaphroditic and a bisexual form. Such a factor in some individuals of a mixed male and hermaphroditic population might confer selective advantage on mutations which would convert the hermaphroditic individuals into females, thus ending with two separate sexes.

All of these autosomal mutations which have been reported affect the 2X:2A individual, which would be a female in the absence of an intersex factor. These factors either stimulate or fail to inhibit the production of the additional male reproductive system. They therefore indicate the presence of autosomal genes which are critically related to sex determination. Alleles of the genes for maleness may occur which have greater effect than the normal gene. Genes for femaleness may mutate to alleles which are not effective in suppressing the differentiation of the male system. In 2X:3A intersexes, the frequency relation is such that neither system has the proper genic milieu to stimulate and/or inhibit the development of either reproductive system completely. Therefore mixtures of varying parts of male and female systems are produced. This unbalanced

system is particularly susceptible to the modifying effects of environment, etc. This seems to be the case for crosses between species in some instances.

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IX. DISTRIBUTION OF THE VIRILIS GROUP IN THE UNITED STATES

J. T. PATTERSON

In a recent paper the writer (Patterson, 1941) has given an account of the occurrence of the *virilis* group of Drosophila in Texas. This group now contains five known species, as follows: *Drosophila virilis* ,Sturtevant, *D. americana* Spencer, and three new ones found by us, *D. texana*, *D. novamexicana* and *D. montana* (Patterson and Wheeler, 1942). As indicated in the first article of the present publication, the various strains of *D. virilis* have puparia which vary in color from gray to black, while the basic color of the puparia of the other four species is reddish. The point of interest is that the strains of *D. virilis* are found almost exclusively in stores and produce houses which handle fruits and may be designated the domestic forms. The other four species are found in the country unassociated with such habitats and may be designated the wild forms.

Since the 1941 paper was sent to press we have had opportunities to collect *Drosophila* in several other states, and consequently we have secured much additional data on the distribution of the members of this group. It is the purpose of the present article to summarize all available facts which have any bearing on the problem of the distribution of these flies in the United States.

GEOGRAPHICAL DISTRIBUTION OF DOMESTIC FORMS

Both the domestic and wild forms have sparce populations. The domestic forms have become adjusted to conditions prevailing in produce houses, especially those found in commission houses which handle bananas. These houses are equipped with temperature-regulated vaults for maturing bananas for the retail market and it is in and about these vaults, together with certain fruit stores, that *D. virilis* breeds and frequently builds up colonies of considerable size. Of the 253 specimens collected by us, 169 came from commission houses, 78 from stores handling fruits and only six from the country. The total number of 247 specimens from towns and cities is not large, but had there been any real point in securing larger numbers we could have done so easily by making repeated collections at stores and commission houses where they were known to occur.

In the upper part of Table 1 are listed sixteen towns and cities in which we have found *D. virilis*. The letter "C" or "S" in parenthesis after each place indicates whether the collection was made in a commission house or in a store. In the second vertical column is given the number of specimens of *D. virilis* which was collected at each place, while the succeeding columns list other species of *Drosophila* which were taken at the same time.

In this article we shall not attempt to analyze the data on the other species, except to point out a few facts of especial interest. The species

D. melanogaster, D. hydei, D. repleta and *D. busckii* are common and widely distributed and usually are found in all stores and commission houses. The species *D. simulans* is also widely distributed, but is more common in the southern than in the northern parts of the United States. We therefore did not find this species in many such places when we collected in the north during June and July, 1941. The species *D. immigrans* and *D. funebris* are much more common in the north than in the south and this fact is reflected in the difference in the number of specimens listed in the table for the two regions. The species *D. mulleri* is restricted almost entirely to the state of Texas, and consequently it appears only in collections from places located within its distribution area.

In the last column of the table are grouped together all other species which were collected at the same places. Their number (1053 is not large, amounting to only slightly more than one per cent of the entire collection of 88,100 specimens listed in the table. This group is composed of fourteen species as follows, with their numbers given in parentheses: *D. ananassae* (216), *D. mercatorum* (232), *D. hamatofila* (222), *D. pseudoobscura* (137), *D. longicornis* (85), *D. affinis* (124), *D. melanica* (16), *D. macrospina* (10), *D. carbonaria* (4), *D. similis* (2), *D. robusta* (2), *D. nebulosa* (1), *D. cardini* (1), *D. transversa* (1).

The per cent of *D. virilis* in the population of *Drosophila* varies greatly for the different collected places. At a store located on the western edge of New Orleans, Louisiana, the per cent was 55.1; at the commission house in Austin, Texas, it was 8.14; and at a fruit store in Brooksville, Florida, it amounted to 3.7. The other extremes were found at Dallas and Fort Worth, Texas, where the per cents were 0.04 and 0.03, respectively

On the basis of our collection records, it would seem that the main distribution area of *D. virilis* occupies that portion of the United States which lies south of the 35th parallel. Of the 247 specimens collected in stores and commission houses, all except one came from this area, and the six captured in the country are also from this region. In order to make this point clear I have plotted on an outline map all of our records as near as possible to the points of capture (Fig. 1). As the table and map indicate, fourteen specimens were collected in California. These were taken by Mr. G. B. Mainland in August and September in a large wholesale fruit store located at Santa Barbara. The total number collected in Texas over a period of three years is 170, incuding six from the country. A single speciman was taken by Dr. G. M. Mickey in May, 1939, at Baton Rouge, Louisiana, and our stock collectors obtained 55 specimens at New Orleans during the month of June, 1941. They also collected on the same trip five specimens each at Brooksville and Tampa, Florida. The writer captured two specimens on June 20, 1941, in a commission house at Memphis, Tennessee.

In the lower part of Table 1 are listed fifteen cities in which collections were made at commission houses. All of these cities are located north of

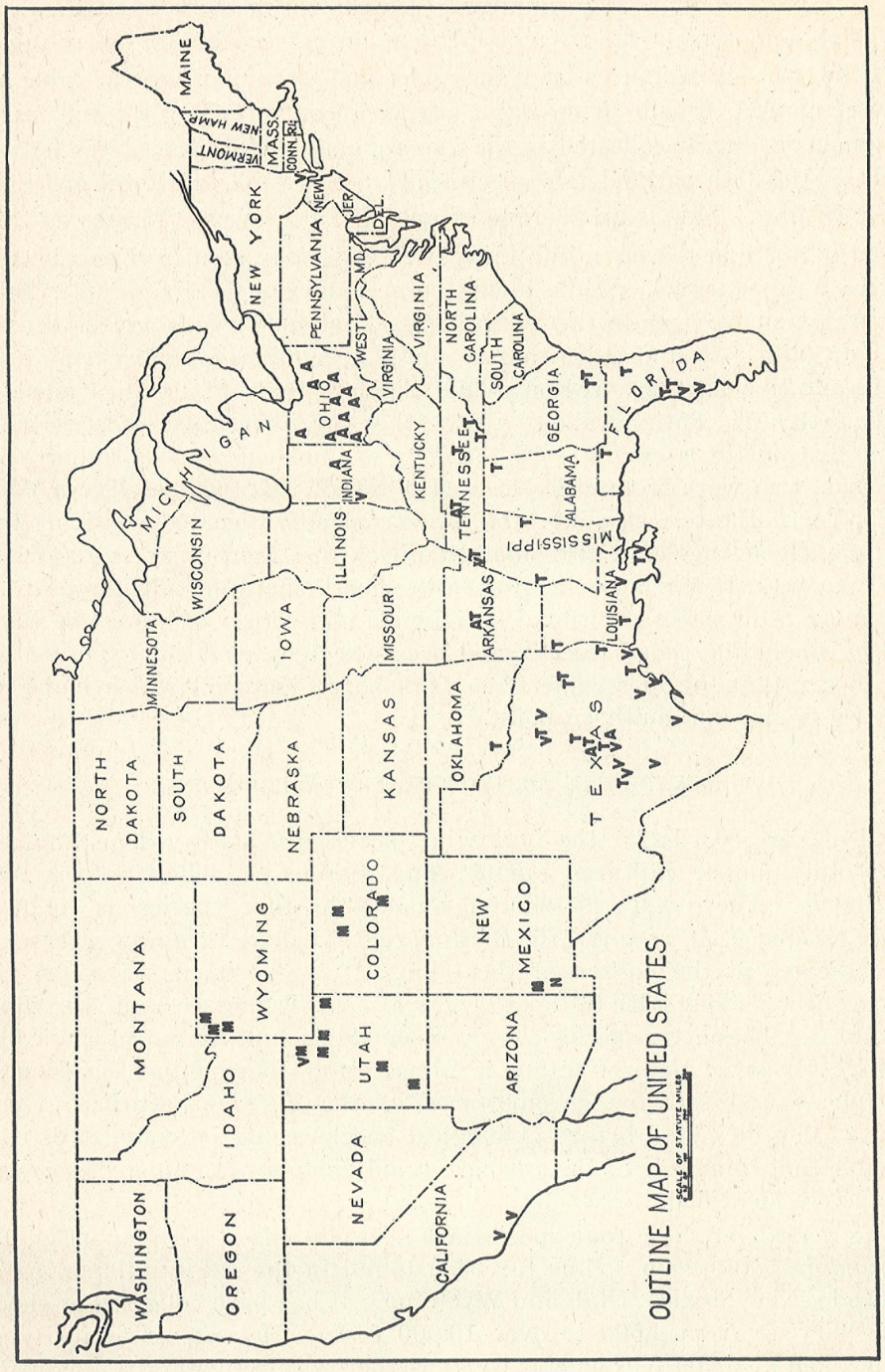

Fig. 1. Map showing the distribution of the virilis group in U.S. The letters on the map indicate the points at which the different species were collected, as follows: A, *D. amercana;* M, *D. montana;* N, *D. novamexicana;* T, *D. texana;* V, *D. virilis.*

the 35th parallel. The total number of flies taken in the fifteen cities was 27,003, among which was a single *virilis* male, captured at Ogden, Utah.

If the two sets of figures from the upper and lower parts of the table are now compared, it will bring out a striking contrast in the numbers of specimens of *virilis* collected in the two regions. From the places located south of the 35th parallel, 246 specimens were present in a total of 61,097 collected flies. This is an average of one *virilis* in every 248 flies as compared to a single specimen in a total of 27,003 flies taken in places located north of this parallel. While it will be necessary to obtain samples from a more extensive area in the north before final conclusions can be drawn, yet our failure to find more than a single specimen of *virilis* in a fairly representative northern region would indicate that this species must be rare north of the 35th parallel. A few other specimens have been recorded from this northern region. Of the four established records, other than our own, two were from cities located north of this parallel (New York, N.Y., Terre Haute, Indiana), and two from cities located south of this latitude (Los Angeles, California, New Orleans, Louisiana; see author's 1941 paper). If further collecting in the north should result in failure to find more than a few additional individuals of *virilis*, one would be justified in concluding that its occasional presence there could be explained on the theory that the flies came from fruit-borne material which had been shipped from some southern point.

Geographical Distribution of Wild Forms

All of our records for the four wild species are displayed on Table 2. The total number captured during four seasons of collecting was 557 specimens. These were distributed among the four species as follows: *D. americana* 9, *D. texana* 276, *D. montana* 271, and *D. novamexicana* 1. As indicated at the bottom of the table, a few specimens of *americana* were collected along with those of *texana* at Eva, Tennessee, and Morrilton, Arkansas. These two species closely resemble each other morphologically, so that for exact determination it is necessary to examine the somatic metaphase and salivary chromosomes of the different strains. Since some of the flies died before cytological checks could be completed, it is possible that some few of the *americana* individuals were among those not completely identified.

The records of these four species are also plotted on the map (Fig. 1). *Drosophila montana* has thus far been found in the Rocky Mountains of Colorado, New Mexico, Utah and Wyoming. It has been collected at elevations varying from 4,500 to over 10,000 feet, with a large majority of them taken at 6,500 feet or above. It is therefore a mountain inhabiting form. Four of the points of capture indicated on the map are from records supplied by Professor Th. Dobzhansky, who kindly sent me the flies and the data on localities. A single specimen was taken at each of the following places: Soapstone Camp east of Kamas, Utah; Little Brush

Canyon at Vernal, Utah; Pikes Peak, Seven Falls Canyon, Colorado; Park Range, near Rabbit Ears Pass, Colorado.

On the basis of our collection records, *D. novamexicana* is the rarest member of the group. Only one specimen has been captured up to the present time. It was received in the laboratory on November 9, 1940, and was found in one of three dozen trap bottles which had been exposed for several days near Silver City, New Mexico. We are very grateful to Superintendent A. B. Cutler of the Soil Conservation Service, who kindly exposed the baited traps and returned them to the laboratory. Apparently, the distribution range of this species is limited to a relatively small area in southwestern New Mexico, although it is possible that it may extend across the border into Mexico.

Drosophila texana has been collected in Texas, Oklahoma, Arkansas, Louisiana, Mississippi, Tennessee, Alabama, Georgia and Florida (Fig. 1). Its distribution area covers therefore the greater part of the southeastern quarter of the United States. This region has a rather low elevation and warm climate, and the flies are able to breed for the greater part of the year. This species breeds in certain favorable localities and in some of them is able to build up toward the end of the main breeding season fairly large colonies (Table 2).

Drosophila americana has been collected in Texas, Arkansas, Tennessee, Indiana and Ohio. The main area of its distribution seems to center in Ohio, where Professor W. P. Spencer has taken it at a number of different places. Eleven of these places are plotted on the map (Fig. 1), including one near Piqua where the writer trapped two specimens on July 1, 1941. We are greatly indebted to Dr. Spencer for his kindness in supplying the data on these localities. In September, 1941, Mr. W. K. Baker, one of our graduate students, collected a pair of flies near Anderson, Indiana, which constitutes the single record from that state. Between Ohio and Texas several scattered specimens of this species have been found. In Texas a single female was collected at San Gabriel Park near Georgetown, and a second one at the Aldrich farm near Austin. Among the fifty-nine specimens of the wild form taken from traps near Morrilton, Arkansas, two have been identified as *americana,* and three out of the twenty-five from near Eva, Tennessee were also found to be *americana.*

SUMMARY

The five known species of the virilis group have various types of "isolating mechanisms" (Patterson 1942). In this article we shall consider only those which relate to geography and ecology, since both of these are concerned with the distribution pattern of the group. In the first place it should be stated that the four wild species live in moist or even wet habitats in the country.

Drosophila montana and *D. novamexicana* are definitely separated geographically from *D. texana* and *D. americana*. Both of these species have been collected at relatively high altitudes in the Rocky Mountain Ranges, but *montana* has been taken mainly in the montane forests and lives in regions characterized by short summers and severe winters. It must therefore have a short breeding season. In contrast to this, the single specimen of *novamexicana* came from the upper sonoran type of forest, surrounded by xerophytic areas. The climate is considerably milder, thus permitting a longer breeding season for this species. So far as known at present, the distribution areas of these two species do not overlap, but future collecting may very well result in extending these areas. This is especially true for the range of *montana* which probably extends in a northwestery direction into Idaho and Montana, and perhaps even into Canada.

Drosophila texana occurs in the southeastern section of the United States, and, as stated above, its distribution range extends from central Texas to Florida. It not only includes all of the states bordering on the Gulf of Mexico, but also Oklahoma, Arkansas, Tennessee and Georgia, and it probably extends east into North and South Carolina. This region has a rather heavy rainfall and is traversed by many rivers and streams which are lined with forests and other types of natural vegetation. It is further characterized by a fairly low altitude and has a mild to warm climate throughout most of the year. It therefore furnishes excellent breeding sites for forms like *texana* and provides for a prolonged breeding season. The main distribution of *D. americana* lies to the north of this region. It has cooler summers with longer winters which would consequently shorten the breeding season for this species.

In the next article it will be shown that *americana* arose by hybridization from *texana* and *novamexicana*. The exact place where the original hybridization occurred is not known, but apparently it was at some point here in the Southwest. After its origin the new hybrid species moved northeast into a new environment in Ohio. On the basis of this interpretation, the few scattered specimens collected in central Texas, Arkansas and Tennessee represent the descendants of the remnants left along the trail over which it moved in its northward expansion. The evidence supporting this suggstion was brought to light by a study of the salivary chromosomes, which were found to be different in gene order from one another among the different species involved. The nature of the inversions in these chromosomes makes it clear that *americana* is not a homogeneous species, because its population still contains several of the combinations of chromosomes of the two parent species. Since recombinations would occur in the descendants of their hybrids, it is not surprising to find that different strains of *americana* occurring along this trail and in Ohio show a limited number of these combinations.

Drosophila virilis is isolated from the four wild forms, although its main distribution area overlaps that of *texana*. The type of isolation is ecological and is due to differences in habitats. The fact that nearly all of the captured specimens of *D. virilis* have been taken in stores and commission houses where wild forms have never been found, makes it certain that the two types of the group are effectively isolated. This conclusion is strengthened by the observation that five of the six individuals of *virilis* taken in the country were captured at places like roadside parks which are frequented by picnic parties. Such parties usually carry out bananas or other kinds of fruit from stores in which *D virilis* may be present. It is therefore not improbable that this is the source of the few individuals which are found out in the country.

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TABLE 1

Populations of Species of Drosophila in fruit stores and commission houses

Species / Cities	virilis	mel. +sim.	hydei	repleta	buskii	immigrans	funebris	mulleri	other species
Austin, Texas (C)	84	814	62	72					0
Beaumont, Texas (C)	2	669	177	151	3				15
Blanco, Texas (S)	2	1,312	835	1				126	57
Dallas, Texas (C)	1	1,606	1,117	157	88	7	1	1	3
Fort Worth, Texas (C)	5	10,792	1,132	66	39		2		9
Galveston, Texas (C)	17	5,507	767	219	1			2	112
Henly, Texas (S)	3	2,768	829					215	13
Houston, Texas (C)	8	1,963	1,248	575			1		3
San Antonio, Texas (C)	35	12,652	2,417	611	24		9	401	395
Victoria, Texas (S)	7	788	139		1			199	6
Baton Rouge, La. (S)	1	2,039	183		1	12			34
New Orleans, La. (S,C)	55	2,475	236	1	20	1	1		17
Memphis, Tenn. (C)	2	201*	18	1	2		1		1
Brooksville, Fla. (S)	5	102	23	6					2
Tampa, Fla. (C)	5	360	510	37	25				5
S. Barbara, Calif. (S)	14	2,330	310	48	73	242			310
Totals	246	46,378	10,003	1,945	322	262	15	944	982
Amarillo, Texas (C)		5,658*	251	4	114	181	4	3	16
Knoxville, Tenn. (C)		422*	205	7	1,682	3	1		1
Dayton, Ohio (C)		838*	20	1	73	4	58		0
Cincinnati, O. (C)		99*	40	10			1		2
Owensboro, Ky. (C)		698*	28	17	24	1	10		0
Paducah, Ky. (C)		482*	45		23		20		0
Springfield, Mo. (C)		775*	62	16	19		17		6
McAlester, Okla. (C)		154	173	165	36		1		1
Pueblo, Colo. (C)		3,051*	160		45	1,251	1,164		8
Col. Springs (C)		414*	26		7	13	23		8
Denver, Colo. (C)		1,525	201	1	175	59	50		16
Cheyenne, Wyo. (C)		165*	39		20		15		2
Ogden, Utah (C)	1	3,699	154		63	9	73		5
S. Lake City, Utah (C)		229*	2		19	6	22		0
Albuquerque, N.M. (C)		854*	528	17	408	17	18		6
Totals	1	19,063	1,898	238	2,708	1,544	1,477	3	71

*These collections contained *D. melanogaster* only.

TABLE 2

Collection Records: americana, texana, montana, novamexicana

Species	Place	Nearest town	State	Date	Number
D. americana	Aldrich place	Austin	Texas	2/16/40	1
"	San Gabriel Park	Georgetown	"	8/9/40	1
"	Miama River	Piqua	Ohio	7/1/41	2
D. texana	San Gabriel Park	Georgetown	Texas	9/13/38	1
"	In woods	Johnson City	"	9/30/38	1
"	In woods	Fort Worth	"	4/4/39	1
"	Tomato dump	Jacksonville	"	6/11/39	1
"	Roadside Park	Salado	"	8/9/40	1
"	San Gabriel Park	Georgetown	"	8/9/40	2
"	Roadside Park	Round Rock	"	8/9/40	1
"	Roadside Park	Palestine	"	8/12/40	3
"	Swamp	Bon Wier	"	8/14/40	2
"	City Park	Belton	"	8/23/40	1
"	Roadside Park	Devers	"	10/25/40	1
"	Wichita Mountains	Lawton	Oklahoma	4/18/41	1
"	Lake Shore	Lake Charles	Louisiana	6/7/41	1
"	River Bottom	New Orleans	"	6/12/41	9
"	Lake Cross	Shreveport	"	9/5/41	4
"	In woods	Marianna	Florida	6/17/41	2
"	Tsala Apopka Lake	Floral City	"	6/19/41	1
"	Lake McKethan	Brooksville	"	6/20/41	10
"	River swamp	Palatka	"	6/29/41	1
"	Okefenokee Swamp	Ft. Mudge	Georgia	6/30/41	9
"	Walker Lake	Schlatterville	"	6/30/41	5
"	Leroy Percy Park	Hollandale	Mississippi	9/6/41	11
"	Tombigbee River	Columbus	"	9/7/41	50
"	DeSoto Park	Ft. Payne	Alabama	9/8/41	9
"	Hiwassee River	Reliance	Tennessee	9/9/41	20
"	G. S. Nat. Park	Gatlinburg	"	9/11/41	43
"	Cumberland Park	Crossville	"	9/14/41	1
"	Tennessee River	Eva	"	9/15/41	25*
"	Mississippi River	Memphis	"	9/16/41	5
"	Arkansas River	Morrilton	Arkansas	9/17/41	59*
D. montana	Little Thompson R.	Estes Park	Colorado	7/17/41	2
"	Grand Teton Park	Jackson	Wyoming	7/22/41	172
"	Iron Creek	Yellowstone	"	7/25/41	72
"	Madison River	Yellowstone	"	7/25/41	12
"	Ogden River	Ogden	Utah	7/28/41	1
"	Cottonwood Canyon	Salt Lake City	"	7/30/41	8
"	Puffer Lake	Junction	"	7/31/41	2
"	Virgin River	Zion Nat. Park	"	8/1/41	1
"	Gila Nat. Forest	Glenwood	New Mexico	10/20/41	1
D. novamexicana	Gila River	Silver City	New Mexico	11/9/40	1
				Total	557

*A few *D. americana* were present in these collections.

X. GENETIC AND CYTOLOGICAL ANALYSIS OF THE VIRILIS SPECIES GROUP

J. T. PATTERSON, WILSON S. STONE and A. B. GRIFFEN

This paper extends the analysis of the virilis group with the material obtained since 1940. In the paper published that year (Patterson, Stone and Griffen, 1940), we reviewed the findings of Kikkawa and Chino on *Drosophila virilis*, of Spencer, Stalker and Hughes on *virilis* and *Drosophila americana*, and our own work on *virilis*, *americana* and *Drosophila texana*. Certain further information has been presented by Patterson in several published papers (1941, 1942a, 1942b) and in Article VIII of this bulletin. The analyses have centered on the extent and diversity of gene and chromosome differences present in wild populations from various regions. The genetic and cytological relations between the several members of this group, including two new species, have been investigated.

MATERIALS AND METHODS

It is necessary to discuss briefly the composition of the virilis group as it is now known. On the basis of morphological, genetical and cytological relations, Patterson has subdivided these species into two major divisions, the *domestic* form (formerly called *gray*), and the *wild* form (formerly called *red*), and these are subdivided as follows:

(1) *Drosophila virilis* Sturtevant (*domestic* form, dusky amber to black pupae). This includes all tested strains of the *domestic* form both Asiatic and American. Despite the fact that these strains proved to be of different genetic architecture, when tested to the several members of the wild group, they showed no isolation *inter se*.

(2) *Drosophila texana* fully described by Patterson and Wheeler (1942) (*wild* form, red pupae). The genetic and cytological conditions of this species were described in 1940. Further information is included in this paper.

(3) *Drosophila novamexicana* fully described by Patterson and Wheeler (1942) (*wild* form, red pupae). This species is known from a single male caught near Silver City, New Mexico. He was tested to *virilis*, *texana* and *americana*. Numerous offspring were produced in the cross to *americana* females, but very few in crosses to *texana* and *virilis*. The cytological relations were determined in the F_1 and F_2 hybrid offspring. The hybrid stock of the cross to *americana* has been tested further.

(4) *Drosophila americana* Spencer (*wild* form, red pupae). This was classed as a subspecies of *virilis* by Spencer. The evidence that *americana* is a derivative of hybridization between *texana* and *novamexicana* is presented in this paper. For this and other reasons Patterson classifies it as a separate species, not a subspecies of *virilis*.

(5) *Drosophila montana* Patterson and Wheeler (1942) (*wild* form, red to black pupae). This is a new species collected in the Rocky Mountains during the summer of 1941.

Dr. M. Chino was kind enough to send us a number of strains of the *domestic virilis* from Japan and the adjacent mainland of Asia. We wish to express our appreciation for this material. We are indebted to Dr. W. P. Spencer who kindly sent us several different stocks of *americana* from Ohio, and to Mr. W. K. Baker who sent us a stock from Indiana.

Patterson (1941) has described the origin of the stocks of the virilis group for the southwestern area, and in the preceding article the source of all other stocks is given. Table 1 gives the pertinent information concerning the origin and chromosomal configurations of all stocks used in the experiments. Genetic tests were made to determine the fertility and fecundity of the several stocks, their combinations, and their hybrids. As it was impractical to test all combinations, the two *domestic* stocks, *virilis* and *Henly*, and the two *wild* stocks, *americana* and *texana* were used as standards (see Table 1 for origin). The other tables show the results of the genetic tests; in all tests pair matings were used. Results are given as per cent of pairs fertile and average number of offspring per tube. In the fertility tests one hundred pairs were tested unless otherwise stated. Usually twenty tubes or more were averaged for fecundity (number of offspring).

The chromosomes were checked in the larval salivary glands and brains. In some cases egg counts were made, and fresh eggs from the same fertile females were checked for the presence of sperm. This technique is as follows: The eggs laid during a 2–6 hour period are collected and checked at once. A single egg is placed on a slide and covered with a coverslip. A drop of water is then placed at the edge of the coverslip, and as it is pulled down, the contents of the egg are forced out through a break in the chorion, usually at the micropile region. If the procedure is carefully effected, the contents will flow out into a space sufficiently small to be examined readily under the microscope. The coiled sperm are often recognizable under the low power, and always under the high power of the microscope. In employing this technique only satisfactory smears were counted, as the sperm might have been lost in the few cases where the liquid contents of the egg were washed away, although such preparations were carefully checked to make sure that this selection did not distort the results. In additional cases, egg-hatch counts (in adults) were made.

Several populations of the *domestic* form were checked for visible and lethal mutations by means of the egg-hatch counts. This egg count method better insures the detection of visible mutations as well as lethals.

Certain stocks and hybrid crosses were checked by daily transfer over the period of a generation to determine fecundity as measured on the basis of viable offspring. All experiments were run on the same food in a constant temparture room (22–24C).

Results

The hybrid origin of americana

The cytological configurations, both inversions and fusions, which provide evidence for the hybrid origin of *americana* from *texana* and *novamexicana* are illustrated in Figure 1 and Table 1. The stocks of *virilis*, *texana* and *novamexicana* differ widely in their gene arrangement. If we prefer to postulate the fewest possible changes, the *wild* forms, *texana* and *novamexicana*, must have descended from the same ancestral form. This form differed cytologically from *virilis* only in the two inversions common to the X chromosome of both *texana* and *novamexicana*.

The gene order of the original strain of *americana* is as follows: the X chromosome is similar to that of *novamexicana;* the chromosome 4, which

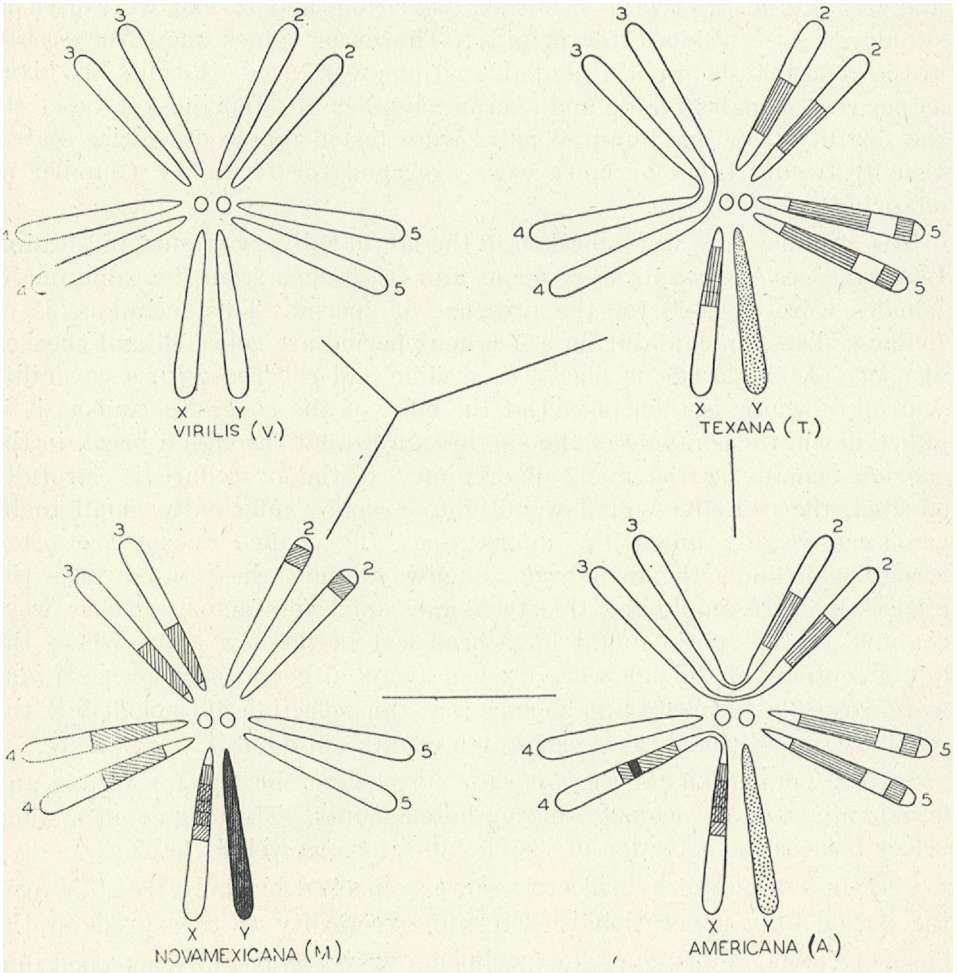

FIGURE 1

is fused with the X, is like that of *novamexicana,* except that it has an additional included inversion which is found in no other stocks; the free male-limited 4 is like *texana;* chromosomes 2, 3, and 5 are similar to those in *texana.* Chromosome 5 varies in gene order in both of these species. Fujii (1940) has reported the single variation found in chromosome 6 of *virilis* strains, but this seems to have no bearing on the determination of the relationship here. *Drosophila novamexicana* has no fusions, *texana* has one (3–4) and *americana* has two (2–3; X–4). It should be noted that each fusion in *americana* may have been derived by single reciprocal exchanges from the 3–4 fusion of *texana.* Considered in this way, neither fusion of *americana* would have necessitated a new fusion of unattached chromosomes. It seems most probable that the two fusions of *americana* were derived separately from the *texana* fusion, and then combined in the stock which gave rise to *americana.* We cannot say that any one of these chromosomes was derived in its entirety from a single stock; only the regions differentiated by the rearrangements can be so designated, and even here the high chiasma frequency in *virilis* might cause some error. The cross-fertility tests showed both *novamexicana* and *texana* to be readily cross-fertile with *americana,* although poorly fertile with each other. This substantiates the theory of the hybrid origin of *americana.* Finally, all hybrid males with the *novamexicana* Y chromosome derived from a cross of *virilis* females to either the *novamexicana* male or the *americana-novamexicana* hybrids were sterile even when the autosomes came from *americana.* No other F_1 *virilis* hybrid males were consistently sterile. It is probable that the Y chromosome of *americana* came from *texana* rather than from *novamexicana,* as the F_1 hybrid males of the crosses of both *americana* and *texana* to *virilis* are fertile. If the Y chromosome of *americana* is the *texana* Y, this would explain why F_2 males from the crosses *V* x *VT* and *V* x *VA* must retain chromosomes 2 and 5 from the *wild* form parent to be fertile. All three chromosomes, Y, 2, and 5 originally came from *texana* according to this hypothesis, and are, therefore, concerned in the previously described fertility determining Y-autosome interrelation (Patterson, Stone, and Griffen, 1940).

Table 1 shows that the several stocks of *americana* do not all have the same gene arrangement; each, however, represents one of the possible combinations which could segregate from a *nova-mexicana-texana* cross. These furnish further evidence that *americana* was derived from the heterozygote between *texana* and *novamexicana.*

Relationship in local populations

Patterson (1941, this bulletin) has described the origin of the local populations of the virilis group. Members from several different populations of the *domestic* form were checked for lethal and visible mutations. This was done with an egg-hatch count from a number of pairs of F_1 offspring from flies taken in their natural habitat, either fertilized females,

unfertilized females crossed to *virilis* males, or males crossed to *virilis* females. The results are given in Table 2. Some of the stocks had lethal or visible mutations present, while others had both types. The presence of the same visible mutation in several stocks from the same collection indicated their close relationship. This also suggests that many of the lethals from the same locality were the same allele, although this was not tested directly, except that one semi-lethal with visible effect appeared in progeny of several individuals. The members of the *domestic* strains have usually been collected in fruit stores or produce houses in the southwestern area. This evidence shows that members of a local colony are very closely related genetically.

Intraspecific variation

There are some genetic differences between strains of the *domestic* species. The following fertility values in per cent were obtained on crossing and inbreeding certain stocks. The F_1 inbred and the F_{15} of these crosses are given for comparison below:

	F_1	F_{15}
Beaumont x H	= 98	94
Blanco x H	= 98	96
V x Blanco	= 100	90
Blanco x V	= 100	97
V x Otaru	= 100	77
Otaru x H	= 90	81
H x Hanchow	= 100	31

These data may be compared with results given in Table 3. Both show a consistent drop from the high F_1 values on inbreeding. The drop was somewhat more pronounced in the crosses between the American and Asiatic strains. This suggested the existence of greater difference in genetic architecture between strains of widely separate geographical origin. Other evidence of the genetic differences between *domestic* strains may be inferred from the data on crosses to the *wild* strains.

Most of the *domestic* strains have high control fertility values although this is less true for the Asiatic strains, notably Tokyo and Kumamoto. The reduction in fertility of the Kumamoto stock is due to the sterility of about half of the males. This sterility cannot be selected out of the stock. Tokyo is less fertile on inbreeding than the ordinary *domestic* stocks, but both P_1 and F_1 outcrosses to other *domestic* forms were highly fertile. Strains from the United States were highly fertile in all tests with other *domestic* stocks.

One disadvantage with the *wild* strains was the fact that their control fertility tests were variable and often low. The results obtained must be due to the genotype of the stock. As proof of this, the control values and cross-fertility tests of the new set of wild stocks crossed to *americana* were low and variable, but the values from the $F_1 \times F_1$ of these crosses were high and fairly uniform, Tables 4, 5, 6. Furthermore, calculations made by Dr. Crow show that the $F_1 \times F_1$ values of the *wild* strains are

statistically different from the control or P_1 values. They, therefore, differed in their ability to cross with each other as well as to the domestic forms due to differences in their genotype.

Interspecific sexual isolation

Sexual isolation exists between all five species to a greater or lesser degree. Many types of crosses showed that the genetic basis of this mechanism is quite different in the several stocks. Isolation in reciprocal matings was often of quite different effectiveness. Therefore, different genes, at least in part, control these reactions in the males and females. The initial (P_1) cross-fertility, measured in number of pairs fertile, was extremely variable, ranging from 0% to 90%, Tables 3, 4, and 7

Some crosses are more easily effected between *wild* males and *domestic* females, while for others the converse is true. *Henly* is the *domestic* strain with the most extreme and consistent isolation to all *americana* and *texana* strains tested; however, it crossed fairly readily to *montana* males. Among the Asiatic *domestic* strains only Hiroshima approached it in degree of isolation. The geographical origin of the stocks was not consistently correlated with the degree or direction (in reciprocal crosses) of the sexual isolation.

The genes that control isolation are not always equivalent alleles. This was shown by crossing two *domestic* strains, then mating the heterozygotes to *americana* and *texana*, Table 8. That the same sort of differences exist in the *wild* forms was demonstrated in the crosses of their heterozygotes to the *domestic* strains, Table 6. As an example of such differences in the *domestic* forms, strains were isolated from the inbred heterozygotes between *virilis* and *Henly* which had quite different cross-fertility relations than those of *virilis*, *Henly*, or their hybrids when tested to *americana* and *texana*.

As it is difficult to measure quantitatively the degree of sexual isolation between all the strains, we have measured it on the basis of the cross-fertility percentage. The accuracy of that value was tested in this way. Crosses were put up in pairs and left 25 days. At the end of this period the vials were examined for signs of fertility. The females from the pairs that had not produced offspring in the 25 days were dissected and their genetalia were checked for the presence of sperm. The results are given below.

Cross	Fertile	Sterile	
		sperm absent in ♀	sperm present in ♀
V x A	38	10	6
A x V	22	7	20
T x Shengking	19	10	10
A x Shengking	2	2	14
Shengking x A	9	20	10

The reliability of this measure of sexual isolation varies with the cross to some extent, as other factors, such as sperm mortality, enter. The per cent of cross-sterility is, therefore, a measure of the sum of the effectiveness of several isolating mechanisms.

Comparative fecundity

The relation between members of the virilis group and their hybrids is further expressed by their fecundity. In this instance, fecundity refers to the number of viable offspring produced by a pair of flies, rather than the number of eggs produced. It is possible to determine the effectiveness of isolation of parent stocks or of hybrids by such tests. Table 9 records the results of a few tests of this nature. Results are given beginning with the seventh day, as none of the pairs produced offspring until they were seven days old. In this experiment pairs of flies were mated the day they emerged. These pairs were transferred daily to fresh food vials and their progeny counted upon emergence. The data obtained provided a rough measurement of the number of progeny produced by each pair during one complete life cycle; that is, in the period following their own emergence until their offspring began to emerge.

In crosses between *americana* and *virilis* and their *AV* hybrids, there is a marked difference in fecundity. During a twenty-three day period (a span corresponding to the *virilis* life cycle, being a few days less than the *americana* life cycle) one pair of *virilis* produced 884 offspring, while a single offspring was derived from one pair mating of *(AV) (A)*. Although very considerable difference was noticed between pairs of the same cross as regards fecundity, yet a more decided difference was observable between the several crosses.

Another test of comparative fecundity was the hatch from ordinary pair matings of pure stocks and hybrids. In general the *wild-domestic* hybrids fall between their parent forms in fecundity both on inbreeding and backcrossing (Tables 4, 5, 7, and 10).

In some ways a more critical test was the use of egg-hatch counts from fertilized females for the several combinations. A count from pairs of the pure stock of *americana* gave 450 adults/952 eggs or 47.3% hatch. *Drosophila virilis* gave from 82% (Table 12) to 92% (Patterson, Stone and Griffen, 1940). Several backcrosses of their hybrids are listed below:

(1) AV x V = 203/454 = 44.7%
(2) VA x V = 304/594 = 51.2%
(3) AV x A = 64/436 = 14.7%
(4) VA x A = 87/544 = 16.0%
(5) V x AV = 150/425 = 35.3%
(6) A x AV = 139/269 = 51.7%

The absence of appreciable differences between the percentage of egg hatch of the reciprocal hybrids backcrossed to either parent type, ((1) and (2),

(3) and (4)) indicated that there was no effect of maternal inheritance on fecundity. (1) and (2) compared to (3) and (4), and to (5) and (6) showed the differences between *americana* and *virilis* in the backcrosses. The poor hatch in the control tests of the *americana* stock made it somewhat difficult to evaluate the hybrid hatch counts properly.

Numerous other variations in fecundity appear in the data. P_1 crosses between the two types have fewer offspring by far than the *domestic* forms and usually considerably less than the *wild* forms. The fecundity was variable, and a few crosses gave progeny nearly equal in number to the control values of some *wild* forms. The fecundity was variable, and a few crosses gave progeny nearly equal in number to the control values of some *wild* stocks. It was much more often the case that of the reciprocal crosses, the few *wild* females that were fertilized by *domestic* males gave a higher average number of progeny, even though sexual isolation was greater in this direction.

In F_1 x F_1 crosses the fertility was more often high. In these crosses fecundity, when measured as hatch from a pair in a vial, was almost always as high as that of the usual *wild* stock. F_1 hybrids when backcrossed to either parent type were most often fertile. In these cases the fertility values were as a rule as high or higher than those of the *wild* controls (Table 11). The same general relation held for fecundity in the backcrosses. In most instances backcrosses to the *wild* parent strain were both less fertile and less fecund than those to the other parent. Although a few backcrosses were exceptions, the initial cross, rather than crosses involving F_1 hybrids, showed lower fecundity.

In order to determine the cause, or causes, of the reduction of fecundity in the P_1 matings between the *wild* and *domestic* forms, the following crosses were made: *V* x *V*, *V* x *A*, *A* x *A*, and *A* x *V*. Pairs of ten day old flies were placed in fresh food vials without etherization, and watched until they mated. The duration of mating was recorded, and the pairs of flies separated immediately. Some of the eggs laid by these females at different intervals of time were checked for sperm. Others were counted, and kept to determine how many hatched. At the end of the test the females were dissected and their ventral receptacle and spermathecae were examined for sperm. Table 12 gives the data for all females that had sperm present in their genitalia.

The first point of interest is the mating time in the V x V crosses, in which all the females were fertilized, this varied from 2.2–9.0 minutes with an average of 3.6 minutes in copula for 23 pairs. In *A* x *A* the time varied from 2.0–4.7 minutes with an average of 3.1 for eight pairs. In *V* x *A* this time ranged from 2.5–4.8 minutes with 3.6 the average for 19 pairs. In the *A* x *V* matings tested, only one pair produced offspring (mating time 3.0 minutes). In the eggs checked from this cross, one out of six which were smeared had sperm; one egg out of six, which were counted on the day of mating, hatched; and only one egg out of twenty-two, which were counted on the next day, produced a progeny. No other

eggs were laid although sperm were present when the female was dissected on the tenth day. Other *A* x *V* crosses mated for 1.5, 3.0, 1.0, 0.5, 0.8 and 2.5 minutes respectively. No eggs from these females hatched, and no sperm were present when they were dissected at the end of ten days. These matings were, therefore, ineffective for some unknown reason.

The data from Table 12 indicates that sperm are affected by the environment in the spermathecae and ventral receptacle of the females. In homologous crosses sperm survived and effected fertilization of the eggs for a number of days after mating. If the smears were made within a sufficiently short time after the eggs were laid, the sperm were often still motile. In heterologous crosses sperm were not effective in fertilizing eggs except for a short period after copulation, even though inactive sperm were later found in the sperm receptacles of the females.

Relationship in the virilis group

The division into the *domestic* form, consisting of the strains of *virilis*, and into the *wild* forms, consisting of *americana, texana, novamexicana* and *montana* was made on ecological grounds, and has been considered fully elsewhere (Patterson, this bulletin). Comparatively little is known about *montana*. The gene order differs among the several strains and from all other members of the virilis group, according to the unpublished evidence of Miss Mary Warters, who is working on the extent of diversity in gene order of several forms. Chromosome 2 has a pericentric inversion which changes the number of long euchromatic arms in the salivary gland nuclei to six. This pericentric inversion proves the non-terminal position of the centromere for chromosome 2 of the virilis group (Stone, Griffen and Patterson, 1942). We do not know the gene orders of these stocks.

A few crosses with *montana* have been made, Table 13, but unfortunately *montana* does not breed well in pair matings. The female hybrids of crosses to *virilis, Henly* and *Shengking* are fertile when backcrossed, but no additional information is now available. In so far as these crosses may be considered evidence, *montana* seems more closely related to *virilis* and *Henly* than to *americana* and *texana*.

Even less is known about the genotype of *novamexicana*. It has a genic balance which differs from *americana* and *texana*. This is proven by the sterility of hybrid males from crosses between *virilis* and the hybrid *americana-novamexicana* stock. The Y chromosome must be involved in this sterility. The chromosome configuration and gene order of the *novamexicana* male was accurately determined, and has proven of much interest.

We have expressed the opinion that *americana* is a species established from hybridization between *texana* and *novamexicana*. We cannot hope to prove the actual events that occurred in the past history of these forms. We can only infer from the evidence that is now available.

There is some evidence from the fusions. *Drosophila texana* has chromosomes 3 and 4 fused. These same two chromosomes are involved in the

X–4 and 2–3 fusions of *americana*. It is, therefore probable that the fusions of the *americana* were derived from replacements of arms from the 3–4 fusion of *texana*. They might be independent as chromosome 4 has been shown cytologically in *virilis* to have a small heterochromatic arm across the centromere from the large euchromatic arm. The presence of the pericentric inversion in *montana* proves that chromosome 2 is also a J with a very short arm.

The gene arrangements afford further evidence concerning the relationship. Figure 1 illustrates the chromosome configurations of the four species. In comparison to *virilis,* two inversions in the X are shared by the three other species as nothing else differs consistently. The ancestral stock of all three species differed from *virilis* cytologically only in this way. The *texana* stocks from the southeast have an inversion in chromosome 2, two inversions in chromosome 5, and the fusion of 3 and 4. *Drosophila novamexicana* has a third inversion superimposed on the first two in the X, and different inversions in 2, 3, and 4. The *americana* strains are a composite of these two. The X chromosome is similar to *novamexicana.* In some stocks the 4 chromosome fused to the X is like that of *novamexicana;* in others it is like *texana* in gene order while the free 4 is like the *texana* 4 in all strains tested. The 2 and 3 chromosomes, which are fused in *americana,* are like those of *texana.* Chromosome 5 is a mixture, and is often heterozygous in single stocks; it may have both inversions, one inversion, or no inversions of *texana.* In Texas, where the western boundary of the distribution of *texana* occurs, stocks of this species also share with *americana* the diverse gene order found in chromosome 5. The presence of one inversion part of the time must be due to crossingover.

Genetic evidence is as follows. Both *texana* and *americana* have the same Y–2–5 complementary chromosome balance in crosses to the *domestic* forms. The Y chromosome of *novamexicana* is different since the male hybrids produced in the crossing of this species with the *domestic* form are sterile. Although *Drosophila texana* and *americana* crossed readily, as did *americana* and the one male *novamexicana, texana* and this male crossed very poorly. Several females were used in each test.

Certain data on the distribution of *texana* and *americana* are pertinent here. These two forms occupy the same area in central Texas, Arkansas, and Tennessee without losing their identity by too frequent crossing; therefore, sexual isolation must be sufficiently effective under the natural conditions of population size and cycle.

There are two possible assumptions for the origin of these three species. One is that the ancestral *virilis* species evolved as one species until it had acquired the several different gene orders which are present in *texana* and *novamexicana* and shared by *americana;* then it was separated into three groups which proceeded to diverge genetically. The alternative hypothesis is that the precursor *virilis* which had the two X chromosome

inversions and the red pupa color became separated. They evolved separately until they acquired at least all the differences shared in *americana,* and perhaps all the differences now present in the chromosomes. They subsequently hybridized, and *americana* was sorted out from the hybrid stock.

The fact that both *texana* and *novamexicana* cross readily to *americana,* but poorly to each other, although *americana* and *texana* occupy in part the same area, is somewhat more difficult to explain on the first hypothesis. Dr. Ernst Mayr, of the American Museum of Natural History, N.Y., has suggested the following explanation for the observed facts from analogy with other known cases in animals. The hypothetical original *wild* form (with the two X inversions) became separated by the last advancing ice sheet into two parts which finally retreated into Florida and Mexico where they evolved differences. After the ice age, the Florida strain—*texana*—expanded over the low wooded area of the southeast. The Mexican form—*novamexicana*—advanced north. These met and hybridized giving rise to *americana* in this region of central Texas which represents the juncture between the moist forest zone and the arid western zone. This is at least a plausible explanation for the facts known at present.

Drosophila americana spread from this region up the Mississippi valley to Ohio where it is extensively distributed, but did not penetrate the southeast territory occupied by *texana* to any great extent. It now occupies for the most part a different ecological niche from the other two species, a situation which is analogous to that of many hybrid plant forms, as reported by Clausen, Keck, and Hiesey (1940).

Isolation in the virilis group

Since Patterson (1942b) has reviewed the isolating factors known in *Drosophila,* this account will be somewhat restricted. Patterson (this bulletin) also has discussed ecological distribution in detail, and, therefore, it is unnecessary to comment on that phase of isolation further.

In addition to ecological isolation, the genetic mechanisms now known for this group are sexual isolation, gamete mortality, zygote inviability, and hybrid sterility.

There seems to be no isolation between members of the *domestic* species. All cross readily, producing quite Fertile F_1 which show hybrid vigor. However, the inbred heterozygotes (ten to fifteen generations) sometimes show reduced fertility. It may be concluded that there is enough difference in the organization of the genomes of several stocks so that not all recombinations between them are normal.

Several strains of *texana* and *americana* were tested. The results are given in Tables 4, 5, 14, and 15. Certain of these have been considered elsewhere in this bulletin (Stone, Heterosis, this bulletin). In Tables 14 and 15 the results of crosses between several *americana* strains are seen. In no case did the P_1 cross between them go as readily as the two controls.

The F_1 x F_1 were often more fertile and fecund than the P_1 crosses. As there seems to have been no reduction in cross-fecundity in *americana* or *texana* or their crosses, the low P_1 cross-fertility must have been the result of sexual isolation. Stalker (1942) has reported sexual isolation between strains of *americana* also. The F_1 from crosses between various *texana* strains, *americana* strains, or between *texana* and *americana* strains show the effect of heterosis for at least some crosses.

Sexual isolation and gamete mortality occur in crosses between *domestic* and *wild* forms while hybrid sterility and zygote mortality occur in the progeny of hybrids only.

These factors all sum to keep the several species from exchanging genes. If ecological isolation fails to keep these forms in separate places, sexual isolation reduces the incidence of mating. If this is insufficient, gamete (sperm) mortality reduces the number of egg-fertilizations in cross matings. Finally, the few hybrids have reduced fecundity and certain combinations are sterile.

The genes influencing sexual isolation in this group seem to be autosomal recessives in many cases, or at least they are not effective in isolating *wild-domestic* hybrids from either parent form, Tables 6, 11, 16. If any of the genes were in the X or the Y, hybrid males should show sexual isolation in some tests, and very seldom this seemed to be the case. These isolating factors, present in both *domestic* and *wild* forms, are not the same in different strains of the same species. In the first place the direction and extent of crossing varied markedly in the different combinations. Reciprocal crosses often gave very different cross-fertility values. When different stocks are compared, they may differ in degree or direction of greatest isolation. When two *wild* strains are crossed, the F_1 may cross much more readily to a *domestic* strain than either parent. For example, both *americana* and 841.10 were sterile to *virilis* males, but 24% of their heterozygotes were fertile to this type of male, see Table 6.

In this connection, Silow (1941) reports that *Gossypium anomalum* crossed much more readily to a *Gossypium barbadense-Gossypium hirsutum* hybrid than to either of the pure species. The same situation is encountered in tests of the *domestic* strains of *virilis*, Tables 4, 6, 7, 8. Sometimes a heterozygote between two *domestic* forms crossed more readily to *americana* or *texana;* other heterozygotes were intermediate; and still others crossed even less readily. Table 17 gives the averages of the crosses of the pure strains from Table 7, and their heterozygotes from Table 8, to *americana* and *texana*. Only crosses to the wild males averaged higher for the heterozygotes. Heterozygotes with *Henly* did not increase in fertility nearly as much as heterozygotes with *virilis*. This shows that the genes in *Henly* which are responsible for its extreme isolation from the *wild* species are at least semi-dominant. It also illustrates the difference in isolating factors that affect the male and female.

Tests for fecundity, to be an accurate measure of ability to produce offspring, must be run so that there is no factor of crowding, which reduces laying or decreases adult hatch. This is most easily accomplished by daily transfer of pairs to fresh food vials. This was done in a few instances, Table 9. In ordinary matings a pair was placed in a vial and left ten to twelve days. A comparison of Table 9 with other tables in which average hatch is given shows that this factor, average hatch, is not an absolute measure of fecundity. In fact, in some cases a smaller hatch might come from a stock which was not really less fecund. However, average hatch does measure vigor and, in part, fecundity of a stock. Ideal fecundity tests, i.e., egg-hatch counts for complete life cycles of a number of representative pairs from stocks or crosses, are too tedious and time-consuming to attempt for all these tests; therefore, analogy will be drawn from the few tests that are reported.

Results given in Table 12 prove that sperm from *virilis* males survive only a short period (24± hrs.) in *americana* females. The reciprocal cross showed the same effect although the reduction in effectiveness of the sperm was not so pronounced. Reference to the tables of crosses show that these relations are generally true. The average hatches were smaller when *texana* or *americana* males were crossed to the *domestic* type females, either stock or heterozygotes, than in the reciprocal crosses; see, for example, the summary, Table 17. The inadequate tests with *montana* cannot be interpreted to determine whether or not fecundity was reduced in these crosses. Tests between *americana* and *texana* stocks showed no sign of such an isolating mechanism. Reduced fecundity appeared in all crosses between members of these two species and all *domestic* strains.

This sperm mortality in crosses between different species is due to recessive genes, usually, if not always, autosomal. These genes, recessive in their ability to effect cross-sterility for both male and female hybrids, crossed back to both parent types with good hatches. There were a few matings with hybrids which may have been exceptions. Also, the egg-hatch suggests that hybrid females crossed back to the *wild* forms may still have reduced fecundity although the experiments are not sufficient to prove it. It is possible that part of the egg mortality is due to the gene combination of the sperm, but we have not found unequal ratios in the chromosome combinations in backcrosses.

All things considered, it seems most probable that the sperm mortality is restricted to initial crosses between species. As we know that the sperm is effective in P_1 crosses for less than twenty-four hours, we can infer that the production of offspring on several days in the daily transfer experiments, Table 9, proves that mating is repeated. The reaction which inactivates the non-homologous sperm must be due to conditions which are normal to the sperm receptors of the female and favorable to homologous sperm as replacement is readily achieved.

Only a few experiments were designed to distinguish between the effect of sexual isolation and sperm immobilization. For this reason most of the results for the P_1 crosses represent the sum of the effects of these two primary isolating mechanisms.

Some of the hybrids produced, as a result of the failure of sexual isolation and sperm immobilization to prevent effective crossing, are completely sterile. In a few inadequate tests, F_1 male hybrids from crosses to *montana* produced no offspring. The F_1 *virilis-novamexicana* hybrids with the Y from *novamexicana* were sterile. No other F_1 hybrids were consistently sterile, however. The F_1 hybrids were most often quite fertile, but certain complications effected the fertility of some F_2 males. Comparisons of Table 4 with 5, and Table 7 with 10 show that on inbreeding the F_1 hybrids of *domestic* and the two *wild* forms, *texana* and *americana*, were much more fertile and fecund than the P_1 crosses. Hybrids with *Henly* were few and may not follow this general rule in some cases. Usually the fertility and the fecundity of these $F_1 \times F_1$ hybrids equalled or exceeded that of their *wild* parents. Backcrosses of the F_1 hybrids also showed high fertility and fecundity. The daily transfer experiments, Table 9, showed that *AV* hybrids are about as fecund as their *americana* parents. Egg counts showed that the *americana* stock produced about 50% normal zygotes and both male and female *AV* hybrids produced only about 50% normal gametes. In the experiment, Table 9, *AV* x *AV* proved as vigorous a combination as *A* x *A*.

Table 16 summarizes the backcrosses of hybrids between *domestic* strains and both *texana* and *americana* of the *wild* species. Backcrosses to both parent types are included. These data prove the existence of genetic differences between the American and Asiatic strains of the *domestic* species. Higher fertility and fecundity values were obtained for crosses involving the American strains. This was true for each type of backcross with the exception of the *A* (*A* x American) which involved only two tests.

The F_2 crosses in Table 11 usually showed high fertility and fecundity. The fertility of hybrid males is dependent on the presence of complementary Y-autosome factors. The fertility of hybrid males in subsequent generations derived from crossing *virilis* females to *texana* or *americana* males is contingent on the presence of the Y, 2 and 5 chromosomes from the *wild* ancestor (Patterson, Stone, and Griffen, 1940). This was not only the case with different strains of the *domestic* form which were tested, but also with various strains of *texana* and *americana*. A similar complementary factor relationship exists between the Y, 2 and 5 chromosomes of the *domestic* species. Two strains of *texana* were utilized for tests, the original strain from Texas and one from Lake McKethan, Florida. These *wild* females were crossed to *virilis* or Blanco males. Generation after generation hybrid males were backcrossed to the same strain of *wild* females. When these males proved fertile, a cytological check in the salivary glands of their offspring proved that chromosomes 2 and 5 from

the *domestic* ancestor were invariably present. The *domestic* Y chromosome must be complemented by genes in chromosomes 2 and 5 not present in the *wild* strain.

Species relations and degrees of divergence

Dobzhansky (1941) and Patterson (1942b) have treated in some detail isolating mechanisms, those genetic or other conditions which reduce or prevent gene exchange between populations. Only a few additional comments will be made here. These mechanisms are cumulative in effect, but vary widely in the several species that have been studied. For example, *Drosophila pseudoobscura-miranda* hybrid females very seldom produced offspring because of the abnormalities of their eggs (Kaufmann, 1940). Even if offspring occurred, there would be little or no recombination of the genes within the chromosomes due to the extensive difference in their gene order (Dobzhansky and Tan, 1936). The hybrid sterility might be said to be superimposed on a cytological condition which would prevent any recombination except between whole chromosomes, even though it does not seem probable that this represents the historical sequence of events.

Nowhere among the *Drosophila* can chromosome differences, as such, be demonstrated to be an isolating factor, except in preventing gene recombination through crossingover. The primary factors all seem to be genic. Even in virilis forms the poor egg hatch of *americana* (47.3%) was as low as that of the hybrid combinations *VA* x *V* (51.2%) and *AV* x *V* (44.7%) so that it is not possible to ascertain that low egg hatch from the hybrids was due to chromosome difference, even with two fusions present heterozygous.

Sexual isolation or failure to mate is one of the primary isolating factors in *Drosophila*. Dobzhansky (1941) has correlated the degree of sexual isolation between two species with their distribution. Strains of *pseudoobscura* and *miranda* are less likely to cross if they come from the same or neighboring localities than if they are from widely separated places.

This is by no means the universal rule. In the macrospina group, Mainland (this bulletin) has shown that sexual isolation usually increased with distance between points of origin of the strains. In the melanica group (Griffen, this bulletin) and in the virilis group, the degree of sexual isolation between strains of two species seemed to vary in an unpredictable fashion. No single explanation for these diverse situations, except fixation of mutations which, perhaps incidentally, effect sexual isolation to other species, might be universally true. It is possible that in the rather small virilis populations mutations which effect sexual isolation, in some instances even within the species may be fixed by chance as suggested by the data for the *americana* strains.

Although we do not know the effective size or structure of the breeding populations of *virilis* in Asia, one of the stocks suggests some populations must be small. In the Kumamoto stock approximately half the males were

sterile. In crosses made with the fertile males, or with the females, the F_1 males were fertile. Therefore, the fertility does not seem to depend on genes in the sex chromosomes. This condition cannot be eliminated from the stock by selection. Egg counts showed it is not connected with a lethal. No fusions or any other cytological abnormalities were present. Fertility of the males seems to depend on heterosis of the type proposed by East (1936) due to difference in alleles, and similar to the female sex-determining mechanism demonstrated by Whiting (1935) in *Hymenoptera.*

The simplest assumption is that fertility of the males depends on the heterozygosity of two recessive autosomal genes, which are presumably alleles, as no recombination takes place between them. Either gene produces sterility in males, if present homozygous, but does not affect the females. This is similar in effect to the two *yellow* alleles, y^2 and y^{99b}, which, heterozygous, produce the normal gray body color in *melanogaster,* although either gene produces a yellow body if homozygous. The sperm of these sterile Kumamoto males are aberrant, resembling the earlier undifferentiated stages, and the testes never develop to the normal size.

Genes which cause sexual isolation between members of the virilis group are known to be present in *virilis, texana,* and *americana.* It is possible to say that certain of these isolating factors are recessive or dominant within a species. Some examples have been given. *Drosophila virilis* females were crossed to *Henly* males and the F_1 males were backcrossed each way. Females from the parent stocks, their F_1, and both backcrosses were tested to *americana* males with the following results in pairs fertile: $V \times A = 79\%$; $H \times A = 1\%$; $VH \times A = 85\%$; $(V \times VH) \times A = 88\%$; $(H \times VH) = 27\%$. In this case certain isolating factors in *Henly* must have been recessive to their alleles in *virilis.* However, it is not certain that these isolating factors are recessive in all crosses between species. They are recessive in effect, in that the hybrids are most often fertile to both parent species, but this might be due part of the time to the presence of dominant genes from both parent species in the hybrid which enables it to cross to each parent. The same argument might apply concerning the recessive nature of the factor or factors which cause immobilization of the sperm in crosses between *virilis* and *americana.*

Tables 3, 18, and 19 give us some more information about isolating factors—the genes which affected fertility and fecundity could not always be separated. Table 19 shows that inbred hybrid stocks did not often lose their ability to cross to their P_1's. On the other hand, Tables 3 and 18 show that the inbreeding of heterozygotes between two *domestic* forms did not often reduce their isolation to *americana* or *texana,* and, in several cases, isolation increased. The data suggest chance loss or accumulation of these factors in the stocks, although accumulation seems more frequent.

We cannot say that sexual isolation is effective to the same degree in laboratory tests as it is in nature. In fact the tests give the greatest opportunity to mate. Probably sexual isolation is much more effective in nature as indicated by the fact that species *pseudoobscura* A and B occur

together without the detection of hybrids, and *texana* and *americana* occur together in several areas without losing their identity.

The reduction in fecundity in crosses must be as effective in nature as in the laboratory. This, coupled with the tendency to polyandry of the females, must reduce the effectiveness of the few cross-matings that do occur.

Hybrid sterility or inviability in *Drosophila* is more often found to involve the heterogametic sex, the male. However, the gene in *Drosophila aldrichi* 2, found by Crow (this bulletin), caused the death of hybrid females. In this case the effect was restricted to the female hybrids by the sex linked nature of the gene so that we cannot know how it would affect hybrid males. Male sterility has by no means always been conditioned by X-autosome balance in the several cases studied. In *Drosophila micromelanica* it depends on an X-Y relation, Sturtevant and Novitski (1941), and in the macrospina group there is a Y-autosome relation. In *virilis* a Y-autosome relation makes *virilis-novamexicana* hybrid males sterile. Here fertility of a male with the *novamexicana* Y depends on a gene or genes in the autosomes which are recessive to their alleles in *virilis*. In crosses between *virilis* and *texana* or *americana*, the Y, 2 and 5 chromosomes from the same parent species must be present for the males to be fertile. Since the autosomes may be heterozygous, the genes involved in the complementary action must be dominant. Therefore, male sterility of hybrids does not appear in these crosses until F_2.

Muller (1942) is of the opinion that this form of complementary action is the result of translocation of the chromosomes of the species in the past. We prefer the hypothesis that it is due to transfer of function in the genome through gene drift (Wright, 1940). Certainly gene drift has changed the genome of the several members of the domestic group. In this respect they resemble to some degree the difference between the diploid species *Gossypium arboreum* and *herbaceum* which intercross freely and give vigorous F_1 hybrids, but subsequent generations of hybrids have such reduced vigor, due to disharmonic recombinations, that the two species can be grown in mixed plots without losing their identity (Silow, 1941).

It is not difficult to separate the virilis group into three distinct divisions. One is the *domestic* type, *virilis*, found in both America and Asia. The second is *montana*, which has achieved both major morphological and physiological differences from all the others. However, in the third group the relations between *novamexicana*, *texana*, and *americana* are much closer than those involving any other combination.

Spencer originally classed *americana* as a subspecies of *virilis*. The genetic and cytological relationships within this group, which have already been discussed, indicated that *virilis* is a distinct species from any of the wild forms. Patterson and Wheeler (1942) have described *novamexicana*, *texana*, and *montana*. We are of the opinion that each of the members of the virilis group so far described should be considered as separate species.

Sturtevant (1942) is of the contrary opinion and considers them all subspecies. His reasons and criteria for determining the status of a form are quoted below (from pages 32 and 33), italics ours.

> (a) *Distinct species must be separable on the basis of ordinary preserved material.* This is in order to make it possible for a museum man to apply a name to his material. The necessity for such a provision seems to be obvious, since only in this way can effective use be made of the whole technique of taxonomy.
>
> (b) *Cross fertility between distinct species is in general absent or so slight as to make unlikely any transfer of genes from one to the other in nature.* This criterion is difficult to apply, and seems to me of secondary value for that reason. Geneticists are likely to emphasize its importance, taxonomists to minimize it. It is clearly of first importance for evolutionary theory, but even in the best understood cases it is still difficult to judge how much actual transfer of genes occurs.
>
> (c) *Subspecies usually replace each other geographically, species may do so but are more likely to show extensively overlapping distribution areas.* This criterion is one that taxonomists usually emphasize. It is clearly helpful, but can never be decisive (unless made so by artificial definition). Our knowledge of distribution areas of Drosophila is still too imperfect in most cases to make possible a rigorous use of this principle. It should also be pointed out that this criterion alone is not adequate. Drosophila pseudoobscura is gradually replaced by athabasca as one travels northward in British Columbia, and by affinis as one travels eastward in central Texas. Both replacement zones are typical of those recorded for subspecies; but they concern wholly distinct types, that are very different morphologically, are certainly wholly cross-sterile, and that have geographical forms within themselves that show much less sharp replacement zones.

These criteria merit careful consideration so each one will be discussed briefly. Whatever may be the philosophical connotations, (a) implies that species have reality only if certain trained men, often using only part of the available evidence, i.e., morphology, can easily separate them from closely related forms. This attitude is certainly not justified by the knowledge now available about living forms, past and present. It should be noted that this is not applied to the affinis complex by Sturtevant himself. Certainly *montana* males or females differ from all other members of the virilis group more than any of the affinis complex differ from one another. Even *virilis* can be separated from the *wild* forms of its species group more easily than most of the affinis group can be separated. In fact Sturtevant and Dobzhansky (1936) state concerning the eight members of the affinis group they describe: "The eight forms concerned here are so similar in appearance that we have been unable to devise satisfactory methods of distinguishing pinned females." The males of several

of these species cannot be separated with certainty in all cases. In this same complex *pseudoobscura* A and B males or females cannot be separated, while *miranda* is not very different from either.

Point (b) is so qualified that it is difficult to apply as Sturtevant has indicated. Certainly cross-fertility is very different in various types of organisms. Numerous accepted plant species cross readily. The extent of gene transfer is a most important consideration. When it may be applied, it certainly should be taken into account.

Point (c) is again of variable worth. Sturtevant himself cites examples where species replace each other in geographic regions. This would seem to be a question of habitat selection (c.f., Miller, 1942) and competition rather than a test of relationship.

In the virilis group, *texana* and *virilis* occupy the same general area in the south, while *montana* is widely separated from them. Nevertheless, *montana* seems as far removed genetically from the other two as they are from each other. We accept, as the most satisfactory available, the definition of a species given by Wright (1940), p. 162, as follows: "The ideal has been to apply the specific name to groups within which all subdivisions interbreed sufficiently freely to form intergrading populations wherever they come in contact, but between which there is so little interbreeding that such populations are not found." We differ with Sturtevant on what may be considered sufficient and critical evidence to determine whether a form is close enough to that ideal to be called a species.

Certain other phases concerning gene exchange between populations should be commented upon. Stebbins (1940) has pointed out that hybridization followed by polyploidy, which is common in plants, has the effect of breaking down isolating mechanisms and bringing two genomes together. This raises an interesting question concerning the evolutionary advantages of strict autopolyploidy, strict amphidiploidy with only bivalents formed, and an intermediate condition. Strict autopolyploidy affords only the advantages that might accrue from this change in genic balance which follows change in absolute, but not relative, gene frequency. Strict amphidiploidy can only give a type of prolonged hybrid vigor without recombination within the diverse duplications of the genome.

An intermediate condition, where recombination could occur by occasional crossingover between the not completely homologous chromosomes, would seem to be a most favorable condition, provided this recombination were not so frequent that it would seriously reduce the reproductive efficiency of the form. This would seem to be a particular type of gene combination and recombination between species, differing, in degree, from the combination that produced *americana*. Muntzing (1936), has reviewed at length the situation in polyploids, and has pointed out that the general situation falls between the strict auto- and allopolyploid. This intermediate condition certainly suggests that a sampling between the possible combinations of the heterogeneous genotype has been an important factor in the development of natural polyploids.

Polyploidy, because of the cushioning effect of duplicate genes, allows greater genetic variability than diploidy. This variability does not necessarily lead to greater flexibility than is possible in a diploid. In fact, only the recombination by crossingover and the possible rapid divergence in function through mutation of the duplicate genes allow the sexually reproducing polyploid to achieve some of the flexibility characteristic of the sexually reproducing diploid.

Anderson (1939) has discussed character recombination in species crosses. He pointed out that functional recombinations more often resemble most closely one or the other parent type or their F_1. Drosophila americana is a combination between *texana* and *novamexicana*, whether or not these last two were differentiated species at the time of their hybridization. In this case, *americana* resembles *texana* most closely, both in phenotype and chromosome structure. Also it inhabits the moist forest lands, as does *texana*. However, its center of distribution is Ohio which is much colder and has, as a consequence, a more restricted breeding season than *texana*, which is centered in the deep south. Both have rather extensive distributions, but these overlap only along the edge of their distribution. They, therefore, occupy different habitats, replacing each other in space. *Drosophila americana* has achieved a certain amount of physiological dissimilarity from *texana* in tolerance to environment.

Following our suggestion of this mechanism (Patterson, Stone, Griffen, 1940), Muller (1942) has developed at some length the beneficial effect of a restricted exchange of genes between species when isolating mechanisms are not completely effective. This process must be of some importance in nature, for many different kinds and combinations of isolating mechanisms have been found which are very often not completely effective in preventing gene exchange between species. Of course, this is a special type of grouping of peak combinations of genes (Wright, 1940). Here the valley between peaks is made more difficult to cross by isolating factors which prevent the descendant of a group at one peak from moving over to the other. Here the crossing is similar to migration between semi-isolated populations of the same species. Wright points out that migration may be treated similarly to mutation pressure. In the case under discussion this would amount to a burst of mutations which may be considered to be more probably useful than random mutation because they have already been selected to active participation in one genome. The amount of crossing that is most useful between two species is restricted by the amount of reproductive wastage that they can tolerate to gain variability.

The cyclic nature of the variations in the size of populations may have some effect in such cases. Wright has pointed out that in these instances the effective breeding size of the populations is more nearly characterized by the minimum number of breeding individuals representing any generation in the cycle with the interval between generations equivalent to the length of the cycle. The *Drosophila* show many examples of this type of variation. Patterson (unpublished) has numerous data on the number

of flies of the several species per collection throughout the year at a particular locality. For example, the mixed *melanogaster-simulans* population varied from an average of one fly in twelve collections in February to 1098 per collection during its peak in September; *hydei* went from one in twelve in February to 469 per collection in the peak month, April; and *meridiana* had a peak of some 32 flies per collection in October, although no flies of this species had been caught in March, April, May, June, and July of that year. Other forms showed the same general relations.

The reproductive potential of the peak population is, therefore, impossible of realization. At this season of largest population size there is the greatest chance of contact and crossing between species. Therefore, even if this does reduce the reproductive efficiency of the hybrids, it does not particularly reduce that of the population. The valuable recombinations that may be formed have some chance of passing through some of the few individuals that carry over for the next generation.

These population cycles, besides increasing the flow of migrants between peak populations, must have another effect. The growth and large numbers of the population that occur through the cycle must allow many mutations to occur. The reduction of population numbers must be accompanied by an increase in intensity of selection and would tend to favor mutations both dominant and recessive (as the population size was reduced) which increased vigor as well as eliminating the deleterious mutations. This mechanism would seem to simulate a selective increase of beneficial mutations for the benefit of the population. The presence of different harmonious combinations of genes in the several populations of the domestic form, and the presence of heterosis in *hydei* and the virilis group (Stone, Heterosis, this bulletin) shows that different, dominant, beneficial genes are very often present in local populations even though it does not prove that population cycles contributed to their frequency.

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TABLE 1

Origin and Chromosome Configuration of Stocks

Form	Origin	Number	Designation	Chromosomes					
				X	4	3	2	5	6
Domestic	New York	------	*virilis* = V	V	V	V	V	V	V
"	Texas	86.4	*Henly* = H	V	V	V	V	V	V
"	"	290.3	Blanco	V	V	V	V	V	V
"	"	718.7a	Victoria	V	V	V	V	V	V
"	"	725.9f	San Antonio	V	V	V	V	V	V
"	"	754.9a	Beaumont	V	V	V	V	V	V
"	"	472.4	Galveston	V	V	V	V	V	V
"	"	494.5a	Galveston	V	V	V	V	V	V
"	California	863.b	Santa Barbara	V	V	V	V	V	V
"	"	863e	Santa Barbara	V	V	V	V	V	V
"	Japan	------	Tokyo	V	V	V	V	V	V
"	"	------	Kumamoto	V	V	V	V	V	V
"	"	------	Otaru	V	V	V	V	V	V
"	"		Hiroshima	V	V	V	V	V	V
"	"		Sendai	V	V	V	V	V	V
"	Manchuria		Mukden	V	V	V	V	V	V
"	"		Kirin	V	V	V	V	V	V
"	"		Shenking	V	V	V	V	V	V
"	China		Peking	V	V	V	V	V	V
"	"		Hanchow	V	V	V	V	V	V

Wild									
"	Texas	84.7	*Texana* = *T*	T	V	V	T	V or T	V
"	"	812.12b	Georgetown	T	V	V	T	T	V
"	"	812.12c	Georgetown	T	V	V	T	V	V
"	"	822.14 ♀ x 821.12c ♂	Roundrock	T	V	V	T	T	V
"	"	825.13b ♀ x T ♂	Palestine	T	V	V	T	(V or T)?	V
"	"	825.13c	Palestine	T	V	V	T	T	V
"	"	841.10	Bon Wier	T	V	V	T	(V or T)?	V
"	"	849.11	Belton	T	V	V	T	(V or T)?	V
"	Florida	1148.9	Lake McKethan	T	V	V	T	T	V
"	New Mexico	968.7	*Novamexicana* =N (Silver City)	T + N	N	N	N	V	V
"	Ohio		*americana* = *A* (Smithville)	T + N	N ± A or V	V	T	(T or V)?	V
"	Texas	821.12a ♀ x A ♂	Georgetown	T + N	N + A	V	T	(T or V)?	V
"	Texas	552.5 ♀ x T ♂	Aldrich (Austin)	T + N	N + A	V	T	(T or V)?	V
"	Ohio		Oxford ♂	T + N	V	V	T	T	V
"	Ohio		Cedar Swamp ♂	T + N	V	V	T	T	V
"	Ohio		Overton	T + N	V	V	T	T or V	V
"	Ohio		Piqua ♂	T + N	N + A	V	T	T	V
"	Ohio		Killbuck	T + N	N + A	V	T	T	V
"	Ohio		Pee Wee	T + N	N + A or V	V	T	T or V	V
"	Indiana		Anderson	T + N	V	V	T	T	V

TABLE 2

Visible and lethal mutations in domestic populations

Stocks	Tests for lethals; count of adults/eggs	Visibles
722.4 i ♀ Austin	32/33, 34/42, 19/26, 69/87, 60/68=214/256 =84%	small bristles; abnormal abdomen; crossveinless
772.4 l ♀ Austin	43/73=60%=lethal. 34/45, 40/45, 52/58, 61/61, 22/25, 71/83= 280/317=88%	small bristles
777.4 f ♂ x V ♀ Austin	63/69, 69/75, 88/102, 91/101, 77/84, 54/64= 442/495=90% 37/84=44%=lethal.	round wing; abnormal venation; small bristles
754.9 a ♀ Beaumont	23/23, 40/40, 83/87, 56/60=202/210=96%	abnormal wing; eyeless
754.9 b ♂ x V ♀ Beaumont	96/109, 101/103, 119/123, 85/102, 104/108, 51/62, 79/80=645/687=94% 65/101=64%=lethal.	eyeless; small bristles
739.8 ♀ x V ♂ Dallas	102/109, 62/62, 55/55, 94/95, 57/57=370/387 =98%	
713.11 ♀ Ft. Worth	22/23, 46/58, 38/46=106/127=83%	rough eye; abnormal pupae
738.9 c ♂ x V ♀ Ft. Worth	43/62, 42/74, 19/32, 31/67=135/235=lethal 45/56, 72/80, 57/57, 97/103, 50/58, 50/55= 371/409=91%	clipped wing; abnormal abdomen
738.9 d ♂ x V ♀ Ft. Worth	64/67, 36/42, 125/130, 119/123=344/362 =95%	
730.8 b ♂ x V ♀ Houston	25/28, 35/35, 68/69, 58/74, 63/70, 34/35= 283/311=91%	hairless abdomen
730.8 d ♂ x V ♀ Houston	75/90, 113/131, 65/75, 88/110, 92/105, 91/108, 96/135=810/973=83%	small bristles; dark; pointed wing with broken crossvein; abnormal ocelli
730.8 f ♂ Houston	96/135=71=lethal (possibly) 96/105, 65/79, 72/84, 87/91, 18/20, 74/77, 60/63=472/519=91%	small bristles; clipped wings
725.9 h ♂ x V ♀ San Antonio	53/53, 100/105, 98/107, 109/120, 45/47, 30/30, 52/52, 62/63, = 549/577=95% 44/60=73%=lethal (possibly)	small bristles; sex-linked light color
725.9 k ♂ x V ♀ San Antonio	46/48, 65/69, 80/89, 58/61, 40/46, 60/64, 45/50=394/427=92%	extra wing veins; small bristles
725.9 l ♂ x V ♀ San Antonio	81/86, 67/70, 64/65, 87/102, 61/66, 56/61= 416/450=90% 40/55, 50/63, = 90/118=76%=semilethal	abnormal legs and body; rough eyes; 5th vein broken; many died in pupa cases
725.9 m ♂ x V ♀ San Antonio	95/98, 123/138, 77/81, 86/90, 59/65, 109/118, 113/119, 81/82=743/791=94%	bubble wing; small bristles; sex-linked light color; same semilethal as 725.9 l.
725.9 o ♂ x V ♀ San Antonio	88/98, 74/76, 76/80, 71/84, 30/32, 13/15, 47/54, 49/50=448/489=92% 72/108=67%=lethal	rough eyes; clipped wings
725.9 p ♂ x V ♀ San Antonio	45/50, 28/30, 83/94, 65/66, 79/81, 29/32, = 329/353=93% 63/87=72%=lethal.	small eyes
725.9 q ♂ x V ♀ San Antonio	15/16, 25/28, 92/99, 50/52, 91/105, 78/78, 17/19, 33/34, 33/34=434/465=93% 33/47=70%=lethal.	small eye; extra bristles
725.9 r ♂ x V ♀ San Antonio	16/17, 115/120, 51/55, 98/106, 72/76, 92/94, 101/114, 84/96, 98/111=727/789=92% 63/97=65%=lethal.	small eye; small bristles; crossveinless; clipped wings
785.6 b ♀ San Antonio	51/69=73% 17/43, 48/91=65/134=lethal.	small wing; small bristles; abnormal abdomen

TABLE 3

Cross-sterility factors; crosses with F_1 and F_{10} hybrids

Cross	Initial		americana				texana			
			female		male		female		male	
	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}
H x J*	100%	78%	3%	Sterile	20%	26%	38%	8%	12%	9%
J x N*	100%	87%	Sterile	2%	35%	2%	33%	2%	45%	Sterile
C* x N	98%	88%	Sterile	30%	17%	Sterile	no test	70%	Sterile	6%
V x C	100%	98%	7%	18%	83%	36%	49%	34%	94%	50%
H x C	98%	93%	no test	Sterile	no test	Sterile	no test	4%	no test	Sterile
V x J	100%	65%	15%	Sterile	75%	20%	50%	Sterile	25%	Sterile
C x J	98%	87%	22%	Sterile	5%	Sterile	66%	2%	14%	2%
J x C	94%	76%	no test	4%	38%	25%	no test	11%	no test	13%
V x H	100%	90%	Sterile	53%	94%	82%	Sterile	70%	92%	79%
			Virilis				Henly			
T x A	58%	70%	91%	73%	9%	21%	13%	4%	4%	13%

*Strains J, N and C are from Japan, New Orleans and China, respectively—for their origins see Patterson, Stone and Griffen, 1940.

TABLE 4

P_1 Crosses of Red Forms

Stocks	Controls	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
V	100% 67.2								
H	98% 82.9	96% 90.4	98% 80.2						
A	74% 18.7	79% 2.7	Sterile	1% 1.0	3% 1.7				
T	54% 25.6	44% 4.8	11% 7.4	8% 1.4	1% 6.0	44% 22.0	28% 18.8		
821.12a	47% 51.2	32% 5.4	3% 9.0	4% 1.1	Sterile	34% 77.6	59% 59.7	67% 79.3	34% 70.4
821.12b	44% 48.0	33% 17.1	9% 28.0	6% 1.3	2% 10.0	81% 46.0	66% 68.9	71% 61.4	86% 53.2
821.12c	55% 35.4	30% 6.7	3% 12.6	12% 1.4	Sterile	35% 67.3	89% 50.9	55% 47.7	23% 35.3
822.14	46% 45.3	50% 14.5	7% 17.7	6% 1.3	5% 9.0	26% 72.6	57% 53.7	43% 57.4	73% 51.9
825.13b	56% 34.4	33% 15.9	6% 34.2	3% 1.1	2% 10.0	67% 80.8	75% 61.2	75% 69.5	56% 60.4
825.13c	67% 24.6	51% 6.5	8% 17.1	2% 2.5	3% 8.6	22% 49.8	45% 42.3	45% 46.3	62% 35.0
841.10	72% 26.4	56% 7.6	Sterile	2% 3.0	1% 2.0	66% 52.1	25% 55.1	57% 22.1	54% 27.7
849.11	44% 46.7	57% 15.7	8% 18.2	2% 2.0	5% 11.4	68% 53.5	66% 62.8	78% 53.7	90% 65.2

TABLE 5

F_1 X F_1 Crosses of Red Forms

Stocks	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
821.12a	60% 63.9	56% 40.6	1, tested 15.0	No test	92% 70.2	100% 67.9	96% 52.3	58% 54.0
821.12b	70% 60.6	30% 35.0	2, tested 1, fert. 8.0	5, tested Sterile	100% 47.7	94% 76.1	92% 75.4	90% 58.8
821.12c	74% 82.4	80% 45.9	3, tested Sterile	No test	94% 77.6	90% 63.4	86% 73.5	96% 65.1
822.14	84% 53.7	54% 56.4	1, tested Sterile	30, test. 11, fert. 27.4	94% 80.8	92% 77.8	92% 56.4	96% 49.0
825.13b	78% 61.1	88% 92.9	1, tested Sterile	8, tested Sterile	94% 78.3	100% 91.6	70% 49.7	88% 73.7
825.13c	66% 55.7	58% 74.9	1, tested Sterile	4, tested Sterile	86% 71.7	78% 56.9	54% 49.8	64% 37.7
841.10	64% 48.2	No test	No test	No test	96% 60.4	88% 66.4	74% 34.5	64% 42.0
849.11	96% 87.0	34% 66.7	2, tested Sterile	29, test. 4, fert. 10.5	96% 94.6	90% 77.3	90% 90.2	98% 85.7

TABLE 6

Texana males x americana females; F_1 hybrids x virilis

Tested stocks	Initial crosses to			Hybrid crosses to	
	A♀	V♀	V♂	V♀	V♂
821.12b	81% 46.0	33% 17.1	9% 28.0	86% 5.5	10% 21.2
821.12c	35% 67.3	30% 6.7	3% 12.6	91% 6.2	28% 9.8
825.13c	22% 71.7	51% 6.5	8% 17.1	75% 8.1	27% 11.0
841.10	66% 52.1	56% 7.6	Sterile	82% 8.3	24% 9.2
849.11	68% 53.5	57% 15.7	8% 18.2	62% 4.9	12% 11.7
americana	74% 18.7	79% 2.7	Sterile		

TABLE 7

P_1 Crosses of Gray Forms

Stocks	Controls	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
Beaumont, 754.9a	95% 72.8	94% 78.5	97% 93.9	100% 181.0	94% 70.1	2% 17.0	17% 4.4	43% 13.7	6% 3.0
Blanco	95% 70.3	95% 92.1	99% 74.0	95% 68.4	98% 55.3	20% 23.2	27% 3.1	22% 19.1	46% 1.4
Galveston, 472.4	100% 93.6	95% 121.9	95% 110.6	85% 88.2	78% 82.2	47% 4.6	42% 3.6	35% 24.0	50% 9.1
Galveston, 494.5a	98% 11.12	96% 92.3	96% 81.5	97% 77.7	96% 92.9	14% 24.5	47% 1.9	27% 24.9	36% 1.9
San Antonio, 725.9f	91% 95.5	93% 100.0	86% 130.0	84% 102.5	90% 131.1	46% 15.7	15% 1.6	67% 19.1	10% 35.5
Victoria, 718.7a	100% 107.9	98% 126.6	94% 106.7	99% 126.1	97% 148.9	4% 13.3	9% 1.4	2% 12.0	1% 3.0
California, 863b	80% 96.0	87% 94.4	91% 65.3	87% 123.0	82% 142.5	21% 10.0	14% 2.4	39% 29.4	27% 4.0
California, 863e	93% 71.4	79% 85.1	93% 72.4	96% 79.9	65% 83.7	10% 5.9	1% 1.0	53% 26.5	67% 5.5
Hanchow	92% 70.5	89% 94.4	80% 94.9	92% 122.3	93% 107.9	27% 13.7	54% 5.3	44% 11.3	22% 6.6
Hiroshima	94% 65.8	91% 96.4	97% 87.5	92% 89.5	87% 76.8	2% 7.0		5% 15.2	31% 1.7
Kirin	79% 81.9	93% 115.2	94% 84.7	89% 87.1	91% 91.7	25% 12.8	25% 2.2	47% 27.9	5% 1.5
Kumamoto	50% 75.9	52% 91.1	99% 85.9	48% 90.1	94% 107.7	15% 10.7	36% 5.9		46% 5.3
Mukden	85% 98.2	90% 79.6	88% 57.8	94% 81.3	92% 112.0	3% 21.0	17% 5.6	23% 6.3	22% 3.6
Otaru	75% 89.4	85% 98.4	92% 93.6	80% 66.7	97% 80.3	9% 7.2	19% 3.1	41% 13.0	20% 2.2
Peking	88% 130.0	99% 117.6	99% 138.8	93% 92.4	98% 154.0	3% 4.0	36% 4.8	36% 20.6	15% 2.7
Sendai	86% 86.0	71% 81.9	96% 93.9	78% 87.8	57% 73.8	6% 7.8	40% 3.1	5% 11.6	21% 4.2
Shengking	89% 65.6	91% 97.5	94% 72.2	93% 81.4	92% 71.1	35% 27.0	10% 1.4	45% 44.9	8% 3.5
Tokyo	52% 91.3	96% 89.3	94% 81.4	87% 80.4	93% 62.9	18% 6.6	34% 2.2	20% 25.0	33% 2.5

TABLE 8

Tests of isolating factors

Crosses	americana						texana					
	female			male			female			male		
	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial
Hanchow x V	42	33	13.9	55	78	6.5	49	25	14.6	51	92	15.3
V x Hanchow	41	42	4.7	43	37	5.4						
Hanchow x H	33	49	25.1	50	32	3.3	50	42	18.2	48	35	3.8
H x Hanchow	27	41	13.5									
Hiroshima x V	125	30	22.5	121	83	8.0	99	40	16.1	98	31	11.7
V x Hiroshima	117	46	16.2	117	87	7.3	101	44	16.1	115	91	7.3
Hiroshima x H	119	17	10.3	112	70	2.4	121	69		105	52	2.7
H x Hiroshima	66	14	8.1	120	58	3.7	115	67	40.9	96	60	3.1
Kirin x V	45	9	4.3	42	26	4.6	58	67	51.2	86	81	5.8
V x Kirin	50	16	6.3	55	75	5.7	34	21	5.9	53	60	4.5
Kirin x H	57	11	7.5	47	9	4.0	54	22	15.0	50	42	5.4
H x Kirin	55	6		101	29	3.5	54	6	10.7	54	57	5.5
Kumamoto x V	51	8	10.3	51	77	8.8	55	44	8.3	39	59	6.3
V x Kumamoto	54	19	12.8	48	73	7.2	44	21	17.6	46	94	9.1
Kumamoto x H	54	9	12.6	54	22	2.1	54	7	5.3	55	26	2.7
H x Kumamoto	27	11	7.0	44	11	2.6	52	27	12.3	52	42	2.6
Mukden x V	39	Sterile		58	66	6.0	53	45	7.4	41	61	8.0
V x Mukden	40	10	10.5	52	64	3.8	54	48	17.7	47	45	5.6
Mukden x H	39	3	1.0	60	12	2.0	61	49	18.7	57	32	3.4
H x Mukden	51	31	9.3	58	12	1.7	50	48	12.9	50	32	2.9
Otaru x V	47	49	10.2	53	43	6.6	52	94	15.0	54	63	5.3
V x Otaru	55	62	11.3	53	70	6.8	56	91	8.1	56	64	9.4
Otaru x H	46	50	29.0	53	23	3.0	52	54	16.8	53	34	3.2
H x Otaru	46	33	4.3	59	56	6.0	50	36	10.6	54	48	3.5
Peking x V	49	2	1.0	44	30	5.1	49	6	15.6	52	73	8.5
V x Peking	25	Sterile		39	28	4.7	28	4	3.0	53	62	10.4
Peking x H	49	Sterile		54	15	3.4	42	2	6.0	53	36	2.6
H x Peking	32	Sterile		38	5	2.5	36	3	12.0	49	16	3.9
Sendai x V	46	17	13.4	52	77	15.1	51	24	11.0	48	88	6.8
V x Sendai	56	13	13.5	47	85	10.0	61	39	18.6	56	29	7.7
H x Sendai	38	13	6.8	36	28	2.9	50	64	11.5			
Shengking x V	47	9	16.0	53	78	10.8	45	29	21.3	99	88	11.4
V x Shengking	50	10	23.7	52	64	6.5	24	21	9.3	27	59	9.5

TABLE 8—(Continued)

Tests of isolating factors

Crosses	americana						texana					
	female			male			female			male		
	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial	No. pairs tested	Per cent fertile	Hatch per vial
Shengking x H	112	40	4.0	117	33	3.0	104	52	17.2	102	25	4.6
H x Shengking	102	33	14.3	112	43	4.5	113	85	9.7	108	32	6.6
Tokyo x V	44	7	3.0	35	87	12.8	39	5	3.0	41	88	11.7
V x Toyko	59	10	7.5	54	83	9.2	46	28	9.4	48	90	8.6
Tokyo x H	41	15	3.8				55	7	6.3	23	13	3.5
H x Tokyo	54	13	7.2	59	56	3.9	50	8	3.8	41	51	5.3
863b x V	49	23	9.9	50	30	3.7	50	24	19.4	50	48	6.5
V x 863b	49	53	15.3	39	56	5.0	28	64	23.7	50	50	4.7
863e x V	49	57	37.0	50	66	8.2	50	66	28.4	50	26	9.8
V x 863e	50	44	11.8	50	54	4.7	50	56	21.6	50	58	9.5
494.5a x V	54	6	32.6	51	94	8.8	51	6	8.3	49	76	10.0
V x 494.5a	52	27	16.3	54	80	5.8	36	6	5.3	50	74	8.7
472.4 x V	54	24	15.2	50	64	4.4	50	38	21.8	52	89	10.5
V x 472.4	54	17	15.5	54	79	12.1	52	19	5.1	55	87	13.0
863b x H	53	23	11.0	48	35	2.0	98	50	12.6	55	33	2.0
H x 863b	49	12	6.4	42	26	1.3	49	45	27.0	55	36	2.6
863e x H	55	35	17.3	50	8	2.0	50	76	27.9	38	13	3.0
H x 863e	47	9	4.0	51	10	5.0	45	58	15.1	38	16	4.1
494.5a x H	52	27	3.0	49	51	2.4	52	4	18.0	50	66	6.5
H x 494.5a	30	Sterile		26	19	1.2	30	20	25.2	28	54	3.7
472.4 x H	53	15	15.0	49	49	2.0	55	33	4.8	32	81	5.4
H x 472.4	27	41	6.8	51	46	6.8	51	18	6.8	52	52	6.5

TABLE 9

Fecundity—daily production of offspring

Cross \ Days	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total
V♀ x V♂	1	51	28	70	52	55	48	31	51	48	41	37	49	51	32	59	36	740
	39	38	34	40	44	65	30	27	21	49	60	41	80	113	24	79	40	824
	71	30	30	53	9	45	27	37	45	46	41	21	58	28	48	70	38	697
	48	59	49	31	41	61	37	61	57	59	40	38	64	83	44	69	43	884
A♀ x A♂	0	0	0	0	0	20	26	11	11	24	12	0	0	8	28	7	0	147
	0	0	0	0	0	0	11	0	18	20	31	19	30	35	30	36	32	262
	0	0	0	0	0	11	37	36	32	47	16	24	39	43	13	3	0	301
	0	0	0	0	0	0	0	6	5	25	12	7	23	43	26	31	20	197
V♀ x A♂	0	0	0	0	0	4	1	2	4	4	0	3	0	1	0	1	0	20
	0	0	0	0	0	0	0	3	1	4	0	0	1	1	1	0	0	11
	0	0	0	5	0	1	1	2	2	1	0	0	1	1	3	0	0	17
A♀ x V♂	0	0	1	1	0	0	0	2	3	1	0	0	3	1	0	0	3	15
	0	0	0	0	0	0	0	0	0	0	0	5	4	0	2	0	0	11
	0	0	0	0	0	3	0	0	1	1	0	0	0	1	0	1	0	7
	0	0	0	0	0	0	0	0	0	2	7	1	4	2	1	6	6	29
V♀ x AV♂	8	0	0	0	0	0	11	9	0	10	0	0	0	13	19	0	1	71
	4	3	5	13	0	11	10	14	0	4	21	23	2	16	21	12	7	166
	0	17	0	3	15	36	1	24	15	20	14	28	19	25	48	43	41	349
	21	6	7	21	10	17	17	10	12	12	1	0	25	5	32	14	18	228
AV♀ x V♂	0	1	2	14	4	13	18	27	29	22	21	39	32	20	21	8	17	228
	5	15	8	5	14	10	7	14	17	32	26	26	25	31	17	21	17	290
	2	0	13	10	14	23	20	24	30	28	14	21	28	37	39	34	17	354
	3	7	4	14	10	9	13	9	7	29	2	12	21	25	10	19	11	205
A♀ x AV♂	0	1	1	0	0	0	0	4	5	5	2	0	1	2	1	0	0	22
	0	2	15	17	11	20	3	27	4	13	15	4	28	30	29	35	31	284
	0	2	17	13	0	1	0	0	0	0	0	1	0	0	0	0	0	34
	0	3	4	6	3	21	17	8	5	16	11	12	6	15	10	0	0	137
AV♀ x A♂	0	0	0	0	6	17	28	21	16	17	3	11	18	14	6	17	10	184
	0	0	0	0	3	7	10	16	9	9	7	5	7	13	5	3	5	99
	0	0	0	0	0	0	1	1	6	12	2	3	7	18	3	11	7	71
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
AV♀ x AV♂	5	4	0	14	5	4	11	12	9	8	6	9	11	14	10	15	14	152
	3	13	6	17	11	5	9	14	9	13	7	19	10	15	10	6	8	175
	21	13	6	3	1	7	16	11	17	15	11	16	26	23	28	13	15	242
	9	15	20	27	31	34	45	32	30	39	25	21	25	23	10	23	24	423

TABLE 10

F_1 x F_1 Crosses of Gray Forms

Stocks	V♀	V♂	H♀	H♂	A♀	A♂	T♀	T♂
Beaumont, 754.9a	100% 88.0	100% 124	100% 73.1	98% 67		89% 32.1	92% 59.6	
Blanco	100% 108.2	100% 99.8	98% 88.2	98% 98.2	80% 22.8	100% 28.0	60% 20.5	82% 43.1
Galveston, 472.4	94% 64.8	96% 44.3	94% 46.6	98% 71.2	100% 28.6	90% 35.0	100% 96.9	100% 44
Galveston, 494.5a	100% 100.5	98% 102.1	96% 84.5	58% 57.6	94% 32.0	70% 25.8	72% 37.3	84% 49.9
San Antonio, 725.9f	98% 103.0	100% 101.3	98% 72.0	96% 98.8	67% 20.7	80% 12.7	92% 65.8	75% 35.5
Victoria, 718.7a	94% 54.1	100% 142.9	96% 57.6	98% 74.8	88% 45.1		80% 53.0	92% 42.7
California, 863b	100% 104.9	98% 94.3	100% 74.8	98% 80.4	72% 27.5		76% 62.2	92% 32.8
California, 863e	100% 121.2	100% 121.2	98% 144.3	100% 116.9	88% 43.4	71% 43.1	88% 25.4	100% 31.0
Hanchow	92% 58.8	100% 134.4	100% 82.4	98% 80.4	24% 36.7	58% 51.5	78% 35.3	86% 48.6
Hiroshima	98% 47.9	88% 80.4	82% 70.9	82% 54.4	56% 30.9	71% 24.8	74% 67.0	54% 42.3
Kirin	90% 104.8	100% 117.2	96% 40.7	100% 65.1	78% 29.3		62% 10.7	80% 34.6
Kumamoto	92% 69.4	100% 84.7	84% 99.6	88% 65.0	20% 37.8	90% 22.5	50% 40.2	80% 42.3
Mukden	98% 88.4	100% 77.5	88% 87.4	100% 103.6	68% 27.3	40% 11.0	68% 54.2	60% 67.5
Otaru	100% 89.5	100% 104.4	98% 71.0	90% 73.4	42% 30.0	43% 13.6	80% 89.0	74% 43.2
Peking	98% 130.4	100% 59.9	98% 71.7	98% 82.6	72% 28.6	42% 12.2	44% 38.9	
Sendai	88% 114.9	74% 56.0	100% 89.0	96% 157.3	66% 18.1	66% 29.3	74% 35.0	
Shengking	98% 100.7	98% 78.6	92% 68.7	92% 76.0	20% 24.9		28% 27.2	
Tokyo	100% 57.8	98% 75.0	84% 81.2	94% 44.2	90% 40.0	48% 29.5	82% 53.7	

TABLE 11

F_1 and F_2 Backcrosses

Cross	Number of Tubes	Per Cent Fertile	Average Per Tube
(T x Hanchow) x Hanchow	25	84.0	47.9
Hiroshima x (A x Hiroshima)	34	70.6	40.4
(A x Hiroshina) x Hiroshima	38	42.1	30.4
Kirin x (T x Kirin)	54	37.0	19.9
(T x Kirin) x Kirin	53	94.3	22.2
T x (T x Kirin)	50	30.0	5.5
(T x Kirin) x T	54	79.6	16.5
A x (Peking x A)	53	30.2	12.0
Peking x (T x Peking)	44	68.2	38.9
(T x Peking) x Peking	48	64.6	49.3
T x (T x Peking)	55	30.9	23.5
(T x Peking) x T	38	34.2	25.2
(A x Kumamoto) x Kumamoto	13	7.7	—
Kumamoto x (Kumamoto x T)	43	86.0	58.3
(Kumamoto x T) x Kumamoto	50	34.0	56.8
(Kumamoto x T) x T	24	58.3	24.4
Kumamoto x (T x Kumamoto)	52	75.0	48.9
(T x Kumamoto) x Kumamoto	52	46.2	34.5
Mukden x (A x Mukden)	49	53.1	24.0
(A x Mukden) x Mukden	44	81.8	27.0
A x (A x Mukden)	35	62.9	23.7
(A x Mukden) x A	27	59.3	24.8
Mukden x (T x Mukden)	27	40.7	
(T x Mukden) x Mukden	27	85.2	
T x (T x Mukden)	15	66.7	
A x (Otaru x A)	9	55.5	15.4
Otaru x (T x Otaru)	56	82.1	—
(T x Otaru) x Otaru	60	20.0	—
T x (T x Otaru)	55	78.2	33.1
(T x Otaru) x T	50	90.0	36.8
A x (A x Sendai)	50	34.0	14.9
(A x Sendai) x A	50	92.0	17.8
Shengking x (A x Shengking)	53	69.8	47.4
(A x Shengking) x Shengking	49	83.7	42.6
A x (A x Shengking)	44	72.7	33.5
Shengking x (T x Shengking)	35	28.6	21.9

TABLE 11—(Continued)

F_1 and F_2 Backcrosses

Cross	Number of Tubes	Per Cent Fertile	Average Per Tube
(T x Shenking) x Shengking	54	92.6	36.2
T x (T x Shengking)	19	10.5	5.0
(T x Shengking) x T	41	73.2	12.1
(Tokyo x A) x Tokyo	12	75.0	21.8
(Tokyo x T) x T	52	84.6	37.5
Tokyo x (T x Tokyo)	48	79.2	44.5
(T x Tokyo) x Tokyo	47	74.5	33.1
T x (T x Tokyo)	52	63.5	40.7
(494.5a x A) x 494.5a	13	92.3	48.3
(494.5a x A) x A	63	20.6	2.8
494.5a x (494.5a x T)	12	91.5	50.2
494.5a x (T x 494.5a)	47	93.6	73.5
(T x 494.5a) x 494.5a	51	82.4	70.9
T x (T x 494.5a)	38	76.3	23.1
(T x 494.5a) x T	43	81.4	27.2
(472.4 x A) x 472.4	8	100.0	49.0
T x (472.4 x T)	19	94.7	59.2
T x (T x 472.4)	24	79.2	81.4
T x (T x Beaumont)	93	81.7	39.1
(Beaumont x T) x T	87	70.1	23.2
Beaumont x (T x Beaumont)	45	97.7	99.5
(T x Beaumont) x Beaumont	50	96.0	63.1
T x (T x San Antonio)	50	66.0	44.2
(T x San Antonio) x T	47	83.0	20.8
San Antonio x (T x San Antonio)	50	90.0	76.4
(T x San Antonio) x San Antonio	32	90.6	51.6
863b x (A x 863b)	36	72.2	20.6
(A x 863b) x 863b	37	97.3	19.4
(A x 863b) x A	32	34.4	23.0
863b x (T x 863b)	51	72.5	35.9
(T x 863b) x 863b	62	72.6	41.7
863e x (T x 863e)	53	84.9	32.8
(T x 863e) x 863e	57	94.7	28.8
(T x 863e) x T	46	54.3	13.4
(V x 821,12b) x V	50	90	73.0

TABLE 11—(Continued)

Cross	Number of Tubes	Per Cent Fertile	Average Per Tube
V x (V x 821.12b)	50	76	48.7
V x (821.12b x V)	50	34	20.7
(821.12b x V) x V	50	10	15.0
(V x 821.12c) x V	50	78	82.8
V x (V x 821.12c)	50	79	86.3
(V x 822.14) x V	50	90	66.6
V x (V x 822.14)	50	55	76.8
(V x 825.13b) x V	50	84	91.3
V x (V x 825.13b)	50	70	53.1
(V x 825.13c) x V	50	100	83.5
V x (V x 825.13c)	50	98	61.1
(V x 841.10) x V	50	88	83.9
V x (V x 841.10)	50	56	39.8
(V x 849.11) x V	50	94	118.0
V x (V x 849.11)	50	92	100.3
(V x 821.12a) x V	50	53	42.9
(Kumamoto x T) x Kumamoto x Kumamoto	54	37.0	51.7
Shengking x Shengking x (A x Shengking)	41	95.1	33.2
Shengking x (T x Shengking) x Shengking	36	16.7	29.6
494.5a x 494.5a x (494.5a x T)	33	39.4	55.0
(T x 494.5a) x 494.5a x 494.5a	15	86.7	84.4
494.5a x 494.5a x (T x 494.5a)	47	61.7	62.3
494.5a x (T x 494.5a) x 494.5a	55	94.5	45.1
494.5a x (T x 494.5a) x 494.5a	56	66.1	32.1

TABLE 12

Egg hatch from females mated once

Cross		First day		Second day		3–10 day	total
		smear	hatch	smear	hatch	hatch	hatch
VxV	Sperm in eggs	15/15	47/66	8/10	30/34	82/93	159/193
	%	100	71	80	88	88	82
AxA	Sperm in eggs	20/22	49/70		38/43	145/167	232/280
	%	91	70		88	87	83
VxA	Sperm in eggs	8/52	7/215	0/13	0/71	0/964	7/1250
	%	15	3	0	0	0	0.6

TABLE 13

P_1 Crosses of Montana Stocks

Stocks	Controls	A ♀	A ♂	T ♀	T ♂	H ♀	H ♂	V ♀	V ♂	L McK ♀	L McK ♂
1211.51	5.8% 5.2	st.	st.	3% 4.2	st.	1% 1	st.	16% 6.4	st.	30% 9.3	st.
1218.8d	24% 17.2	st.	st.	18%	st.	13% 7.1	st.	62% 23.0	12% 16.8	30% 8.4	
1210.98	19% 10.2	st.				4.7% 3.3	st.	46% 6.5	st.	st.	
1212.5c	12.7% 17.4	st.	9% 11.1	13% 7.7	st.	13% 1.8	st.	19% 6.6	st.	7% 5.2	
1210.83	11% 8.6	st.		18% 7.8	st.	25% 3.7	st.	32% 11.3	st.	5% 8.6	st.
1211.58	15% 22.4	st.	st.	10% 9.5	st.	17% 6.0	st.	61% 11.1	st.	4% 2.5	
1221.2	13% 28.0	st.	st.			35% 7.0	st.	50% 19.0	st.		

TABLE 14

P_1 Crosses between americana stocks

♀ \ ♂	A	Indiana	Overton	Pee Wee
A Smithville, Ohio	74% 18.7	33% 34.9	41% 34.1	20% 34.7
Anderson, Indiana	38% 31.9	45% 37.2	4% 30.3	36% 16.1
Overton, Ohio	71% 24.2	30% 42.2	66% 20.0	82% 21.9
Pee Wee, Ohio	41% 26.3	63% 35.2	38% 23.8	86% 33.2

TABLE 15

F_1 x F_1 Crosses between americana stocks

♀ \ ♂	A	Indiana	Overton	Pee Wee
Americana, Smithville, Ohio	------ ------	39% 50.2	85% 49.7	19% 45.9
Anderson, Indiana	80% 41.0	------ ------	------ ------	20% 23.7
Overton, Ohio	67% 39.7	15% 22.3	------ ------	81% 31.9
Pee Wee, Ohio	82% 29.2	75% 27.2	87% 20.1	------ ------

TABLE 16

Cross	Per Cent	Average Hatch
(A x asiatic) A	59.4	20.1
(A x asiatic) asiatic	60.5	29.2
(T x asiatic) T	58.3	21.4
(T x asiatic) asiatic	64.2	30.1
Sum (*wild* x asiatic) bc	$\frac{2682.3}{44} = 61.0$	$\frac{11484}{44} = 26.1$
T (T x american)	76.3	36.8
(T x american) american	87.9	56.8
A (A x american)	27.5	12.9
(A x american) american	90.4	34.3
Sum (*wild* x american) bc	$\frac{2070.0}{26} = 79.6$	$\frac{11191}{26} = 43.0$

TABLE 17

F_1 Heterozygotes crossed to *texana* and *americana*

Heterozygote	americana ♀		americana ♂		texana ♀		texana ♂	
	Per Cent	Av. Hatch	Per Cent	Av. Hatch	Per Cent	Av. Hatch	Per Cent	Av. Hatch
V x H	0	-----	94	3.8	0	-----	92	4.8
Average of american stocks from Table	20.5	14.3	21.5	2.43	36.0	21.1	30.4	7.93
Average of asiatic stocks from Table 3	14.3	11.8	30.1	3.74	29.6	19.5	22.3	3.38
Average of both from Table 3	17.1	12.9	26.1	3.1	32.6	20.3	25.9	5.4
Asiatic x V from Table	19.6	10.1	65.6	7.55	36.6	19.2	69.3	8.57
Asiatic x H from Table	20.5	9.10	30.2	3.21	36.0	13.4	37.2	3.84
American x V from Table	31.3	19.2	65.4	6.59	34.9	16.7	63.5	9.10
American x H from Table	20.3	7.94	30.5	2.84	38.0	17.2	43.9	4.23
Average of 4 from Table	21.7	10.8	48.9	5.30	36.4	16.6	54.1	6.44

TABLE 18

F_1 and F_{15} of crosses between two *domestic* strains tested to *texana*

Cross	F_1 in per cent	F_{15} in per cent
T x (V x Blanco)		10
T x (Blanco x H)		6
(Blanco x H) x T		2
T x (Hanchow x H)	42	4
(Hanchow x H) x T	35	40
(Otaru x H) x T	34	0
T x (Otaru x H)	54	16

TABLE 19

Hybrids and inbred hybrids back-crossed to parent strains

Cross	Inbred		virilis				americana			
			female		male		female		male	
	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}	F_1	F_{10}
V x A	64% 19.5	75% 38.3	55% 25.1	63% 39.0	90% 43.5	27% 10.0	26% 13.6	78% 33.4	88% 35.4	90% 29.0
								tex	ana	
V x T	20% 20.2	63% 22.9	75% 43.5	67% 62.9	95% 41.5	86% 41.4	54% 35.6	56% 24.2	65% 28.6	75% 33.7
T x V	66% 31.5	72% 46.2	70% 59.6	84% 60.0	91% 47.5	92% 60.7	42% 31.8	83% 15.8	89% 29.2	75% 24.0