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V. ISOLATING MECHANISMS

Directed by

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Professor of Zoology
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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

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PREFACE

The ten articles included in this bulletin constitute number V of the series of publications to be issued from our genetics laboratory. As indicated in the subtitle, the majority of these articles deal with types of isolating mechanisms which operate in bringing about evolutionary divergence, either by reducing or preventing the exchange of genes between the different forms. Such studies now in progress in this and other laboratories are certain to shed much light on the mode of evolution as it occurs in groups of living organisms.

We wish to express our appreciation to the University Research Institute, which, under the direction of Dr. A. P. Brogan as Dean of the Graduate School, has furnished the funds for meeting the expenses of publication.

J. T. PATTERSON

Austin, Texas,
February 21, 1947.

I. SEXUAL ISOLATION BETWEEN MEMBERS OF THE VIRILIS GROUP OF SPECIES

J. T. PATTERSON, LINDA WHARTON MCDANALD, WILSON S. STONE

INTRODUCTION

Dobzhansky (1937) proposed the term "isolating mechanisms" as a generic name for the various agents which prevent interbreeding of groups of individuals. These mechanisms therefore play a very important rôle in the evolutionary process. As he points out, it is not necessary to assume that a single mechanism is involved in preventing the interbreeding of a given pair of species. As a matter of fact it is usually the total effect of several such mechanisms which finally brings about the more or less complete isolation of two forms, by reducing or preventing the exchange of genes between them. It has been abundantly demonstrated that this is true in the genus Drosophila. Various mechanisms have been found to occur among members of this genus, but the one most frequently encountered is what has been termed sexual isolation.

Sexual isolation is sometimes referred to as psychological isolation, since it involves behavioristic phenomena. In this type of mechanism complete copulation resulting in insemination does not occur frequently between individuals of different species, and sometimes it may not occur between individuals of different geographic strains of the same species. The failure to mate may be due to differences in odor, courtship, behavior, sexual recognition signs, or other possible types of stimulation. It is the purpose of the present article to present the results which the authors have obtained on tests between members of the virilis species group.

Three different methods have been employed to determine the possible occurrence of sexual isolation in Drosophila. One of these involves direct observation. By watching cultures containing males and females of different species, it is possible to determine whether crossbreeding is taking place. This method, however, is too tedious and too time-consuming where it is desired to obtain data sufficient for statistical purposes. This method was employed in many of the early studies on the mating habits of Drosophila.

A second method of testing for sexual isolation is by the use of pair matings. Under this method a number of cultures are made up with each containing a male and a female belonging to different forms (species, subspecies, or strains). In due time the cultures are checked and the number failing to produce offspring recorded. From the data thus obtained the degree of sexual isolation may be determined. This method has been used extensively in the Texas laboratory, and from time to time reports have been made on a series of tests carried out on forms belonging to several different species group (virilis group, Patterson, Stone, and Griffen, 1940, 1942, Patterson and Griffen, 1944; repleta

group, Wharton, 1942; mulleri subgroup, Crow, 1942; funebris group, Mainland, 1942; quinaria group, Sears, 1947 in this publication).

The third method of testing for sexual isolation is what has been termed the multiple choice technique. In employing this method, the usual procedure is to place two kinds of females in a container with males belonging to one of the two types of females, and, after a certain elapsed time, the females are dissected to determine whether or not spermatozoa are present in their seminal receptacles. If the length of exposure of the sexes is properly regulated, and provided that not all of the females have been fertilized, it is possible to determine whether the insemination of the two kinds of females has taken place selectively, or at random. If it has occurred selectively, and not at random, sexual isolation will be indicated (Dobzhansky and Mayr 1944).

The following investigators have used this method in studying sexual preference among several different Drosophila forms: Dobzhansky and Koller 1938, *pseudoobscura* subgroup; Stalker 1941, 1942, *virilis* and *americana;* Dobzhansky and Mayr 1944, strains of *willistoni;* Dobzhansky and Streisinger 1944, strains of *prosaltans;* Dobzhansky 1944, strains of *sturtevanti;* Mayr and Dobzhansky 1945, *pseudoobscura, persimilis,* and strains of *prosaltans;* Levene and Dobzhansky 1945, *pseudoobscura* and *persimilis;* Mayr 1946a, 1946b, *pseudoobscura* and *persimilis;* Wallace and Dobzhansky 1946, *pseudoobscura, persimilis,* and *subobscura.* As indicated in these references, this method has been used for the study of sexual preference both between different species and between geographic strains of the same species. Sturtevant (1920, 1921) had previously obtained evidence that in mixed cultures of *D. melanogaster* and *D. simulans* the flies exhibited preference for mating with members of their own species, and Lancefield (1929) found the same was true for races of *pseudoobscura.*

Further comments on the second and third methods of testing for sexual isolation seem desirable. There are several factors which must be considered in any attempt to evaluate the results obtained by the pair-mating method. Not all sterile cultures, upon which the magnitude of sexual isolation is based, are due to a lack of copulation, for at least some of the females from such cultures have been inseminated, as has been shown by the presence of sperm in their seminal receptacles. The failure of inseminated females to produce offspring may be due to any one of several different causes. In interspecific inseminations the sperm may be killed or inactivated in the reproductive tract of the alien female, either immediately or shortly thereafter (Patterson, Stone, and Griffen 1942). Even though in some cases the sperm may succeed in penetrating the egg, yet development does not take place. This represents an initial form of zygotic mortality. Zygotic inviability is very common in interspecific crosses of Drosophila, due to genic unbalance (Patterson 1942), or to complementary gene systems (Crow 1942, Patterson and Griffen 1944).

The third method of multiple choice technique is a test designed for the detection of sexual preference, rather than one intended primarily for use in the discovery of sexual isolation. Sexual isolation if present will usually be revealed in the results obtained in the experiment. Sexual preference may reduce the exchange of genes between two forms, but will not necessarily prevent such exchanges, whereas sexual isolation as strictly defined will prevent these exchanges. In tests between two species with the use of the pair-mating technique, only heterogamic matings are possible, and if none of the females has been inseminated it is evident that complete sexual isolation exists. On the other hand, in sexual preference tests both homogamic and heterogamic matings are possible.

In view of these facts we decided to carry out a series of tests in which the results from the pair-mating and multiple choice techniques could be compared. Members of the virilis group were selected for the experiments, because we already had accumulated a considerable amount of data on these forms from our pair-mating tests. We also hoped that it might be possible to isolate and study some of the factors underlying sexual isolation, since it seems clear that this phenomenon must be due to genetic factors rather than to extrinsic environmental influences.

EXPERIMENTAL METHODS

In this study we have used principally the multiple choice technique, and wherever possible have compared the results obtained with those derived from the pair-mating tests for the corresponding crosses. As indicated in the introduction, the multiple choice method has been used by several different workers for studying sexual preference between different species or different geographic strains of the same species.

In the recently reported investigations of Dobzhansky and co-workers the practice was followed of placing ten virgin females of each of two species or strains in a food vial together with ten males of one of the forms. After the females had been exposed to the males for a certain period of time they were dissected and their seminal receptacles examined under the microscope for the presence of sperm. From the results thus obtained it is possible to determine whether sexual preference is present.

In general we have followed this same procedure, with but one important exception. In making up the cultures we have used only five females of the two forms under test and a single male of one of the forms. We adopted this method after considerable experimentation with other methods, not only because the results were more consistent, but also because they revealed the performance of a single male. We suspected that in mass cultures not all of the males might possess the genetic factor or factors responsible for sexual preference. Such individual differences, if they exist, would be masked in the results from tests in which several males had been used. It was not considered practical to make similar tests of females, although it could be done by the use of direct observa-

tions. Even in that case the aggressive rôle of the male in courtship might conceal any preference on the part of the female. However, the results derived by our technique will show the effect of female preference to some degree, as well as male preference. If all of the females were equally responsive to the courtship of the male, only male preference would determine the results. Any rejection of the courtship of the male by one type of female will reduce the number of matings of that type, especially in the presence of several females of the other type.

In our experiments we used flies which were mature at the beginning of the test. The length of time for the flies to reach maturity varies in different species, and sometimes even in the two sexes of the same species. In the virilis group, for instance, the females on the average mature and are ready to mate about one day earlier than the males. This fact was taken into consideration in making up the cultures for the tests. Once the age of maturity is determined, it becomes a matter of importance to ascertain the proper length of time to expose the sexes before dissecting the females. This period of time is especially important in intraspecific tests between geographic strains, and in those interspecific crosses which yield fairly high degrees of homogamic and heterogamic matings. It is somewhat less important in interspecific crosses in which heterogamic matings either do not take place or occur only rarely. In recording the data derived from the experiments, we used long mimeographed sheets ruled with horizonal lines for listing the individual females, and vertical columns for showing the type of cross, age of the female, age of the male, length of exposure, presence of the sperm in the receptacles, and the motility and quantity of spermatozoa present in the receptacles. The age of the flies at the start of the experiment was given in hours, as was the length of time the males and females were exposed to each other. In attempting to determine whether or not the sperm were alive and motile, it was sometimes necessary to tap the cover glass with the point of a pencil. To determine the exact quantity of sperm present is difficult, and any method adopted to estimate the amount will not be entirely satisfactory. We finally established three categories, under the terms "many," "moderate," or "few."

INSEMINATION AND THE DISTRIBUTION AND MOTILITY OF THE SPERM IN THE SEMINAL RECEPTACLES OF WILD STRAINS OF DROSOPHILA FEMALES

As a preliminary study to our proposed experiments on sexual isolation, we desired to know more about the degree of insemination in wild strains of Drosophila. We also wanted to learn more about the distribution and motility of the sperm in the seminal receptacles of such females. Accordingly, we collected several of the species which are common in the vicinity of Austin, especially those found at the nearby Aldrich farm. The only species in the list that was not collected in the country was *D. repleta.* This form rarely frequents wild habitats, and we there-

fore collected it at one of the produce houses in Austin where it was known to live and breed. As soon as the collections were brought into the laboratory, the females were isolated and placed separately in food vials. No selection was practiced in isolating these females, other than to exclude specimens which had been injured by the collecting net.

These cultures were held long enough to allow development to occur in case the females had been inseminated in the field. The length of time to hold the vials would obviously depend on the length of the life cycle of any given species. At the end of this period the cultures were examined and the number containing offspring was used as a basis for determining the per cent of fertile females. The females from the sterile cultures were then dissected and their seminal receptacles examined for the presence of sperm. The number containing sperm was added to the number fertile to give the per cent inseminated. This procedure obviates the necessity for large-scale dissections, and in addition to giving the per cent inseminated, also shows the per cent of fertile females.

In Table 1 are listed the data for thirteen wild-type species, or rather species collected in the wild. The first column, following the name of the species, gives the total number of females isolated and tested. The second column gives the per cent of fertile females, that is, females which actually produced offspring. The third column shows the number of dissected females from sterile cultures. The fourth column gives the number of these females which contained sperm, and the fifth gives the per cent inseminated. The last column gives the per cent of each species in the population at the time the collections were made.

TABLE 1

Per cent of inseminated females in wild strains of Drosophila

Species	females isolated	per cent fertile	females dissected	females with sperm	per cent insem-inated	per cent in popu-lation
D. busckii	51	96.0	2	1	98.0	1.8
D. putrida	203	92.1	16	1	92.6	15.4
D. melanogaster	547	95.0	27	3	95.6	23.2
D. simulans	302	92.0	24	3	93.0	18.1
D. affinis	88	84.1	14	0	84.1	5.5
D. pseudoobscura	331	96.6	11	5	98.5	27.0
D. tripunctata	42	92.8	3	0	92.8	0.89
D. macrospina	105	83.8	19	3	86.6	4.8
D. repleta	152	73.0	41	32	94.0	18.7
D. hydei	552	80.8	106	55	90.7	23.4
D. mulleri	201	50.2	100	40	70.1	12.7
D. longicornis	130	30.8	88	48	67.7	11.6
D. meridiana	63	85.7	9	5	93.6	3.2

In the first eight species listed in the table the percentage fertile is very close to the percentage inseminated, due to the fact that only a few of the females from sterile cultures possessed sperm. In two cases *(affinis, tripunctata)* all inseminated females produced offspring. In the next four species, all members of the repleta group, there is a wide

divergence between the per cent inseminated and the per cent fertile. Just why so many of these females with sperm in their receptacles should fail to produce offspring is not clear, unless it be due to the laboratory food. It should be stated that every chance was given these females to breed. In most cases they were held for three weeks and over, with several changes to fresh food, and still no offspring appeared in the cultures. The last species on the list is *D. meridiana,* also a member of the repleta group. The difference between the two percentages of this form is not as striking as for the other four members of the group, but it is still greater than that of any of the first eight species.

Drosophila longicornis shows the greatest difference between the two percentages of any of the thirteen species. It also has the lowest fertility percentage. The fact that only about half of the females brought in from the field were found to be inseminated may be due to the long maturing period of this species.

Another point of interest is the apparent lack of correlation between the per cent inseminated and the per cent in the population of this group of species. There seems to be very little correlation between these two percentages. For example, for *D. busckii* the percentages are 98 and 1.8, respectively, whereas for *D. pseudoobscura* they are 98.5 and 27; again, for *D. meridiana* they are 93 and 18.1. Although in some cases the percentages are nearly equal (e.g., *simulans* and *repleta*), yet on the whole there is very little correlation between the density of population and the degree of insemination.

In Table 2 are presented the facts concerning the distribution and motility of the sperm in inseminated females. The data are presented under two headings for each of the thirteen species, for fertile and sterile females. The data in the fertile series were obtained from dissections of about fifty specimens, whenever that number was available. The only exception is *D. pseudoobscura* for which dissections were not made. The data for the sterile series are rather meager, since so many of the females from the sterile cultures did not contain sperm.

In the fertile series sperm were present both in the spermathecae and the ventral receptacle in all except two species. The two exceptions were *D. mulleri* and *D. longicornis,* in which sperm were present in the ventral receptacle only. These two forms belong to the mulleri subgroup of species in which the spermathecae do not function as sperm receptacles, but have evolved into glandular structures.

At the bottom of the table are given the totals for the fertile and sterile cultures, together with their grand totals. These figures present some interesting facts with reference to the distribution and motility of the sperm. In the fertile series there are 281 cases of motile sperm as against 214 non-motile in the spermathecae, while in the sterile series there were 12 motile and 94 non-motile cases for the same organ. The numbers of motile and non-motile cases in the fertile series were 510 and 47, respectively, for the ventral receptacle, or a ratio of about

eleven to one. In the sterile series there were 99 motile and 30 non-motile cases for the ventral receptacle, or a ratio of slightly more than three to one.

TABLE 2

Distribution and motility of sperm in fertile and sterile females

Species	Type of culture	Spermathecae motile	Spermathecae non-motile	Ventral receptacle motile	Ventral receptacle non-motile	Sperm absent	Totals
D. busckii	fertile	35	13	43	5	0	48
	sterile	0	1	0	1	1	2
D. putrida	fertile	53	6	58	1	0	59
	sterile	1	0	1	0	15	16
D. melanogaster	fertile	29	43	65	7	0	72
	sterile	1	2	3	0	24	27
D. simulans	fertile	38	37	64	11	0	75
	sterile	1	2	2	1	21	24
D. affinis	fertile	25	10	36	2	0	38
	sterile	0	0	0	0	14	14
D. pseudoobscura	fertile	not dissected					
	sterile	2	3	5	0	6	11
D. tripunctata	fertile	11	2	13	0	0	13
	sterile	0	0	0	0	3	3
D. macrospina	fertile	54	2	49	6	0	57
	sterile	2	1	3	0	16	19
D. repleta	fertile	8	44	37	5	0	52
	sterile	1	31	7	6	9	41
D. hydei	fertile	0	52	10	15	0	53
	sterile	1	54	5	5	51	106
D. mulleri	fertile	0	0	49	1	0	50
	sterile	0	0	28	14	58	100
D. longicornis	fertile	0	0	36	4	0	40
	sterile	0	0	40	3	45	48
D. meridiana	fertile	28	5	50	0	0	50
	sterile	3	0	5	0	4	9
Total, fertile series		281	214	510	47	0	607
Total, sterile series		12	94	99	30	267	460
Grand total		293	308	609	87	267	1,067

These figures establish the fact that in fertile females there is a larger proportion of motile sperm in the ventral receptacle than is the case for the spermathecae. In contract to this condition, in sterile females the proportion of motile to non-motile cases is reversed for the spermathecae (12 vs. 94), while for the ventral receptacle the proportion is still in favor of the motile cases, yet the ratio is much less than for fertile females. We shall delay any further discussion of the possible significance of these data until a later section where larger numbers will be available.

TESTS FOR SEXUAL PREFERENCE AMONG MEMBERS OF THE VIRILIS SPECIES GROUP

There are six known members of the virilis species group, and five of these were available for the study of sexual preference. The five forms are *D. virilis* Sturtevant, *D. americana americana* Spencer, *D. americana texana** Patterson, Stone, and Griffen, *D. montana* Patterson and Wheeler, and *D. lacicola* Patterson. Two different series of tests were carried out, one on interspecific crosses between the five forms, and one on intraspecific crosses between a selected list of geographic strains of three of the forms. There are twenty possible reciprocal crosses between the five forms, using a single strain for each form (Table 4). For each cross we dissected at least one hundred females for each of the two types of matings. The numbers dissected for the two types were usually not equal, due to the fact that some few females die during the course of the experiment, or are otherwise lost.

The terms homogamic and heterogamic matings are employed in the same sense as was used by Dobzhansky and co-workers. The former term includes all matings occurring between the male and the females of his own species, subspecies, or strains, while the latter term includes all matings between the male and the alien females. The strains used for the five different species were, *virilis,* Pasadena strain, *americana,* a strain from Anderson, Indiana, *texana,* strain 1128.10 from New Orleans, Louisiana, *montana,* strain 1318.8a from Hart Prairie, Arizona, and *lacicola,* strain 1360.2 from Fairbanks, Minnesota. The values of χ^2 were calculated by the use of the fourfold table, and those for the isolation index by the formula developed by Stalker (1942).

It was found necessary to adjust the length of exposure and the age of the flies in making tests for sexual preference, otherwise the results will not be entirely consistent. Two different series were carried out for the purpose of demonstrating this fact. Reciprocal crosses of *virilis* and *americana* were made and the cultures divided into two groups, one of which was kept at a temperature of 72° F., the other at a temperature of 62° F. The former represents the temperature of the air-conditioned laboratory. The flies were collected once each day and aged in the laboratory for ninety-six hours before making the crosses. Five females of each species were placed with one male of one of the forms in food vials. The females were dissected at 24-hour intervals (using ten cultures for each interval), and their sperm receptacles examined for the presence of sperm. Fifty females were therefore dissected for each possible homogamic and heterogamic mating. The number of females inseminated is recorded in per cents (Table 3). The results from the seventy-two degree series are listed in the upper half of the table, and those of the sixty-two degree series in the lower half. In

*We now regard *americana* and *texana* as constituting a pair of subspecies (see Article VIII).

referring to these data, we shall use the term "interval" to cover each of the 24-hour exposure periods. Thus the period from 1–24 hours will be referred to as the 24-hour interval, that of 25–48 hours as the 48-hour interval, and so on through the series.

TABLE 3

Effects of temperature and length of exposure on the per cent inseminated in *virilis/americana* crosses

Temperature	Hours exposed	(5V♀♀ + 5A♀♀) × V♂ per cent of homogamic	(5V♀♀ + 5A♀♀) × V♂ per cent of heterogamic	(5A♀♀ + 5V♀♀) × A♂ per cent of homogamic	(5A♀♀ + 5V♀♀) × A♂ per cent of heterogamic
72 degrees Fahrenheit	24	0	0	0	0
	48	8	0	0	0
	72	10	0	6	0
	96	94	4	62	6
	120	86	12	88	16
	144	100	4	42	16
	168	90	32	42	16
62 degrees Fahrenheit	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
	96	0	0	0	0
	120	0	0	0	0
	144	6	0	0	0
	168	52	0	0	0
	192	56	0	0	0

At seventy-two degrees temperature, with *virilis* used as the male parent the homogamic matings began to appear during the 48-hour interval, whereas the heterogamic matings did not start until sometime during the 96-hour interval. For these three intervals, 56 of the 150 dissected *virilis* females had been inseminated, or 37.3%, while only two of the 150 dissected *americana* females contained sperm, for a percentage of 1.3. For the 120-hour interval there were 86% of homogamic matings and 12% of heterogamic. For the 144-hour interval the per cent of homogamic matings rose to 100, while that of the heterogamic fell to 4. Finally, in the last or 168-hour interval the two percentages were 90 and 32, respectively.

In order to understand these variations in the insemination percentages, it is necessary to refer to our recorded observations made during the course of the dissections. It was observed for the 96-hour interval that a large majority of the females contained many motile sperm in their seminal receptacles. In contrast to this condition, most of the females of the 120-hour interval, especially the *virilis* ones, had very few sperm in these receptacles, frequently making it necessary to examine minutely both the ventral receptacle and the spermathecae in order to determine whether a few sperm might not be present. For the 144-hour interval all of the *virilis* females were fertile, and a majority of them contained many motile sperm. Finally, for the 168-hour interval not as large a proportion of the *virilis* females had as many sperm as those for

the 144-hour interval, but most of the *americana* females did have many sperm in their receptacles.

The inference from these observations is that the *virilis* males first mate with their own kind of female, and do not copulate with the alien females until most of the conspecific females have been inseminated. This is almost certainly true up to the beginning of the 144-hour interval, but during the latter period they again mate with their own kind of females, probably after such females have used up most of the sperm. It is possible that the seven *virilis* females, which had no sperm at the 120-hour interval, had already exhausted their supply of sperm at the time the dissections were made.

In the reciprocal cross, with *americana* used as the male parent, none of the females were inseminated during the first two intervals. This is undoubtedly due to the fact that the flies were too immature, as they were for the first interval of the preceding series. During the 72-hour interval 6% of homogamic matings occurred, and none of heterogamic. Sixty-two per cent of homogamic and 6% of heterogamic matings occurred up to end of the 96-hour interval, and 88% and 16%, respectively, for the 120-hour interval. For the last two intervals these two per cents were identical, 42% homogamic and 16% heterogamic. The same variations in per cents inseminated in this series of tests up to the end of the 120-hour interval were observed as for the corresponding intervals of the first series, and the same explanation may be offered for both. There is considerable difference in the per cent inseminated in the two series for the last two intervals, due in part at least to differences in the two species. Of the two forms, *americana* is slower in maturing, is less fertile, and on the whole is somewhat less vigorous than *virilis*. It was recorded that the *americana* females of the homogamic matings for the last two intervals contained relatively few sperm in their seminal receptacles, indicating that remating had not taken place.

The results from the tests on flies exposed to the sixty-two degrees temperature are listed in the lower half of Table 3. In the cross in which *virilis* was used as the male parent no inseminations was found until the 144-hour interval. During this period homogamic matings alone occurred, to the extent of 6%. The same was true for the 168- and 192-hour intervals, which gave 52% and 56% of homogamic matings, respectively. Not a single inseminated female was found throughout the entire series in which *americana* was used as the male parent.

These results demonstrate that temperature does modify the degree of sexual isolation. The most obvious effect is the striking retardation of copulation. In the cross in which *virilis* was used as the male parent, homogamic matings began during the 48-hour interval at 72°, and not until the 144-hour interval at 62°. Likewise, the heterogamic matings began during the 96-hour interval at 72°, and not at all at 62°. Therefore, at the lower temperature *virilis* males prefer their own kind of female to the exclusion of the alien females. In the reciprocal cross, with *ameri-*

cana used as the male parent, homogamic matings began during the 72-hour interval at 72°, and the heterogamic matings during the 96-hour interval. No matings of either kind occurred at 62° temperature. Since none of the *americana* females in either cross was inseminated at the lower temperature, it is possible that these females failed to respond to the courtship of either type of male.

The above reported tests were carried out for the purpose of augmenting certain results that had been obtained in a series of tests carried out at the beginning of this study. The tests were performed with the view of determining the optimum time of exposure, as well as to determine the best age at which to mate the flies. The results demonstrate that if consistent data are to be obtained, it is necessary to mate the *virilis* flies after they have reached maturity, and then expose the sexes to each other for a definite period of time. Any wide deviations in the lengths of these two periods may modify the results appreciably, especially in intraspecific crosses in which the per cents of homogamic and heterogamic matings are rather high. A single example will make this point clear. If two geographic strains of *virilis* under test show a significant difference in favor of homogamic matings after seventy-two hours of exposure, this difference may be reduced to a point at which it is no longer significant upon prolonging the length of exposure from twenty-four to forty-eight hours. This does not mean that sexual preference is absent, rather it signifies that the males prefer to mate with their own kind first before inseminating the alien females. We finally adopted the plan of aging the flies from 120–144 hours, and then exposing them for seventy-two hours. This plan was followed, with but few minor exceptions, throughout the experiments described below.

The results obtained from the sexual preference tests in interspecific crosses are recorded in Table 4. In six of the crosses (1, 11, 13, 14, 17, 19) the isolation index is 1.00, which indicates that sexual isolation is complete. There are seven other crosses (3, 5, 7, 12, 15, 16, 18) in each of which the isolation index is close to unity and sexual isolation is nearly complete. Even in the cross with the lowest isolation index (8, *lacicola/virilis*) the χ^2 value of 8.02 and the isolation index of 0.29 both indicate that the difference between homogamic and heterogamic matings is significant.

In the *americana/texana* cross (10) the χ^2 value and the isolation index show that the difference is significant. But the isolation index is negative (—0.28), which means that *texana* males prefer *americana* females to their own kind where choice of mate is possible. At the time this result was first obtained it seemed so unusual that the test was repeated. The percentages obtained in the second test were practically identical with those of the first test. We later obtained several cases of negative isolation indices involving geographic strains of the same species, and in some of these the differences were significant (Table 9). Moreover, Dobzhansky *et al.* have reported such indices in crosses between

geographic strains. In this connection it should be pointed out that *texana* and *americana* are now regarded as constituting a pair of subspecies.

TABLE 4

Number of females dissected *(n)* and per cent inseminated (%) in various interspecific crosses of the virilis group

	Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation index
1.	virilis, americana	virilis	102	92.1	103	0.0	175.3	1.00
2.	americana, virilis	americana	105	49.5	106	24.6	14.1	0.33
3.	virilis, texana	virilis	101	74.2	103	2.9	109.9	0.92
4.	texana, virilis	texana	101	58.4	102	26.4	21.2	0.37
5.	virilis, montana	virilis	109	80.7	115	1.8	145.3	0.95
6.	montana, virilis	montana	111	58.5	108	14.8	44.9	0.69
7.	virilis, lacicola	virilis	102	71.5	102	0.98	109.9	0.97
8.	lacicola, virilis	lacicola	102	39.2	108	21.3	8.02	0.29
9.	americana, texana	americana	115	60.8	115	31.3	20.2	0.32
10.	texana, americana	texana	111	30.6	112	55.3	13.9	—0.28
11.	americana, montana	americana	120	50.0	102	0.0	70.6	1.00
12.	montana, americana	montana	109	67.8	115	0.86	112.9	0.97
13.	americana, lacicola	americana	107	50.4	109	0.0	92.6	1.00
14.	lacicola, americana	lacicola	100	75.0	100	0.0	120.0	1.00
15.	texana, montana	texana	107	83.1	104	0.96	145.7	0.97
16.	montana, texana	montana	104	69.0	102	0.98	104.8	0.97
17.	texana, lacicola	texana	101	60.4	102	0.0	88.1	1.00
18.	lacicola, texana	lacicola	100	50.0	100	2.0	61.6	0.92
19.	montana, lacicola	montana	100	52.0	105	0.0	69.6	1.00
20.	lacicola, montana	lacicola	111	73.8	105	6.6	100.6	0.83

The conclusion to be drawn from the results obtained in this series of sexual preference tests is that sexual isolation is a very important isolating mechanism in preventing the exchange of genes between the different members of the *virilis* group.

For purposes of comparison we have brought together certain experimental results which are shown in Table 5. The figures represent percentages and are listed under three main headings of heterogamic, homogamic, and paired matings. The percentages under each heading are given in two columns, in one of which the male had a choice of mate and in the other no such choice was possible. In the heterogamic and homogamic series ten females were exposed to a single male for seventy-two hours and then dissected. In the column under the subhead "with choice" five females each of two forms were used, while under the column "without choice" all of the females belonged to the same form. In the pair mating series the percentages were based on the number of females producing progeny, and in the homogamic column the males and females used belonged to the same form, but in the heterogamic column they belonged to different forms.

A comparison of the results listed in the six columns in the table allows us to determine the extent of variation in mating under the several different conditions. In comparing the same homogamic mating with

and without choice from columns III and IV, we find that usually the results are not statistically different. In fact, only crosses 1, 8, 15, and 20 show a statistically significant difference. Other conditions make it improbable that these differences are due to the choice or lack of choice of mates available. For example, in cross 1 the per cent (92.1) of *virilis* females mated with choice is significantly higher than the per cent (73.0) mated without choice. However, in crosses 3, 5, and 7 the per cents mated with choice (74.2, 80.7, 71.5) are not significantly higher than the per cent mated without choice (73.0). Probably some factor other than choice, or the lack of it, influenced the higher per cent in cross 1. Cross 15 appears to be a similar case with *texana* (compare with 4, 10, 17). Crosses 8 and 20, which are statistically different, probably represent fluctuations of *lacicola* for causes other than choice or lack of choice (c.f., 14, 18).

TABLE 5

Per cents inseminated in homogamic and heterogamic matings in the virilis series of crosses

Type of matings	Ten ♀♀ × one ♂				One ♀ × one ♂	
Crosses	Heterogamic matings with choice	Heterogamic matings without choice	Homogamic matings with choice	Homogamic matings without choice	Pair matings homogamic	Pair matings heterogamic
1. (V♀ + A♀) × V♂	0.0	3.8	92.1	73.0	95.0	8.0
2. (A♀ + V♀) × A♂	24.6	24.0	49.5	52.0	70.0	23.0
3. (V♀ + T♀) × V♂	2.9	10.0	74.2	73.0	95.0	14.0
4. (T♀ + V♀) × T♂	26.4	37.0	58.4	54.0	87.0	36.0
5. (V♀ + M♀) × V♂	1.8	3.0	80.7	73.0	95.0	0.0
6. (M♀ + V♀) × M♂	14.8	37.0	58.5	65.6	87.0	54.0
7. (V♀ + L♀) × V♂	0.98	1.0	71.5	73.0	95.0	0.0
8. (L♀ + V♀) × L♂	21.3	16.5	39.2	55.0	75.0	26.0
9. (A♀ + T♀) × A♂	31.3	26.0	60.8	52.0	70.0	48.0
10. (T♀ + A♀) × T♂	30.6	44.0	55.3	54.0	87.0	25.0
11. (A♀ + M♀) × A♂	0.0	0.0	50.0	52.0	70.0	2.0
12. (M♀ + A♀) × M♂	0.86	0.0	67.8	65.6	87.0	0.0
13. (A♀ + L♀) × A♂	0.0	0.0	50.4	52.0	70.0	0.0
14. (L♀ + A♀) × L♂	0.0	0.0	68.4	55.0	75.0	0.0
15. (T♀ + M♀) × T♂	0.96	1.0	83.1	54.0	87.0	0.0
16. (M♀ + T♀) × M♂	0.98	1.0	69.0	65.6	87.0	2.0
17. (T♀ + L♀) × T♂	0.0	0.0	60.4	54.0	87.0	0.0
18. (L♀ + T♀) × L♂	2.0	0.0	50.0	55.0	75.0	12.0
19. (M♀ + L♀) × M♂	0.0	2.0	52.0	65.6	87.0	14.0
20. (L♀ + M♀) × L♂	6.6	29.0	73.8	55.0	75.0	12.0
Columns	I	II	III	IV	V	VI

In comparing like crosses in columns I and II only two crosses, 6 and 20, show a statistically significant difference. These involve *lacicola* and *montana* which themselves show considerable fluctuation. It is probable, therefore, that no real differences exist between these two columns.

When we compare columns IV and V, we find that in every case the per cent of females producing offspring (V) is higher than the per cent of females inseminated in homogamic matings (IV or III), and is usually

significantly higher. This undoubtedly represents a real difference, and must depend on the longer time of opportunity to mate. In fact, the length of the period of exposure of females to males, usually 72 hours, was held to this short time in order to allow the expression of sexual preference, as longer exposures often resulted in so many females becoming inseminated that no choice could be detected.

A comparison between columns II (or I) and VI does not show any appreciable increase in the pair matings (VI). This point is of considerable interest when we compare it to the comparable results from homogamic matings. Apparently the factors preventing cross insemination are not those of time, choice, or opportunity, such as influence homogamic matings. This makes such measurements of sexual preference or sexual isolation between species of even more evolutionary significance.

When we compare columns I with III, II with IV, and V with VI, there is never any question of the significance of difference between heterogamic and homogamic matings. The only exception to statistically significant difference are those where it is expected, that is, between the subspecies *texana* and *americana*.

At the time the sexual preference tests were being carried out a complete record was kept of the distribution and motility of the spermatoza in the seminal receptacles of all dissected females. These records are tabulated in Table 6, and are shown under two main headings, the paired spermathecae and the single ventral receptacle. Under each of these headings the numbers containing motile and non-motile sperm are given for both homogamic and heterogamic matings. Since none of the females in six of the heterogamic matings had been inseminated, the table lists but thirty-four of the forty possible combinations.

There are some variations in the distribution of the two types of sperm among the different crosses, but we are interested in the totals as given at the bottom of the table. For the paired spermathecae there were 817 females with motile and 645 with non-motile sperm. This gives a percentage of 55.9 in favor of the motile type. For the single ventral receptacle there were 1,327 females with motile and 151 with non-motile sperm, for a percentage of 89.8 in favor of the motile type. The ventral receptacle contains a much larger proportion of motile sperm than the spermathecae.

These totals may be separated into their two natural groups of homogamic and heterogamic. The percentages of motile sperm in the homogamic series were 55.7 for the spermathecae and 91.9 for the ventral receptacle, and those in the heterogamic series were 57.1 and 76.2, respectively. The percentages of motile sperm in the spermathecae for the two sets of data (homogamic, heterogamic) are very similar (55.7, 57.1), but in the ventral receptacle the percentage for the homogamic is significantly higher ($\chi^2 = 46.8$) than that of the heterogamic (91.9 vs. 76.2).

TABLE 6

Distribution and motility of sperm in the female receptacles in crosses between members of the virilis group

Crosses	Female dissected	Spermathecae motile	Spermathecae non-motile	Ventral receptacle motile	Ventral receptacle non-motile
(V♀ + A♀) × V♂	virilis	68	26	93	0
(A♀ + V♀) × A♂	americana	29	14	46	7
	virilis	12	13	20	5
(V♀ + T♀) × V♂	virilis	34	41	71	3
	texana	1	2	1	1
(T♀ + V♀) × T♂	texana	23	29	53	6
	virilis	9	15	21	5
(V♀ + M♀) × V♂	virilis	48	37	79	5
	montana	1	0	2	0
(M♀ + V♀) × M♂	montana	33	32	59	4
	virilis	5	9	7	7
(V♀ + L♀) × V♂	virilis	30	38	67	4
	lacicola	0	1	0	1
(L♀ + V♀) × L♂	lacicola	9	25	32	2
	virilis	18	4	16	6
(A♀ + T♀) × A♂	americana	58	14	69	3
	texana	27	9	30	6
(T♀ + A♀) × T♂	texana	19	13	27	6
	americana	26	26	47	16
(A♀ + M♀) × A♂	americana	31	29	55	4
(M♀ + A♀) × M♂	montana	60	14	64	4
	americana	1	0	0	0
(A♀ + L♀) × A♂	americana	21	20	40	3
(L♀ + A♀) × L♂	lacicola	44	32	61	13
(T♀ + M♀) × T♂	texana	47	40	75	14
	montana	1	0	1	0
(M♀ + T♀) × M♂	montana	42	30	67	3
	texana	0	1	1	0
(T♀ + L♀) × T♂	texana	27	32	57	4
(L♀ + T♀) × L♂	lacicola	22	29	46	2
	texana	1	0	1	1
(M♀ + L♀) × M♂	montana	21	31	46	6
(L♀ + M♀) × L♂	lacicola	43	38	66	10
	montana	6	1	7	0
Totals		817 55.9%	645 44.1%	1,327 89.8%	151 10.2%
Homogamic		709 55.7%	564 44.3%	1,173 91.9%	103 8.1%
Heterogamic		108 57.1%	81 42.9%	154 76.2%	48 23.8%

As we have already stated, the matter of determining the quantity of sperm present in the seminal receptacles of inseminated females is very difficult. To attempt to count the sperm in large scale experiments would have been an impossible task. We therefore established three categories of classification, many, moderate, and few. We placed in the first category all cases in which the receptacle was at least one-third full of sperm; in the second category all cases in which the receptacle was

approximately one-fourth full; and in the third category all cases in which the receptacle was less than a fourth full. In the third category many cases were observed in which the number of sperm might have been counted. At best such a method can give only a rough estimate of the quantity of sperm present in the seminal receptacles.

The data on the quantity of sperm for the thirty-four combinations are tabulated in Table 7. We shall again consider the totals group first.

TABLE 7

Distribution and quantity of sperm in the female receptacles in crosses between members of the virilis group

Crosses	Female dissected	Spermathecae			Ventral receptacle		
		many	moderate	few	many	moderate	few
(V♀ + A♀) × V♂	virilis	85	1	8	86	2	5
(A♀ + V♀) × A♂	americana	33	3	7	37	4	12
	virilis	9	2	12	10	2	11
(V♀ + T♀) × V♂	virilis	62	8	5	57	10	7
	texana	2	0	1	0	0	2
(T♀ + V♀) × T♂	texana	38	7	7	46	5	8
	virilis	11	6	7	8	8	10
(V♀ + M♀) × V♂	virilis	52	18	15	51	17	16
	montana	1	0	0	1	0	1
(M♀ + V♀) × M♂	montana	39	11	15	44	7	12
	virilis	5	2	7	3	4	7
(V♀ + L♀) × V♂	virilis	53	6	8	52	8	9
	lacicola	1	0	0	0	0	1
(L♀ + V♀) × L♂	lacicola	30	3	1	28	2	2
	virilis	18	0	4	6	5	11
(A♀ + T♀) × A♂	americana	67	2	3	69	2	1
	texana	31	2	3	33	2	1
(T♀ + A♀) × T♂	texana	24	4	8	28	4	1
	americana	41	3	8	51	6	6
(A♀ + M♀) × A♂	americana	42	12	6	15	2	0
(M♀ + A♀) × M♂	montana	58	8	8	57	2	9
	americana	0	1	0	0	0	0
(A♀ + L♀) × A♂	americana	32	0	9	33	2	8
(L♀ + A♀) × L♂	lacicola	71	3	2	62	3	9
(T♀ + M♀) × T♂	texana	77	3	7	82	3	4
	montana	1	0	0	1	0	0
(M♀ + T♀) × M♂	montana	49	8	15	52	7	11
	texana	0	1	0	0	0	1
(T♀ + L♀) × T♂	texana	53	3	3	52	3	6
(L♀ + T♀) × L♂	lacicola	50	0	1	40	1	6
	texana	1	0	0	1	0	1
(M♀ + L♀) × M♂	montana	46	3	3	44	4	4
(L♀ + M♀) × L♂	lacicola	71	6	4	61	7	8
	montana	4	2	1	4	3	0
Totals		1,157	128	178	1,114	125	181
		79.1%	8.7%	12.2%	78.5%	8.8%	12.7%
Homogamic		1,022	109	135	996	95	129
		80.7%	8.7%	10.6%	81.6%	7.8%	10.6%
Heterogamic		125	19	43	118	30	52
		66.8%	10.2%	23.0%	59.0%	15.0%	26.0%

These show a very close similarity in the three categories of both the spermathecae and the ventral receptacle. The deviation is less than one per cent for the corresponding categories. The per cents are also very similar for the three categories of the homogamic series, and the deviation is likewise less than one per cent for the corresponding categories. The greatest difference is seen in the heterogamic series. This can be shown by using the number of specimens in the first or many category and the sum of the other two categories for the homogamic and heterogamic series. The totals series may be omitted, since it is a mixture of the other two. The calculations by the chi square method gives χ^2 values of 18.9 for the spermathecae and 49.0 for the ventral receptacles, both of which are significant.

There are three possible explanations for the observed differences between the homogamic and heterogamic series. In the first place, it is possible that in heterogamic matings the males do not deliver as many sperm as in homogamic matings. In the second place, in forms which have the insemination reaction, fewer sperm succeed in entering the seminal receptacles in heterogamic crosses, due to disturbances in the female reproductive tract. Finally, in heterogamic matings the sperm may soon become inactivated and may eventually disappear altogether in the tract of the alien female. The second or third alternatives, or better still, a combination of the two, would seem to offer the best explanation. Repeated matings may also be effective.

It was observed during the course of the dissections that the sperm were not always present in both receptacles. They were sometimes absent from the ventral receptacle or the spermathecae. These data are shown in Table 8 for the thirty-four combinations. The total number of inseminated females was 1,625, of which 1,516 had sperm in both receptacles, 62 in the ventral receptacle only, and 47 in the spermathecae only. In the homogamic series, there were 1,448 specimens, of which 1,357 had sperm in both receptacles, 52 in the ventral receptacle only, and 39 in the spermathecae only. In the heterogamic series these numbers were 159, 10, and 8, respectively. As shown in the last column, there were 79 females which had sperm in only one of the spermathecae. There is one other point that should be mentioned, and this applies equally well to the data given in Tables 7 and 8. The quality of sperm present in the receptacles at the time of dissection must be influenced by the amount of sperm already used up in case the female had been laying eggs. However, this does not detract from the significance of the difference between the amount of sperm present in the homogamic and heterogamic matings. In fact, females in homogamic matings laid more readily than those in heterogamic matings whenever any difference existed.

TABLE 8

Variation in distribution of sperm in female receptacles

Crosses	Dissected female	Both receptacles	Ventral receptacle only	Spermathecae only	One spermathecae only
(V♀ + A♀) × V♂	virilis	94	0	0	1
(A♀ + V♀) × A♂	americana	47	10	0	6
	virilis	24	1	2	2
(V♀ + T♀) × V♂	virilis	75	1	0	1
	texana	2	0	1	1
(T♀ + V♀) × T♂	texana	52	7	0	1
	virilis	23	3	1	1
(V♀ + M♀) × V♂	virilis	80	3	3	3
	montana	1	1	0	0
(M♀ + V♀) × M♂	montana	62	0	2	4
	virilis	12	2	2	1
(V♀ + L♀) × V♂	virilis	65	5	2	2
	lacicola	1	0	0	0
(L♀ + V♀) × L♂	lacicola	140	0	8	2
	virilis	21	0	0	1
(A♀ + T♀) × A♂	americana	69	1	2	5
	texana	36	1	1	2
(T♀ + A♀) × T♂	texana	50	12	1	4
	americana	29	1	0	5
(A♀ + M♀) × A♂	americana	51	1	0	12
(M♀ + A♀) × M♂	montana	68	0	6	6
	americana	0	0	1	0
(A♀ + L♀) × A♂	americana	39	3	2	5
(L♀ + A♀) × L♂	lacicola	75	3	3	0
(T♀ + M♀) × T♂	texana	87	2	0	6
	montana	1	0	0	0
(M♀ + T♀) × M♂	montana	69	0	3	4
	texana	1	0	0	0
(T♀ + L♀) × T♂	texana	59	2	0	3
(L♀ + T♀) × L♂	lacicola	47	1	3	1
	texana	1	1	0	0
(M♀ + L♀) × M♂	montana	52	0	0	0
(L♀ + M♀) × L♂	lacicola	76	1	4	0
	montana	7	0	0	0
Totals		1,516	62	47	79
		93.3%	3.8%	2.9%	4.9%
Homogamic		1,357	52	39	66
		93.7%	3.6%	2.7%	4.6%
Heterogamic		159	10	8	13
		89.8%	5.7%	4.5%	7.3%

TESTS FOR SEXUAL PREFERENCE AMONG GEOGRAPHIC STRAINS OF THE VIRILIS GROUP

For these tests we used four geographic strains each of *virilis*, *texana*, and *montana*. For *virilis* the strains were Pasadena, Florida from Brooksville, Mexico from Mexico City, and Shenking from China. For *texana*, New Orleans from Louisiana, Georgetown and Newton from Texas, and

Okefenokee from Georgia. For *montana* the strains were Yellowstone Park (1211.58) from Wyoming, Cottonwood Canyon (1218.8) and Puffer Lake (1220.2) from Utah, and Bonita Canyon (1324.8) from New Mexico. In recording the data in the table we have listed the strains of *montana* by their stock numbers, as shown here in parentheses. We selected one geographic strain for each species and tested it against the other three strains. The strains selected were Pasadena for *virilis*, New Orleans for *texana*, and Cottonwood Canyon for *montana*. The experimental procedure followed was the same as that for the interspecific tests. We crossed five females each of two strains to one male of one of the strains for each combination. The results obtained in this series of tests are shown in Table 9.

TABLE 9

Number of females dissected (*n*) and per cent inseminated (%) in intraspecific crosses of the virilis group

	Females	Males	Homogamic *n*	Homogamic %	Heterogamic *n*	Heterogamic %	χ^2	Isolation Index
1.	Pasadena, Florida	Pasadena	105	80.9	106	53.6	17.2	0.202
2.	Florida, Pasadena	Florida	104	53.8	100	54.0	0.001	—0.001
3.	Pasadena, Mexico	Pasadena	100	30.0	100	81.0	52.7	—0.459
4.	Mexico, Pasadena	Mexico	100	61.0	100	18.0	38.7	0.544
5.	Pasadena, Shenking	Pasadena	116	90.5	116	43.9	57.1	0.339
6.	Shenking, Pasadena	Shenking	102	33.3	103	65.0	20.6	—0.322
7.	N. Orleans, Georgetown	N. Orleans	103	34.7	101	46.5	2.37	—0.145
8.	Georgetown, N. Orleans	Georgetown	130	40.0	149	32.1	1.52	0.108
9.	N. Orleans, Newton	N. Orleans	104	38.4	104	41.3	0.18	—0.036
10.	Newton, N. Orleans	Newton	104	44.2	112	25.9	8.00	0.261
11.	N. Orleans, Okefenokee	N. Orleans	128	56.2	127	30.7	16.9	0.292
12.	Okefenokee, N. Orleans	Okefenokee	101	28.7	107	48.6	8.64	—0.257
13.	1218.8, 1211.58	1218.8	111	66.7	101	37.6	17.9	0.279
14.	1211.58, 1218.8	1211.58	98	44.9	98	61.2	5.24	—0.153
15.	1218.8, 1220.2	1218.8	104	63.4	106	33.9	18.29	0.303
16.	1220.2, 1218.8	1220.2	114	43.8	114	60.5	6.35	—0.160
17.	1218.8, 1324.8	1218.8	107	51.4	104	56.3	0.40	—0.045
18.	1324.8, 1218.8	1324.8	101	69.3	101	43.5	13.61	0.207

In the *virilis* series the results show that the difference between homogamic and heterogamic matings is distinctly significant in five of the six different crosses (1, 3, 4, 5, 6). This is indicated by the χ^2 values of 17.2, 52.7, 38.7, and 20.6, respectively, as well as by the isolation indices. In the other cross (2) the difference is not significant. The isolation index is negative for three of the six crosses, including the one in which the difference is not significant. In two crosses (3, 6) the negative isolation indices show that the males prefer females of the other strains to their own kinds.

The χ^2 values and the isolation indices in the *texana* series show that the difference between homogamic and heterogamic matings are significant for three (10, 11, 12) of the six crosses. It will be noted that three

of the crosses have a negative isolation index, but two of these (7, 9) are not significant, while the third is (12). In the latter cross, Okefenokee males preferred New Orleans females to their own kind.

In the *montana* series the χ^2 value shows a significant difference for five of the six crosses. In three of these (13, 15, 18), both the X^2 value and the isolation index are rather high, while in the other two (14, 16) they are at or just above the borderline of significance. Here again, three of the isolation indices are negative. In one (17) the difference between homogamic and heterogamic matings is not significant, and in the other two (14, 16) it is low.

Table 10 gives certain information about the inheritance of the genes responsible for the sexual preference. The first eight experiments involved males and females which had been aged 120–144 hours and exposed for 72 hours to each other before the females were dissected. The ninth, where the mating was the same as for the eighth, the flies were aged 240–264 hours and then exposed 24 hours. If they had been exposed 72 hours, all of the females would have been inseminated, making it impossible to detect any sexual preference that might have been present. Nevertheless, crosses 8 and 9 are essentially similar in showing preference.

TABLE 10

Sexual preference tests using Pasadena and Mexico strains of *virilis* and their hybrids. Per cents inseminated based on dissections of 100 ♀ ♀

Male	Per cents inseminated		χ^2	Isolation Index
P ♂	P ♀ ♀ = 30.0	M ♀ ♀ = 81.0	52.7	—0.459
M ♂	P ♀ ♀ = 18.0	M ♀ ♀ = 61.0	38.7	0.544
P ♂	P ♀ ♀ = 26.0	MP ♀ ♀ = 80.0	58.5	—0.509
M ♂	P ♀ ♀ = 29.0	MP ♀ ♀ = 66.0	27.4	0.389
P ♂	M ♀ ♀ = 37.0	MP ♀ ♀ = 64.0	14.6	0.267
M ♂	M ♀ ♀ = 49.0	MP ♀ ♀ = 79.0	19.5	—0.234
PM ♂	P ♀ ♀ = 58.0	M ♀ ♀ = 88.0	22.8	0.205
MP ♂	P ♀ ♀ = 55.0	M ♀ ♀ = 87.0	24.9	0.225
MP ♂	P ♀ ♀ = 30.0	M ♀ ♀ = 63.0	21.9	0.355

If Pasadena and Mexico females are used, all four types of males, (Pasadena (P), Mexico (M), F_1 males from Mexico females × Pasadena males (MP), and F_1 males from Pasadena females × Mexico males (PM)) inseminated more Mexico than Pasadena females. If MP females were used with Mexico or Pasadena females, both Mexico and Pasadena males inseminated more of the heterozygous females. The F_1 females (PM females behave similarly, and PM males give results like those of MP males), therefore, may be said to display hybrid vigor, here expressed as greater per cent of fertility in a limited period of time.

It is still impossible to say how much of a rôle, or how exclusive the activity of one or the other sex is in determining choice. We must assume that dominant genes exist that make MP females more frequently fertilized than either parent in competition. We cannot say whether males

prefer them, or whether they are more receptive than their parent types. No change in preference accompanied hybridization in the males (MP and PM). We can, however, say that gene differences influencing preference exist and can be demonstrated to be inherited in crosses. Certain of these experiments were repeated three times with over six months between the tests, and all gave qualitatively similar results, so it is certain that they represent an expression of a real difference.

Table 11 is included to show the variation between individual males. It is obvious that placing all ten of the males with ten females of each kind in a vial and leaving them for an appreciable period of time, would have given results reflecting the activity of the most aggressive males, provided the females do not control the mating process.

TABLE 11

Test showing mating variability, illustrated from one Pasadena male crossed to five Pasadena plus five Mexico females

Male	Five Pasadena females		Five Mexico females	
	Inseminated	Not inseminated	Inseminated	Not inseminated
1	0	5	5	0
2	5	0	5	0
3	0	5	0	5
4	1	4	5	0
5	1	4	3	2
6	4	1	5	0
7	0	5	3	2
8	3	2	4	1
9	2	3	5	0
10	0	5	2	3

DISCUSSION

Sexually reproducing animals as diverse as fish (Gordon 1947), mice (Blair and Howard 1944), and flies (see below) all exhibit a certain preference in choice of mates. This sexual isolation or sexual preference which may be either interspecific or intraspecific has been extensively studied in the genus Drosophila, as indicated by the list of species and species groups investigated:

(1) *D. melanogaster* and *D. similans* by Sturtevant (1915, 1920) who also made checks on several other species.

(2) *D. pseudoobscura A* and *B (D. persimilis)* by Lancefield (1929); Dobzhansky and Koller (1938); Levene and Dobzhansky (1945); Mayr and Dobzhansky (1945); Mayr (1946a, b); and Wallace and Dobzhansky (1946) who also tested *D. subobscura.*

(3) The melanica group, by Griffen (1942).

(4) The funebris group, by Mainland (1942).

(5) *D. prosaltans,* by Dobzhansky and Stressinger (1944); Mayr and Dobzhansky (1945).

(6) *D. willistoni* by Dobzhansky and Mayr (1944).

(7) *D. sturtevanti* by Dobzhansky (1944).

(8) *D. pallidapennis* by Patterson and Dobzhansky (1945).

(9) The repleta group by Wharton (1942).

(10) The mulleri group by Crow (1942), Baker (this bulletin) and Patterson (this bulletin).

(11) The virilis group, by Spencer (1938); Stalker (1942); Patterson, Stone, and Griffen (1942); Patterson and Griffen (1944).

This list, which is incomplete, indicates that a very representative sample of this genus has been investigated. Sexual preference resulting in failure to inseminate members of the other species seems universally present and complete sexual isolation exists in some cases even within these species groups. It is probably the rule between members of different species groups. These numerous investigators have discussed the situation in the group they investigated, as well as the general problem. The new results of the present investigation of the virilis complex of species, using the presence or absence of sperm in the female as a measure of isolation, give a more complete picture of the situation in this diverse group. Before we discuss the virilis data, there are several results of general application.

One factor all investigators of evolution must evaluate is the reproductive efficiency of the forms investigated under natural conditions. Table 1 shows that under such conditions, the females of the thirteen species of Drosophila investigated are exceedingly efficient in being fertilized. In nine of these thirteen species, more than ninety per cent of the females had been fertilized; in two more about eighty-five per cent, and the last two about seventy per cent. A remarkably high proportion of the females of most species are fertilized, especially considering that a number of the females trapped must be quite young. The difference in fertilization cannot be explained by differences in population density (at least in so far as the numbers of flies in the traps are related to population density) for the four species which showed the lowest amounts of insemination were from rather intermediate population densities. Except for some members of the repleta group (*D. repleta, D. hydei, D. mulleri* and *D. longicornis*) almost all fertilized females produced offspring. In these cases, we can infer that the majority of inseminations were by males of the same species. There is no completely satisfactory explanation for the failure of certain females in the repleta group to produce offspring, although it is known that stocks of these forms are much more temperamental and difficult to test in the laboratory than most of the other species listed.

There seems to be no consistent relation between motility of sperm in the seminal receptacles and production of offspring except that a larger per cent of those failing to produce offspring had non-motile sperm (Table 2). Some of these cases may have been due to abnormality of the sperm or of the fluids in the receptacles. In others the sperm may

have been too old. In some of these cases the non-motile sperm may have been those of a different species, but there is no proof of this point.

The differences in insemination of the same species at different temperatures (Table 3), ages, and length of exposure (see Table 10 and report of results) indicate that isolation is not a single constant thing with a fixed value which is dissociated from metabolic changes and environmental factors. Despite this lack of exact quantitative fixity, we can establish qualitative differences, and even get an approximation of the average quantitative degree of isolation that exists in a particular case.

Table 4 shows that all species of the virilis group show a significant amount of preference for their own kind. The one exception in the table is between the subspecies *americana* and *texana* where (in 10) the isolation index is negative. Four matings show a positive isolation index of 1.0 indicating complete isolation in these experiments, and in a fifth case the isolation is complete in both directions. Other experiments indicate that this isolation can sometimes be broken down in mass matings, or by using other strains. In the other tests varying amounts of isolation were encountered between species. The isolation index is calculated as suggested by Stalker (1942); the difference between the per cent of insemination of the two types of females tested is divided by the sum of the per cent insemination.

Table 5 summarizes data from several sources and gives the same general picture of relations. In this species group choice between their own and alien females or lack of this opportunity to choose mates does not seem to make very much difference in mate preference in homogamic matings, except that the pairs (column V) shows a higher per cent of insemination than the homogamic matings which had only a limited time (columns III and IV) as would be expected with the time factor. The percentage of heterogamic matings did not seem to differ very significantly with varying conditions (choice, lack of choice, pair matings). There seems to be a rather fixed and limited amount of cross mating that will occur, despite the opportunities given. This indicates a qualitative difference in response to members of the opposite sex between species, regardless of opportunities for choice of mate.

There does not seem to be much difference between heterogamic and homogamic matings as to distribution and motility of sperm, although there seems to be more sperm present in homogamic mating. Perhaps this is due in part to remating and various other reasons for reduction in amount of sperm in heterogamic matings, such as the insemination reaction discussed by Patterson and by Wheeler (this bulletin).

Mayr (1946b) has shown that in matings between species, coitus interruptus occurs in a high percentage of attempted fertilizations, so that insemination is not complete and no sperm are found in the females. This did not occur between strains within a species. We do not know how

frequent this mechanism prevents insemination between species of the virilis complex.

If we can generalize from Mayr's results, the number of inseminations represent the net reproduction activity in intraspecific crosses. In the virilis group tested here, intraspecific selection (Tables 9, 10) usually represents a greater efficiency in one type of female in being inseminated, whatever this efficiency depends on. Hybrid vigor of the female seems to be effective here (Table 10) and may even extend to hybrids between species (Mayr, 1946a). This superiority of one genotype of female at attaining insemination seems to be the case in *D. willistoni, D. nebulosa,* and *D. prosaltans,* although *D. sturtevanti* and *D. americana* seem to have a real preference for their own kind. Therefore for most of the cases the intraspecific tests indicate a simple selection for sexual vigor, here represented by insemination, rather than any sort of isolation.

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II. SEXUAL ISOLATION IN THE MULLERI SUBGROUP

J. T. PATTERSON

INTRODUCTION

The mulleri subgroup of species belongs to the large repleta species group, of which more than fifty forms have been described. About sixteen species may be assigned to this subgroup, and eight of these have been selected for this study. These eight species, together with the strains used, are as follows:

D. aldrichi Patterson and Crow 1940; strain from Aldrich Farm, Austin, Texas.

D. arizonensis Patterson and Wheeler 1942; strain 928.5, Tucson, Arizona.

D. buzzatii Patterson and Wheeler 1942; strain 190, Cordoba, Argentina.

D. hamatofila Patterson and Wheeler 1942; strain from Aldrich Farm, Austin, Texas.

D. mojavensis Patterson and Crow 1940; strain from Chocolate Mountains, California.

D. mulleri Sturtevant 1921; strain 1434.4, Aldrich Farm, Austin, Texas.

D. peninsularis Patterson and Wheeler 1942; strain 1148.7, Lake McKethan, Florida.

D. ritae Patterson and Wheeler 1942; strain from Huachuca Mountains, Arizona.

The members of this subgroup have certain characters in common, of which two may be mentioned. While the tergite pattern varies among the different species, yet there is a strong tendency for the abdominal bands to be broken up into spots, either in one or both sexes. The second character, which is constant for the subgroup, relates to the structure of the spermathecae. In most members of the genus Drosophila these paired organs serve as sperm receptacles, but in the mulleri series they are small and have slender stalks, and only rarely are the spermatozoa successful in gaining entrance to them. These organs have become modified into gland-like structures, each surrounded by a thick envelope from which secretions are discharged into its cavity.

In testing for sexual isolation in the mulleri subgroup, the writer has followed closely the experimental procedure used in the preceding article for the virilis group. It is, therefore, unnecessary to give an extended account of the methods employed. The life cycles of the mulleri forms are somewhat shorter than those for the virilis species, consequently, the flies were aged for only four days after emergence before making the crosses. The females were dissected after ninety-six hours exposure

to the males. In the sexual preference tests the same plan was followed as before; five females of each of the two species under test were placed in a vial with a single male of one of the species.

The writer acknowledges his indebtedness for excellent technical assistance, both in this and the following article, to Mrs. Sarah B. Martin who made the dissections, and to Miss Johanna Blumel who bred and separated the flies used in the dissections. He is also indebted to Professor A. H. Sturtevant of The California Institute of Technology for supplying a stock of *D. ritae*.

FERTILITY TESTS AMONG THE EIGHT SPECIES OF THE MULLERI SUBGROUP

Three papers have been published which give the degree of fertility among some of the members of this subgroup. The first of these included but three species (Patterson and Crow 1940), while the second and third dealt with only five species (Crow 1942, Patterson 1942). At most this would involve only twenty different crosses, whereas, the eight species used in the present study give a total of fifty-six different combinations. Moreover, in the three published articles only a few of the exposed females were dissected, and the percentages of fertility were based mainly on the production of hybrids. In the present study we dissected at least one hundred females for each combination, and from these determined the exact number of females which had been inseminated, irrespective of whether or not hybrids had been produced. The number inseminated was obtained by the use of small mass matings of ten females and ten males each. The tests were carried out in this manner because mass matings often yield offspring in crosses in which pair matings fail to do so.

The results obtained from the fifty-six different combinations are given in Table 1. The first column shows the nature of the cross, the second indicates whether or not hybrids were obtained, the third gives the number of females dissected, and the fourth and last shows the number of females inseminated. The different crosses have been numbered from 1 to 56, for ease of reference, and these numbers appear in parentheses in the text. In thirty of the crosses none of the females had been inseminated, and, consequently, hybrids were not obtained. The number of females inseminated in the other twenty-six crosses varied from one to ninety-three. Ten of these yielded some sort of hybrids (1, 3, 5, 7, 9, 15, 21, 27, 31, 32), and sixteen failed to do so. The failure of the females of the latter group to produce offspring was due, in a large measure, to the deleterious effects of the insemination reaction in heterogamic matings (Patterson 1946). This reaction will be fully considered in the next article.

A further analysis of the data displayed in Table 1 may be made by referring individually to each of the eight species.

TABLE 1

Crosses between members of the mulleri subgroup

Crosses	Types of hybrids	♀♀ dissected	Inseminated
1. mulleri ♀ × aldrichi ♂	sterile ♀♀, ♂♂	100	32
2. aldrichi ♀ × mulleri ♂	none	100	18
3. mulleri ♀ × arizonensis ♂	sterile ♂♂	100	8
4. arizonensis ♀ × mulleri ♂	none	100	0
5. mulleri ♀ × buzzatii ♂	abnormal flies	100	2
6. buzzatii ♀ × mulleri ♂	none	101	0
7. mulleri ♀ × hamatofila ♂	sterile ♀♀, ♂♂	100	10
8. hamatofila ♀ × mulleri ♂	none	109	0
9. mulleri ♀ × mojavensis ♂	fert. ♀♀, st. ♂♂	100	21
10. mojavensis ♀ × mulleri ♂	none	105	0
11. mulleri × ♀ peninsularis ♂	none	104	0
12. peninsularis ♀ × mulleri ♂	none	104	0
13. mulleri ♀ × ritae ♂	none	110	0
14. ritae ♀ × mulleri ♂	none	104	0
15. aldrichi ♀ × arizonensis ♂	sterile ♀♀	100	26
16. arizonensis ♀ × aldrichi ♂	none	100	0
17. aldrichi ♀ × buzzatii ♂	none	100	0
18. buzzatii ♀ × aldrichi ♂	none	105	0
19. aldrichi ♀ × hamatofila ♂	none	100	11
20. hamatofila ♀ × aldrichi ♂	none	103	0
21. aldrichi ♀ × mojavensis ♂	sterile ♀♀	100	46
22. mojavensis ♀ × aldrichi ♂	none	100	15
23. aldrichi ♀ × peninsularis ♂	none	102	0
24. peninsularis ♀ × aldrichi ♂	none	105	0
25. aldrichi ♀ × ritae ♂	none	102	0
26. ritate ♀ × aldrichi ♂	none	106	0
27. arizonensis ♀ × buzzatii ♂	larvae only	106	1
28. buzzatii ♀ × arizonensis ♂	none	100	88
29. arizonensis ♀ × hamatofila ♂	none	100	21
30. hamatofila ♀ × arizonensis ♂	none	100	5
31. arizonensis ♀ × mojavensis ♂	fert. ♀♀, st. ♂♂	100	29
32. mojavensis ♀ × arizonensis ♂	fert. ♀♀, ♂♂	100	93
33. arizonensis ♀ × peninsularis ♂	none	102	0
34. peninsularis ♀ × arizonensis ♂	none	100	43
35. arizonensis ♀ × ritae ♂	none	101	0
36. ritae ♀ × arizonensis ♂	none	100	0
37. buzzatii ♀ × hamatofila ♂	none	100	74
38. hamatofila ♀ × buzzatii ♂	none	100	1
39. buzzatii ♀ × mojavensis ♂	none	100	18
40. mojavensis ♀ × buzzatii ♂	none	104	0
41. buzzatii ♀ × peninsularis ♂	none	103	0
42. peninsularis ♀ × buzzatii ♂	none	103	1
43. buzzatii ♀ × ritae ♂	none	100	0
44. ritae ♀ × buzzatii ♂	none	100	0
45. hamatofila ♀ × mojavensis ♂	none	100	19
46. mojavensis ♀ × hamatofila ♂	none	100	33
47. hamatofila ♀ × peninsularis ♂	none	103	0
48. peninsularis ♀ × hamatofila ♂	none	100	10
49. hamatofila ♀ × ritae ♂	none	100	1
50. ritae ♀ × hamatofila ♂	none	100	10
51. mojavensis ♀ × peninsularis ♂	none	103	0
52. peninsularis ♀ × mojavensis ♂	none	100	0
53. mojavensis ♀ × ritae ♂	none	110	0
54. ritae ♀ × mojavensis ♂	none	102	0
55. peninsularis ♀ × ritae ♂	none	103	0
56. ritae ♀ × peninsularis ♂	none	100	0

D. mulleri.—Females of this species were inseminated by the males of five of the other seven species. In four of the crosses (1, 3, 5, 7), such hybrids as were produced were sterile, and in the fifth cross (9) the F_1 males were also sterile, but at least some of the hybrid females were fertile in backcross. In the reciprocal cross, the *mulleri* males failed to fertilize any of the females of six other species, but did inseminate 18% of the *aldrichi* females without producing offspring (2). Several years ago the writer found another member of the subgroup, *D. longicornis,* the males of which fertilized *mulleri* females, producing a few sterile hybrids. The results from this test have been reported by Wharton (1944). So far as the exchange of genes is concerned, the results from these tests show that *D. mulleri* cannot do so except with one other species. In the *mulleri/mojavensis* cross, Crow (1942) has reported that in pair mating tests, 5% of the females produce offspring, and that the F_1 females are fertile in backcrosses to the parent types of males.

D. aldrichi.—Females of this species were inseminated by the males of four other forms, *mulleri, hamatofila, arizonensis,* and *mojavensis.* The first two crosses do not result in the production of offspring (2, 19) while the other two produce sterile females (15, 21). In the reciprocal crosses, *aldrichi* males inseminated *mulleri* females, producing sterile males and females (1), and also *mojavensis* females without the production of offspring (22). This species cannot, therefore, exchange genes with any other member of the subgroup to which it has been tested.

D. arizonensis.—The females of *arizonensis* were fertile to the males of two other species. The first successful cross was to *buzzatii* males and resulted in the production of larvae which died in mid-larval stages (27). The second cross to go was with *mojavensis* males, and yielded fertile females and sterile males (31). In the reciprocal crosses males of *arizonensis* inseminated the females of six other species (3, 15, 28, 30, 32, 34), but only three of these produced hybrids (3, 15, 32). The reciprocal crosses of *arizonensis* and *mojavensis* are two crosses in which an exchange of genes with other members of the subgroup is possible. The *mojavensis/arizonensis* cross was the most successful of the entire series, giving both fertile males and females.

D. buzzatii.—The females of this form were inseminated by the males of three other species, but none of these crosses produced hybrids. In the *buzzatii/arizonensis* cross (28), 88% of the females had been inseminated, and in the *buzzatii/hamatofila* cross (37) 74%. In the third cross, *buzzatii/mojavensis* (39), 18% of the females had been inseminated. In the reciprocal crosses, the males of *buzzatii* had inseminated four types of females, *mulleri, arizonensis, hamatofila,* and *peninsularis.* In the *mulleri/buzzatii* cross most of the resulting zygotes die in larval stages, and only a few reach maturity and are abnormal and sterile (5). The *arizonensis/buzzatii* cross gave a few lethal larvae (27). One female of *hamatofila* and one of *peninsularis* had been inseminated by *buzzatii*

males (38, 42). On the basis of these results this species cannot exchange genes with any other member of the subgroup.

D. hamatofila.—The females of this species were inseminated by the males of *arizonensis* (30), *buzzatii* (38), *mojavensis* (45), and *ritae* (49), but hybrids were not produced. Only *mulleri* females were fertile to *hamatofila* males, but the hybrids were sterile (7). In addition to this cross, the females of the other six species had been inseminated to varying degrees by *hamatofila* males (19, 29, 37, 46, 48, 50). This species is also not capable of gene transfer with other members of the subgroup.

D. mojavensis.—The females of this species were inseminated by *aldrichi* and *hamatofila* males, without the production of hybrids (22, 46), and fertile to *arizonensis* males, giving fertile males and females (32). The males were fertile to *mulleri, arizonensis,* and *aldrichi* females, producing fertile females and sterile males in the first two crosses and sterile females in the third (9, 31, 21). These males also inseminated *buzzatii* and *hamatofila* females, but without producing offspring (39, 45). There are, therefore, three possible chances of gene exchange with this form and other members of the subgroup (9, 31, 32).

D. peninsularis.—The males of *peninsularis* failed to inseminate a single female of any of the other seven species, but males of *arizonensis, buzzatii,* and *hamatofila* did inseminate some of its females (34, 42, 48). However, no offspring was produced, and gene exchange is not possible.

D. ritae.—Offspring were not produced in any of the fourteen possible crosses with this species, but ten females had been inseminated by *hamatofila* males (50), and one *hamatofila* female had been inseminated by a *ritae* male (49). Gene exchange between this species and other members of the subgroup is also not possible.

While some sort of hybrid is produced in ten of the fifty-six crosses, yet there are only three of these in which an exchange of genes is possible. These are the fertile F_1 females from the *mulleri/mojavensis* and *arizonensis/mojavensis* crosses, and the fertile F_1 males and females from the *mojavensis/arizonensis* cross. All other hybrids are sterile and some of them are abnormal, or else the zygotes die in larval or pupal stages. We have no information as to whether the flies which produce fertile hybrids ever mate in nature. So far as known, the distributional areas of *mulleri* and *mojavensis* are widely separated, but there is a possibility that those of *mojavensis* and *arizonensis* may overlap. The most extensive overlapping of distributional areas are those of *mulleri* and *aldrichi* (Patterson and Wagner 1943), but the F_1 flies from the only cross that goes, *mulleri* ♀ × *aldrichi* ♂ are completely sterile. The male hybrids from this cross have been found in nature and definitely identified, and since this is the only known instance in which it has been possible to identify Drosophila hybrids in nature, it seems worthwhile to present the evidence upon which this statement is based.

The hybrids from the *mulleri/aldrichi* cross resemble *mulleri* in most of their characters, but the testis of the male hybrid is rudimentary and

shows little coiling, and, as seen through the abdominal wall, appears in the form of a question mark (?). It is this character which enables one to identify these males in collections brought in from the field. Such males are identical with the hybrid males obtained in the laboratory from this cross. During July and August of 1940 and 1941 collections of Drosophila from the overlap zone of *mulleri* and *aldrichi* in Texas were examined for the presence of these hybrid males. These data are summarized in Table 2. All of the collections were made in the country, and none in towns or cities. In recording the place at which a given collection was made the nearest town is listed, but if no towns were near, it is recorded as from a Roadside Park (R. P., in the table). The number of *mulleri* and *aldrichi* males are given for each collection, followed by the percentage of *aldrichi* males in the male population of both species. The number of hybrid males is shown in the last column. Hybrid females must also be present in some of these collections, but they could not be detected with certainty.

TABLE 2

Per cent of hybrid males found in nature from the cross *mulleri* ♀ × *aldrichi* ♂

Date	Place, County	mulleri males	aldrichi males	Per cent of aldrichi ♂ ♂	Hybrid males
6/13/40	Selma, Bexar	34	188	84.6	1
7/16/40	R.P., Fayette	27	26	49.0	1
7/19/40	R.P., Guadalupe	115	147	56.1	4
8/2/40	Selma, Bexar	100	99	49.7	1
8/2/40	R.P.(A), Comal	279	340	54.8	2
8/2/40	Cactus, LaSalle	5	11	68.7	1
8/2/40	Devine, Medina	24	16	40.0	1
8/9/40	R.P., Williamson	56	212	79.1	9
8/9/40	Troy, Bell	92	50	35.2	2
8/9/40	R.P.(A), Bell	153	148	49.1	3
8/9/40	R.P.(B), Bell	73	41	35.1	1
7/27/41	R.P., Travis	14	36	72.0	3
7/27/41	R.P.(A), Williamson	16	15	48.4	1
6/7/40	San Antonio, Bexar	19	6	24.0	0
8/2/40	R.P., Hays	38	15	28.3	0
8/2/40	R.P.(B), Comal	80	32	28.5	0
8/2/40	R.P., Guadalupe	177	64	26.5	0
8/2/40	Laredo, Webb	19	1	5.0	0
8/9/40	R.P., McLennan	52	3	5.4	0
8/12/40	Franklin, Robertson	190	72	27.4	0
8/12/40	Palestine, Anderson	60	15	20.0	0
7/28/41	R.P.(B), Williamson	68	16	19.0	0

There are twenty-two collections listed in the table. In the first thirteen collections there are 2,317 normal and thirty hybrid males. In the last nine collections there are 927 normal males and no hybrids, although one would expect about twelve such hybrids with this number of normal males, that is, if the populations were alike. Evidently, the size of the collection does not determine the presence or absence of hybrids. For example, a single hybrid male was present in each of the collections from Selma (6/13/40) and Cactus (6/2/40), and yet the first contained 222

normal males and the latter only 16. In so far as the samples in the table are concerned, the presence or absence of hybrids must depend on the proportion of *aldrichi* males in the male population in the two species. In all collections in which these males exceed one-third of this population hybrids are present (first thirteen), but they are absent where it is less than a third (last nine).

SEXUAL PREFERENCE TESTS

As pointed out above, thirty of the fifty-six crosses listed in Table 1 gave no offspring, and none of the females had been inseminated. If in heterogamic tests the male, without choice of mate, does not inseminate any of the females, it is most unlikely that he would do so where a choice is possible. There would, therefore, be no point in carrying out sexual preference tests on these thirty combinations, for the results already indicate that sexual isolation is complete. There were four crosses in which a single female was found to have been inseminated. Such cases cannot be attributed to experimental error, because in each instance the vagina of the dissected female had an old reaction mass of the heterogamic type. There would also not be much point in running sexual preference tests on crosses in which a single female had been inseminated in heterogamic matings. We have, however, tested two of the four such crosses listed in the table. This makes a total of twenty-four crosses to be tested for sexual preference by the multiple choice technique. The results obtained are given in Table 3.

TABLE 3

Number of females dissected *(n)* and per cent *(%)* inseminated in various interspecific crosses of the mulleri subgroup

Females	Males	Homogamic		Heterogamic		χ^2	Isolation index
		n	%	*n*	%		
1. mulleri, aldrichi	mulleri	100	78	100	6	118.4	0.85
2. aldrichi, mulleri	aldrichi	100	63	100	4	78.1	0.88
3. arizonensis, mulleri	arizonensis	100	61	100	5	70.9	0.85
4. buzzatii, mulleri	buzzatii	100	92	100	0	170.4	1.00
5. hamatofila, mulleri	hamatofila	100	77	100	3	114.1	0.92
6. mojavensis, mulleri	mojavensis	100	75	100	11	83.6	0.74
7. arizonensis, aldrichi	arizonensis	100	51	100	10	39.6	0.67
8. hamatofila, aldrichi	hamatofila	100	75	100	3	108.9	0.92
9. mojavensis, aldrichi	mojavensis	100	83	100	20	88.6	0.61
10. aldrichi, mojavensis	aldrichi	100	73	100	9	84.7	0.78
11. buzzatii, arizonensis	buzzatii	100	90	100	0	163.6	1.00
12. arizonensis, buzzatii	arizonensis	100	16	100	58	36.9	—0.56
13. hamatofila, arizonensis	hamatofila	100	63	100	6	74.0	0.82
14. arizonensis, hamatofila	arizonensis	100	74	100	2	110.0	0.94
15. mojavensis, arizonensis	mojavensis	100	89	100	10	124.8	0.79
16. arizonensis, mojavensis	arizonensis	100	60	100	61	0.21	—0.01
17. arizonensis, peninsularis	arizonensis	100	60	100	9	57.5	0.74
18. hamatofila, buzzatii	hamatofila	100	55	100	55	0.0	0.00
19. mojavensis, buzzatii	mojavensis	100	92	100	7	144.5	0.85
20. buzzatii, peninsularis	buzzatii	100	86	100	0	150.9	1.00
21. mojavensis, hamatofila	mojavensis	100	80	100	5	115.1	0.88
22. hamatofila mojavensis	hamatofila	100	64	100	13	54.9	0.66
23. hamatofila, peninsularis	hamatofila	100	72	100	9	82.3	0.77
24. hamatofila, ritae	hamatofila	100	78	100	0	127.8	1.00

The χ^2 values and the isolation indices for a large majority of these crosses show that the differences between the homogamic and heterogamic percentages are highly significant, and any extended comments on them would be superfluous. In four of the crosses (4, 11, 20, 24, Table 3) no heterogamic matings were obtained, although in the corresponding interspecific crosses, in which a choice of mate was not possible, some inseminations were found in each case (5, 27, 42, 49, Table 1). In two of these crosses a few hybrids were produced. In the *mulleri/buzzatii* cross (5) a few abnormal flies were obtained, and in the *arizonensis/buzzatii* cross (27) only inviable larvae were found. Of the other two crosses, the *peninsularis/buzzatii* cross (42) yielded a single inseminated female, and in the *ritae/hamatofila* cross (50) ten inseminated females were found. However, the isolation indices of the sexual preference tests indicate that sexual isolation is complete for these four combinations.

Another point of interest is that *arizonensis* males prefer *buzzatii* females to their own (12). The χ^2 value of 36.9 and the negative isolation index of —0.56 both indicate a very significant difference between the homogamic and heterogamic matings. A negative isolation index is not unusual. Dobzhansky *et al.* have reported several such cases in their studies on different forms, and in the preceding article we found one case between *americana* and *texana*, as well as several others between geographic strains of *D. virilis*. The males of *arizonensis* also show a preference for *mojavensis* females, but the difference of percentages is without significance. Finally *hamatofila* males inseminated *buzzatii* females and their own with equal frequency.

The extent to which sexual isolation is responsible for preventing the exchange of genes between the different members of the mulleri subgroup can be emphasized by reference to the data listed in Table 1. A total of 5,690 females of the fifty-six crosses were dissected, and it was found that 636 had been inseminated. This gives a percentage of slightly over eleven. Therefore, approximately 89% of the opportunities for gene exchange were prevented by sexual isolation. It is well recognized that one cannot measure exactly the effect of sexual isolation in nature. However, these experiments demonstrate the qualitative effectiveness of sexual isolation, and also the quantitative effectiveness within the limits of experimental procedure.

As one would expect, sexual isolation is more effective in preventing gene exchange in some crosses than in others. There were thirty combinations in which no inseminations were found among 3,081 dissected females. Moreover, the different species vary considerably in their reluctance to mate with other forms. In Table 4 are summarized the data which shows these differences. It shows for each species the number of crosses involved, the number of combinations in which its females had been inseminated by other males, and the number of combinations in which its males had inseminated females of other species. The per cents

given are based on two hundred dissected females for each individual cross.

TABLE 4

Species	Number of crosses	Females combinations	Females % inseminated	Males combinations ♀♀	Males %inseminated
hamatofila	11	4	4.1	7	18.4
arizonensis	9	3	11.1	6	34.8
mojavensis	8	3	37.3	5	18.6
buzzatii	7	3	51.6	4	0.5
aldrichi	6	4	17.5	2	15.0
mulleri	6	5	9.6	1	12.0
peninsularis	3	3	12.0	0	0.0
ritae	2	1	5.0	1	0.5

Drosophila hamatofila leads the list in the number of crosses in which it was involved. Its females were inseminated by the males of four other species, and its males inseminated the females of seven different species. The highest per cent of females inseminated by other males was *buzzatti* females, with a percentage of 51.6 in three combinations. But the per cent of females inseminated by *buzzatii* males was one of the two lowest, with a percentage of only 0.5 in four combinations. The six combinations with *arizonensis* males gave the highest per cent (34.8) for inseminations of alien females.

The results obtained in this series of experiments make it clear that sexual isolation is a real barrier to gene exchange between the members of the mulleri subgroup. It represents one of the most important factors in the evolutionary divergence of this group of species. This conclusion is supported by the work of other investigators on several other different groups of Drosophila species. It is not, however, the only barrier to gene transfer. There are several other isolating mechanisms operating in the mulleri subgroup, including the effects of the insemination reaction. All of these will be considered in the next article.

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Other references to the literature on sexual isolation will be found at the end of Article I.

III. THE INSEMINATION REACTION AND ITS BEARING ON THE PROBLEM OF SPECIATION IN THE MULLERI SUBGROUP

J. T. PATTERSON

INTRODUCTION

In a recent article the writer (Patterson 1946) presented a brief account of the changes which take place in the female reproductive tract in some species of Drosophila immediately following copulation. The changes occur mainly in the vagina, and the term "insemination reaction" was used to designate this phenomenon. In the present article it is proposed to give a much fuller account of this reaction and its effect on reproduction among members of the mulleri subgroup of species. The same Drosophila forms are included in this paper as were employed in the preceding article of this publication, namely *aldrichi, arizonensis, buzzatii, hamatofila, mojavensis, mulleri, peninsularis,* and *ritae.* We shall also have occasion to refer to the data included in the preceding article, especially those listed in Table 1 of that paper.

The insemination reaction occurs both in intraspecific and interspecific crosses. It usually follows immediately after coitus, but in some forms it may begin before copulation is completed. The reaction is revealed in the vagina through a rapid secretion of fluid into its cavity, resulting in an increase in size to three or four times normal. In females from homogamic matings the vagina returns to a normal condition, usually within a period of eight or nine hours, but in those from heterogamic matings it may remain swollen for several days. In the latter type of mating this reaction may greatly reduce the number of hybrids produced, and in some crosses may eliminate them altogether, even though the female has been inseminated.

The term "reaction mass" was used in the preliminary paper to designate the contents of the swollen vagina. It is especially appropriate as a descriptive term for heterogamic matings in which the contents of the vagina persist for a long time as a very definite and discrete structure. As will be shown later, the reaction mass in homogamic matings remains soft and is eventually expelled by the female. Consequently, it does not materially affect the production of progeny. Its long retention in the vagina by females of heterogamic matings, accompanied by a change in composition from a soft to a hard, and often, resistant object may prevent the fertilization of the eggs, resulting in a failure of hybrid production.

In this and the succeeding article we are using the term vagina to designate that part of the female reproductive tract which extends posteriorly from the base of the median oviduct to the ovipositor. However, considerable confusion exists in the literature concerning the terminology of the different parts of the female apparatus in Drosophila, especially

with reference to the use of the terms uterus and vagina. Some authors refer to that part mentioned above as the vagina (Snodgrass 1935), others refer to it as the uterus (Pantel 1910), and still others would divide this region into two parts, a uterus proper and a vagina (Nonidez 1920, Wigglesworth 1939, Anderson 1945). Where this division has been suggested, the posterior part of the duct has been called the vagina, and the anterior part the uterus. Some authors have suggested that a uterus is present only in forms in which eggs are incubated (Townsend 1911).

There is need for a study of the histology of the vagina of these forms, not only of the normal condition, but also of the changes which occur during the formation of the reaction mass. We have made sections of a limited number of stages, but the preparations were inadequate for a detailed study. They show many of the histological features described by Nonidez for this organ in virgin females of *D. melanogaster*. It is, therefore, necessary to defer giving an account of the histological changes which accompany the insemination reaction until a later date.

In making preparations of whole mounts of the vagina for study of the insemination reaction, we have used the following procedure. The etherized female is placed on her side in a large drop of saline solution on a clean slide. The dissection is made by means of a pair of very fine needles. One needle is placed through the thorax and the other through the posterior tip of the abdomen, and by a gentle, but steady, pull the entire reproductive tract can be removed except the ovaries, which break off at the upper end of the median oviduct. After the removal of any adhering materials, a cover slip is placed on the preparation, which is then ready for examination under the microscope. The preparation is first examined under the 16 mm. objective, but for a detailed study the 4 mm. objective is used. The use of a large drop of saline is recommended because otherwise the weight of the cover slip may cause the contents of the vagina to be expelled, either through the posterior orifice or the broken end of the oviduct. In the preparation of a large, turgid vagina it is often necessary to support the cover slip by bits of glass in order to prevent the expulsion of the reaction mass. For observations on the movement of the spermatozoa and the formation of the reaction mass, it is desirable to have the vagina somewhat flattened by the weight of the cover glass. If the above procedure is followed the reproductive tract will lie on its side, which is the best position for a detailed examination.

The photomicrographs with which this, and the next article by Mr. Wheeler, are illustrated were made by transmitted light, using a 16 mm. objective and a Leitz 8X Periplan ocular. The films were developed in a strong contrast developer and the prints were made on either No. 2 or No. 3 glossy paper. Neither the negatives nor the prints have been retouched, except in three cases (Figs. 5, 30, 45) in which air bubbles or a piece of tissue in the background, and external to the specimen, were blotted out on the negative with opaque. All of the photographs were

made at the same magnification from freshly dissected, unstained specimens, and as reproduced in the plates are enlarged about seventy-eight times.

The plates are numbered consecutively in the two articles, as are also the figures. The time given for the ages of the different specimens in the legends to these figures in Plates I–IV and XI–XIII in this article were accurately determined by direct observation and for each specimen it represents the elapsed time from the end of copulation to the beginning of the dissection. In Plates V–X, unless otherwise stated, the ages given represent the period during which the males and females were exposed to each other, and consequently indicate the approximate ages of the different specimens.

HISTORY OF THE INSEMINATION REACTION IN HOMOGAMIC MATINGS

The duration of the insemination reaction in homogamic matings is relatively short and does not exceed nine hours for any of the eight species studied. The method followed was to place one female with four or five males in a vial and watch until copulation had taken place. As soon as coitus was completed, the female was separated from the males and a series of such females was dissected from time to time throughout the duration of the reaction. A record of the length of copulation was kept for each female, and from these data the average time was derived. In some species the insemination reaction begins before the end of coitus, and in such forms it was necessary to make dissections before copulation had been completed. This procedure made it possible to determine the exact time at which the vagina begins to enlarge. The facts thus accumulated are rather extensive and too large to present in any great detail. They are, therefore, shown in condensed form in Table 1.

TABLE 1

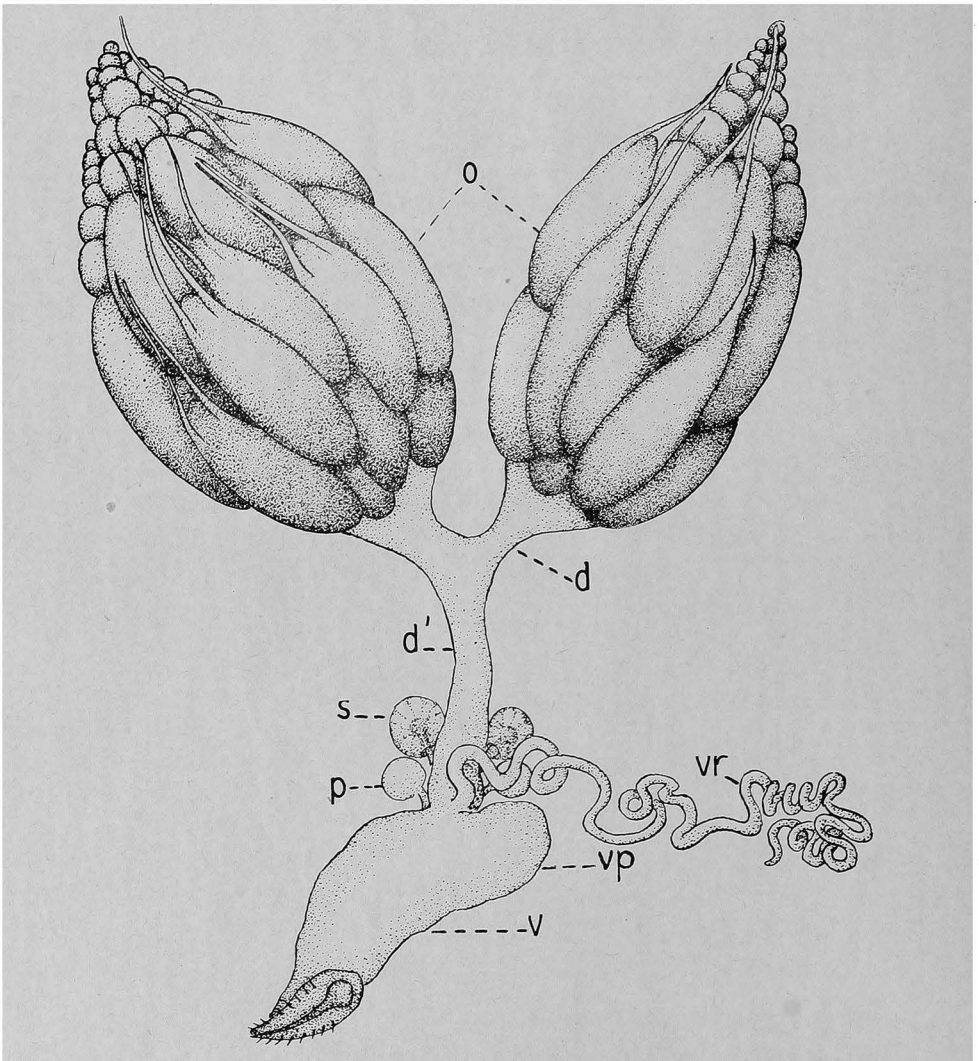
Duration of copulation and the insemination reaction in homogamic matings

Species	Duration of copulation range	Duration of copulation mean	Duration of insemination reaction begins at	Duration of insemination reaction ends at
D. aldrichi	1′20″–5′	3′27″	1′0″*	9 hours
D. arizonensis	30″–2′10″	1′34″	3′50″	9 hours
D. buzzatii	1′–3′10″	2′4″	6′0″	8 hours
D. hamatofila	5′–10″	7′3″	4′30″*	9 hours
D. mojavensis	1′20″–5′	3′27″	2′52″	9 hours
D. mulleri	30″–3′	1′35″	1′30″	8 hours
D. peninsularis	1′20″–2′5″	1′58″	6′0″	6 hours
D. ritae	6′–9′15″	8′0″	5′20″*	8 hours

There is considerable variation in the duration of copulation, both within the same species and among the different species. The shortest time of only thirty seconds was found in *arizonensis* and *mulleri,* which show about the same average time. The longest durations occurred in

hamatofila and *ritae,* and they also have the highest averages. The range and mean times are identical for *aldrichi* and *mojavensis.* In column four is given the time at which the insemination reaction begins in the different species. The figures represent the elapsed time from the beginning of copulation to the start of the reaction. In three species (marked, *) the reaction began before coitus was completed. With the exception of one species, the duration of the insemination reaction was either eight or nine hours. The exception was *peninsularis,* in which the reaction lasted for only six hours. By the end of these periods the vagina has lost its opacity and returned to its original size.

Before giving the more interesting facts observed in the study of homogamic matings, reference to the accompanying text-figure should



Text-figure. Camera lucida drawing of the female reproductive tract of *D. buzzatii.* d, oviducts; d', median or azygos oviduct; o, ovaries; p, parovaria; s, spermathecae; vr, ventral receptacle; v, vagina; vp, vaginal pouch.

be made. The figure illustrates the various parts of the female reproductive system of *D. buzzatii,* and is more or less typical of this system for all members of the mulleri subgroup. The paired oviducts (d) arise from the bases of the ovaries (o) and soon fuse to form the median, or azygos, oviduct (d'). At the lower end of this duct there arise from its dorsal side the paired spermathecae (s) and the paired parovaria (p), the former slightly anterior to the latter. At about the level of the stalks of the parovaria, but on the ventral side of the median oviduct, there arises the long coiled ventral receptacle (vr). Just posterior to the points of origin of the parovaria and the ventral receptacle the duct abruptly enlarges to form the vagina (v). In this, and many other species of Drosophila, the vagina extends antero-ventral to form what is known as the vaginal pouch (vp). The wall of this region is much thinner than that of other parts of the vagina and is capable of great distention. It is here that the swelling caused by the insemination reaction chiefly takes place.

The figures in Plates I and II illustrate the effects of the insemination reaction on the vaginae of the eight species used in this study. Their dark color is due to the opacity of the reaction mass within their cavities. Viewed through a binocular microscope, the specimens appear opaque white, but by transmitted light they photograph black.

Figure 1 shows the reaction in *D. aldrichi* at one hour after copulation. The specimen had been intentionally flattened slightly by pressure on the cover glass in order to bring out more clearly the granular appearance of the reaction mass. This species is one in which the reaction begins before the end of copulation. In a series of dissections of four *aldrichi* females, all made before the termination of coitus, the following observations were recorded: (1) At thirty seconds after the start of coitus the vagina contained no sperm, and was clear and normal; (2) after one minute the vagina contained many sperm in its cavity and was beginning to enlarge; (3) after one minute and thirty seconds it had increased slightly in size, with the sperm surrounding a central core of reaction material; finally, after two minutes and fifteen seconds, the vagina was greatly swollen and some of the motile sperm had been expelled. One female, which was dissected five minutes after the end of copulation, had a very large, turgid and slightly opaque vagina.

Figure 2 illustrates the condition of the vagina of *D. arizonensis* thirty minutes after copulation. In this species the swelling does not begin until after copulation has been completed, and the vagina never becomes as large as in *aldrichi.* It reaches its maximum size in about an hour and fifteen minutes, and soon thereafter begins to shrink and clear up, but does not return to a normal condition until about nine hours.

Figure 3 shows the inseminated vagina of *D. mulleri* at the end of one hour. It was much larger than is indicated in this picture, but "exploded" just as the photograph was being made and expelled a considerable quantity of its contents, which appear as a cloudy mass about the lower end

of the figure. Accidents of this kind occur frequently in handling such large turgid vaginae. Usually, however, any expulsion of the contents takes place through the vaginal orifice, rather than through a rupture of the wall. Another case of this kind is seen in Figure 2.

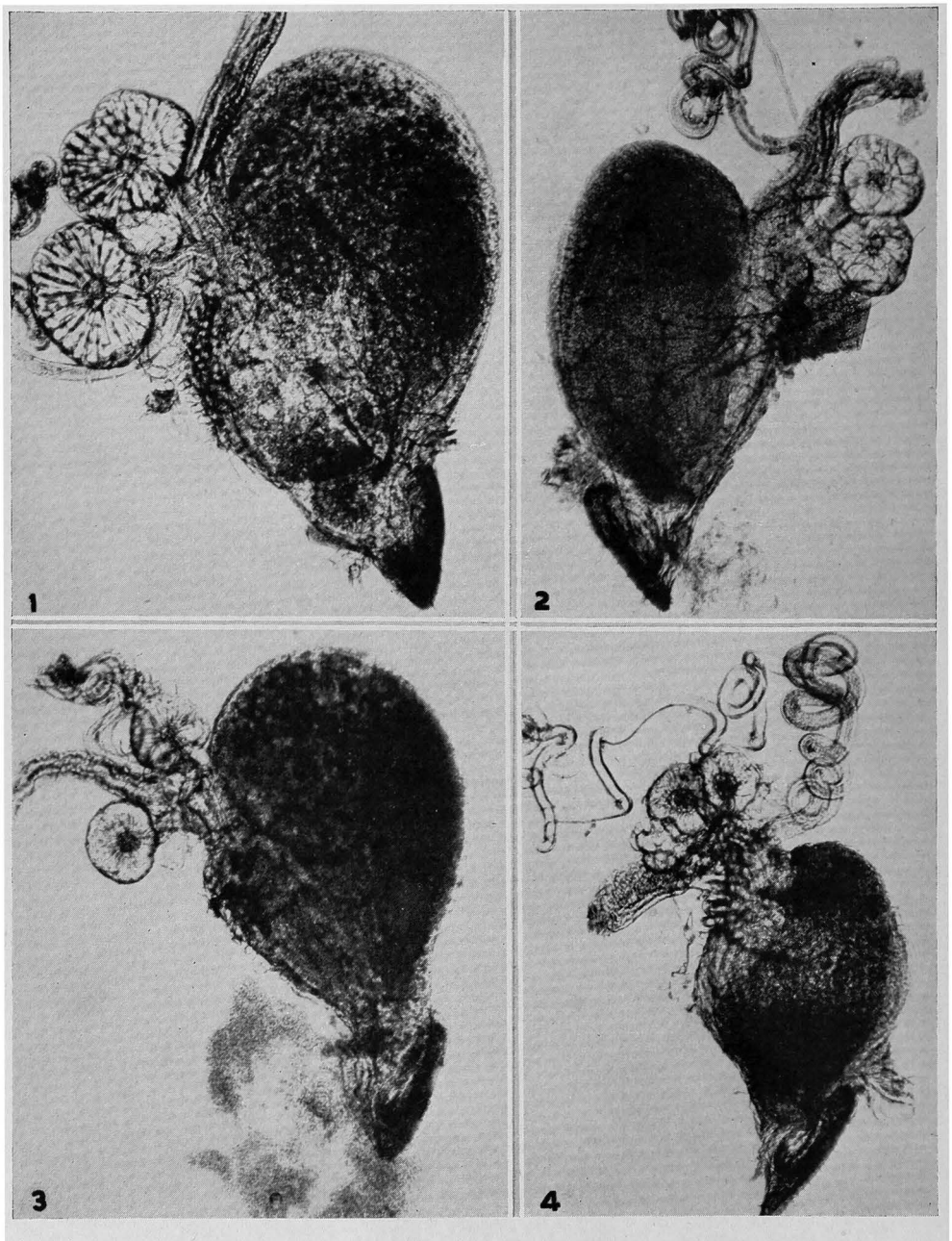


PLATE I. Vaginae from homogamic matings of four species of the mulleri subgroup. Fig. 1, *D. aldrichi* at one hour. Fig. 2, *D. arizonensis* at thirty minutes. Fig. 3, *D. mulleri* at one hour. Fig. 4, *D. peninsularis* at forty minutes.

The inseminated vagina of *D. peninsularis* is illustrated in Figure 4. While the photograph was taken only forty minutes after the end of copulation, yet the vagina had reached approximately its maximum size. It never does become as large as in the other members of the mulleri subgroup, and returns to a normal condition in about six hours.

Figure 5 (Plate II) illustrates the inseminated vagina of *D. mojavensis* at one and one-half hours. It reaches its maximum size one hour after copulation and remains large until some time during the fourth hour. From this time on it gradually clears and decreases in size, and by the end of nine hours it appears entirely normal.

Figure 6 shows the enlarged vagina of *D. buzzatii* one hour after the end of copulation. Forty-seven females of this species in a timed series were dissected, beginning immediately after coitus and extending through the 135th hour. Examinations of the reproductive tracts of these females showed that all except three had been inseminated. After the sixth hour all sperm were found to be located in the ventral receptacle, and none in the rest of the tract. The vagina was cleared and normal by the end of the eighth hour. The females began laying eggs at about seventy-two hours after mating, and offspring developed in a large majority of the retained food vials.

One of the most interesting features of the homogamic mating of *D. hamatofila* is the duration of copulation, which on the average lasts over seven minutes. It is one of three species of the mulleri subgroup in which the insemination reaction begins before the end of copulation. The vagina has reached its maximum size within thirty minutes after copulation, and the finger-shaped reaction mass has become extremely opaque (Fig. 7, Plate II).

The longest time of copulation of any member of the subgroup is found in *D. ritae*. However, a more striking feature of the homogamic mating in this species is the peculiar type of reaction mass. It is also elongated, but as viewed from the ventral side, as shown in Figure 8, it has two constrictions which incompletely divide the mass into three parts of about equal size. The history of the insemination reaction in *ritae* deserves a somewhat fuller description, and the following account is a direct quotation from the notes made at the time the tests were carried out.

"This species is especially favorable for a study of the insemination reaction, both because of the speed at which it occurs and the nature of the opacity of reaction mass. In *ritae* and some other forms this mass soon becomes intensely opaque and appears black by transmitted light. A closely graded series of stages was studied, based on the duration of copulation. The average duration of copulation is about eight minutes. In females dissected immediately following copulation the reaction mass is already well formed, with each end dark and the middle section grayish. In order to observe the beginning of the process, it was necessary to dissect females before coitus had been completed. It was found that sperm had not been transferred up to about five minutes after the be-

ginning of copulation. The first female found with sperm in her vagina had been in copula for five minutes and twenty seconds, and in this specimen it was possible to follow the progress of the formation of the reaction mass. When first examined under the microscope, the semen

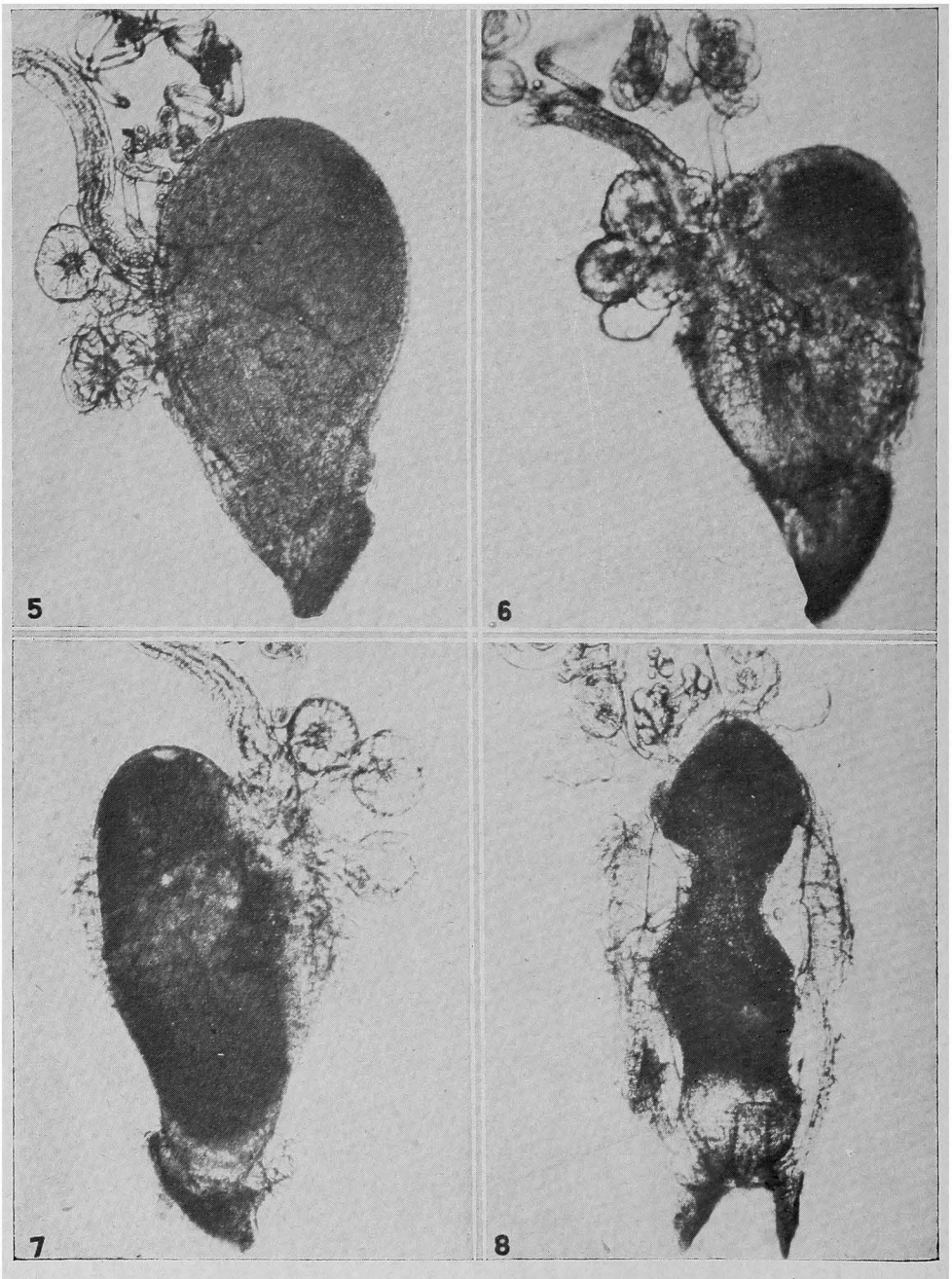


PLATE II. Vaginae from homogamic matings of four other species of the mulleri subgroup. Fig. 5, *D. mojavensis* at one and one-half hours. Fig. 6, *D. buzzatii* at one hour. Fig. 7, *D. hamatofila* at thirty minutes. Fig. 8, *D. ritae* at one hour.

with its sperm was located at the posterior end of the vaginal cavity. Within a very short time the sperm began moving forward through the cavity, and by the end of three minutes they had reached the anterior end, with some of them passing up a short distance into the proximal end of the median oviduct. The formation of the reaction mass followed in the wake of the advancing semen, and by the end of five minutes the appearance of the mass was very similar to that found in females dissected shortly after copulation."

In Plate III are displayed a series of stages showing the changes which take place in the vagina of females from homogamic matings of *D. aldrichi.* Figure 9 is from a virgin female of the same age (five days) as those which were inseminated, and from which Figures 10 to 12 were made. The vagina illustrated in Figure 10 was dissected three and one-half hours after the end of copulation. It was very opaque and represents the maximum size reached by this organ during the history of the insemination reaction. By the end of five hours (Fig. 11) much of the reaction material had been expelled, including any sperm that had failed to enter the ventral receptacle. The condition of the vagina at seven hours after insemination is revealed in Figure 12. The vagina is still slightly enlarged and all but a small amount of scattered material has disappeared. It would have taken about two hours longer, or nine hours in all, for this vagina to return to the condition seen in Figure 9.

The history of the insemination reaction in homogamic matings is essentially the same in all species which have been carefully studied by us. There are, however, certain variations in the process, such as the time at which the reaction begins, the length of its duration, and the character of the reaction mass, but these are minor details. In all species examined the vagina eventually returns to a normal condition, that is, it simulates the transparency and size of this organ in virgin females. In the preliminary article (Patterson 1946) it was pointed out that the contents of the swollen vagina, consisting of excess sperm and reaction material, are expelled in the form of whitish droplets which are easily detected on the food or sides of the container. If examined immediately after deposition under the microscope, such droplets are seen to contain motile sperm. This habit on the part of females has been observed several times in four different species. It may therefore be assumed that this is the principal method of bringing about the return of the vagina to a normal condition.

In most species which have the insemination reaction, the female usually does not remate for some time, and in heterogamic matings she may never remate. This subject will be discussed further in a later section on the mating habits of inseminated females.

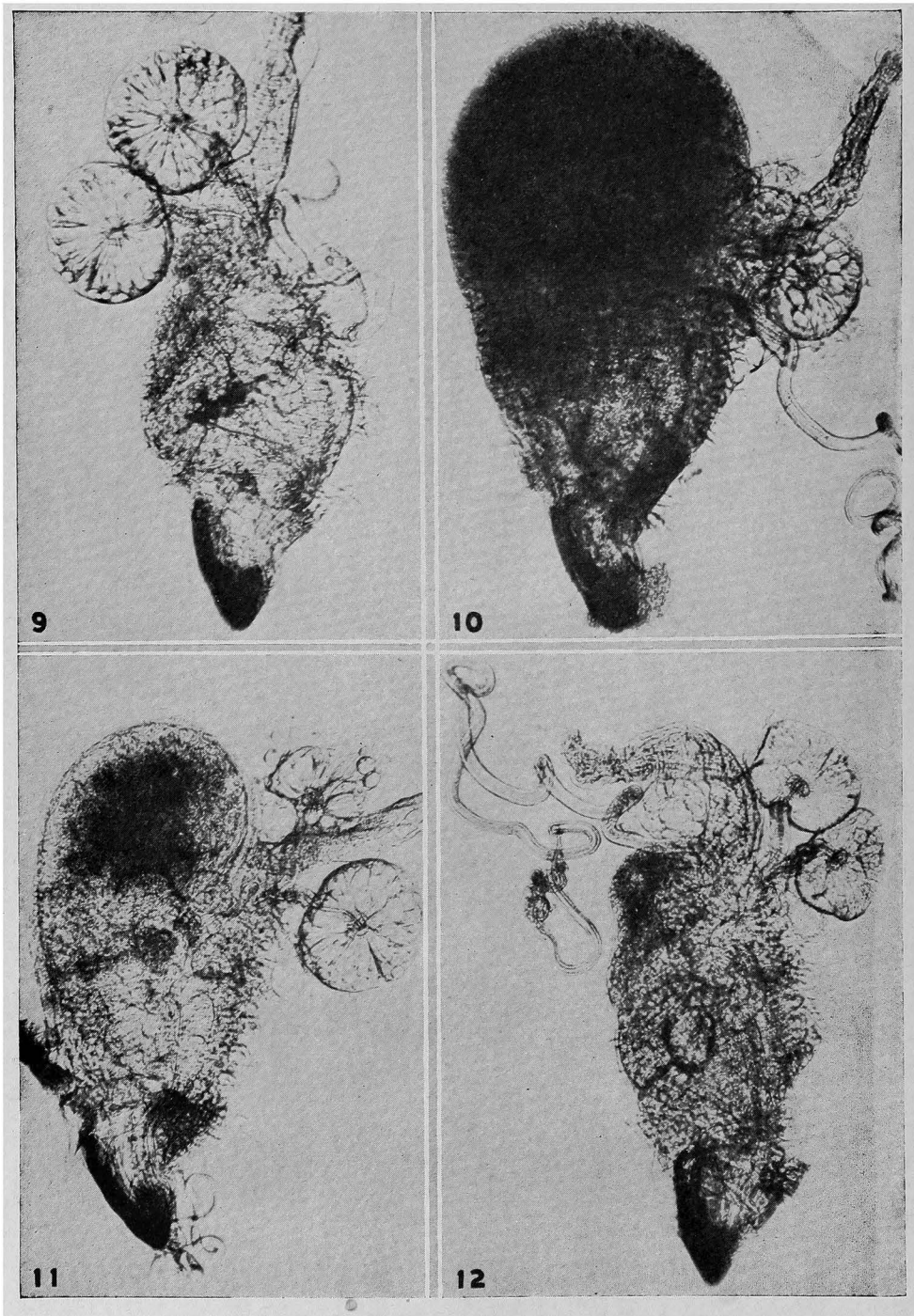


PLATE III. Vaginae of virgin and three homogamic matings of *D. aldrichi*. Fig. 9, virgin. Fig. 10, at three and one-half hours. Fig. 11, at five hours. Fig. 12, at seven hours.

HISTORY OF THE INSEMINATION REACTION IN HETEROGAMIC MATINGS

The early stages of the insemination reaction in heterogamic matings are very similar to those occurring in intraspecific crosses. The vagina undergoes about the same changes in both types of crosses during the first few hours after inseminaton. The reaction progresses somewhat more rapidly in the heterogamic type, and the vagina is frequently more densely opaque. It takes much longer for the sperm to move forward to the point of origin of the ventral receptacle, usually requiring about forty-five minutes to reach this point as compared with five to ten minutes in the homogamic type. Furthermore, the spermatozoa frequently form a tangled mass about the opening to the ventral receptacle. This has the effect of retarding or inhibiting their entrance into the lumen of the receptacle, often resulting in only a few sperm gaining access to this organ, and in some cases, none at all. The sperm which finally succeed in entering the receptacle become located at the distal end of the long, coiled receptacle.

The excess of spermatozoa left in the vagina usually disappears by the end of seven hours, although small groups of non-motile sperm have been observed after this period in a few cases. Their fate has not been definitely determined. We have found no conclusive evidence that they are expelled as in intraspecific crosses. It is possible that these sperm have been digested, and the products either absorbed by the reproductive tract, or else included in the reaction mass. It has been reported that such excess sperm are digested and absorbed in a few other insects. This is said to be the case for the beg-bug *Cimex* (Abraham 1934, Cragg 1920, 1923), and also for the bug *Graphosome lineatum* (Hanrlirsch 1900), as reported by Wigglesworth in his book, *The Principles of Insect Physiology.*

The sperm that are located at the distal end of the ventral receptacle may remain alive for several days. Motile sperm have been observed in the receptacles of females dissected as late as 168 hours after copulation. In many interspecific crosses these sperm never fertilize the eggs, due to the deleterious effects of the insemination reaction and its resulting reaction mass. Even though this mass may finally disappear, the vagina is usually left too severely damaged to permit the passage and insemination of the egg from the oviduct to the exterior. The eggs may disintegrate in the cavity of the vagina, but if they succeed in passing this barrier and are laid, smear preparations show that they have not been inseminated. The consequence is that the female, although fertilized, cannot produce hybrid offspring.

In the series of crosses between members of the mulleri subgroup of species every degree of the severity of this reaction was encountered. At one end of this series, in the *buzzatii/arizonensis* cross, hybrids were never produced, although 88% of the dissected females were found to contain sperm in their receptacles. At the opposite end of the series, in

the *mojavensis/arizonensis* cross, about 75% of the females produced hybrids, while 93% of the females had been inseminated. Here, not only is there a reduction of 18% in the number of females producing offspring, but also the number of offspring per culture is reduced much below that found in homogamic matings. Between these two extremes, various degrees of reduction of hybrid production were found among the different crosses. These will be described in the following pages in conjunction with the photomicrographs.

The data for the twenty-six crosses in which one or more females had been inseminated in heterogamic matings are summarized in Table 2.

TABLE 2

Condition of the reaction mass, vagina, and motile sperm after the males and females had been exposed to each other for ninety-six hours

	Crosses	Inseminated females	Reaction mass	Vagina normal	Motile sperm	Hybrids produced
1.	aldrichi ♀ × arizonensis ♂	36	28	8	24	yes
2.	aldrichi ♀ × hamatofila ♂	14	14	0	0	none
3.	aldrichi ♀ × mojavensis ♂	66	46	20	40	yes
4.	aldrichi ♀ × mulleri ♂	24	24	0	5	none
5.	mulleri ♀ × aldrichi ♂	36	20	16	18	yes
6.	mulleri ♀ × arizonensis ♂	13	9	4	8	yes
7.	mulleri ♀ × buzzatii ♂	2	1	1	1	few
8.	mulleri ♀ × hamatofila ♂	13	12	1	2	yes
9.	mulleri ♀ × mojavensis ♂	32	22	10	18	yes
10.	arizonensis ♀ × buzzatii ♂	1	0	1	1	larvae
11.	arizonensis ♀ × hamatofila ♂	27	27	0	3	none
12.	arizonensis ♀ × mojavensis ♂	39	16	23	19	yes
13.	buzzatii ♀ × arizonensis ♂	156	156	0	97	none
14.	buzzatii ♀ × hamatofila ♂	129	129	0	2	none
15.	buzzatii ♀ × mojavensis ♂	25	25	0	14	none
16.	hamatofila ♀ × arizonensis ♂	7	7	0	2	none
17.	hamatofila ♀ × buzzatii ♂	1	1	0	1	none
18.	hamatofila ♀ × mojavensis ♂	24	11	13	16	none
19.	hamatofila ♀ × ritae ♂	1	1	0	0	none
20.	mojavensis ♀ × aldrichi ♂	24	24	0	10	none
21.	mojavensis ♀ × arizonensis ♂	154	97	57	80	yes
22.	mojavensis ♀ × hamatofila ♂	46	46	0	0	none
23.	peninsularis ♀ × arizonensis ♂	52	52	0	24	none
24.	peninsularis ♀ × buzzatii ♂	1	1	0	0	none
25.	peninsularis ♀ × hamatofila ♂	19	19	0	0	none
26.	ritae ♀ × hamatofila ♂	10	10	0	2	none

These figures were compiled from Tables 1 and 3 of the preceding article on sexual isolation. They show the condition of the reproductive tract ninety-six hours after the males and females had been placed together. The five columns, following the column giving the nature of the cross, indicate respectively the total number of inseminated females, the number with the reaction mass still present in the vagina, the number in which the vagina had cleared, the number with motile sperm in the ventral receptacles, and whether hybrid offspring had been produced.

An examination of these data shows that in all crosses in which hybrid offspring were not produced the vagina still contained a reaction mass. A single exception was the cross *hamatofila* ♀ × *mojavensis* ♂ (18). In that

cross thirteen of the twenty-four dissected females had returned to an apparently normal condition, and a few motile sperm were still present at the tip of the ventral receptacle. This cross never results in the production of hybrids, not even of the inviable larvae. In contrast to this, in all ten crosses which yielded hybrids, including inviable larvae, a large proportion of their vaginae were already cleared and normal by the end of ninety-six hours. In five of the crosses which failed to produce hybrids, the vagina of each contained a reaction mass and all sperm had disappeared from the ventral receptacle (2, 19, 22, 24, 25).

In the interspecific cross of *D. buzzatii* ♀ × *D. arizonensis* ♂, a timed series of sixty matings was obtained, and the dissections revealed that fifty-five of these females had been inseminated. This series gave a complete history of the insemination reaction for a period of ninety-six hours. For the first few hours after insemination the reaction is similar to that occurring in homogamic matings, but instead of clearing up in eight or nine hours, the vaginae retain the reaction mass for at least as long as 192 hours.

Figures 13 to 16 in Plate IV show four stages in the history of the insemination reaction for this cross. The specimen illustrated in Figure 13 was photographed twenty-seven hours after insemination. As the photograph clearly indicates, the reaction mass was very opaque and occupied the entire cavity of the vagina. The vagina remains essentially in this condition for the next twenty-five or thirty hours. At about fifty-six hours the reaction mass becomes distinctly pear-shaped, with its broad end occupying the vaginal pouch and then tapering down to a blunt point at the lower end of the vaginal cavity. By seventy-two hours, some of the reaction material begins to pass out through the vaginal orifice. This material is not suddenly expelled as in homogamic matings, but is best described as draining out gradually. Figure 14 illustrates the beginning of the drainage process. This continues up to ninety-six hours, when the reaction mass becomes a discrete object, sharply outlined, and largely confined to the vaginal pouch (Fig. 15). It remains in this condition through the 120-hour stage (Fig. 16), and, in most cases, for many hours thereafter. A large number of inseminated females have been dissected up to 192 hours after copulation, and in most of them the mass was still present. It may become smaller, either through further drainage or by disintegration, especially should the female begin laying eggs.

In three females, in which the reaction mass had decreased to about one-fourth the volume of the one illustrated in Figure 15, contractions of the dissected vagina in the saline medium had resulted in voiding the mass in two cases and a failure to do so in the third one. In the latter instance the reaction mass had become tightly wedged in the vaginal orifice. The possibility that a female may thus eventually eliminate the remnants of the reaction mass is supported by observations on a fourth case. The female in question had become moribund, and upon making

the dissection it was found that the vaginal orifice had become occluded by the old reaction mass. A few similar cases were observed in females in other interspecific crosses during the course of our dissections.

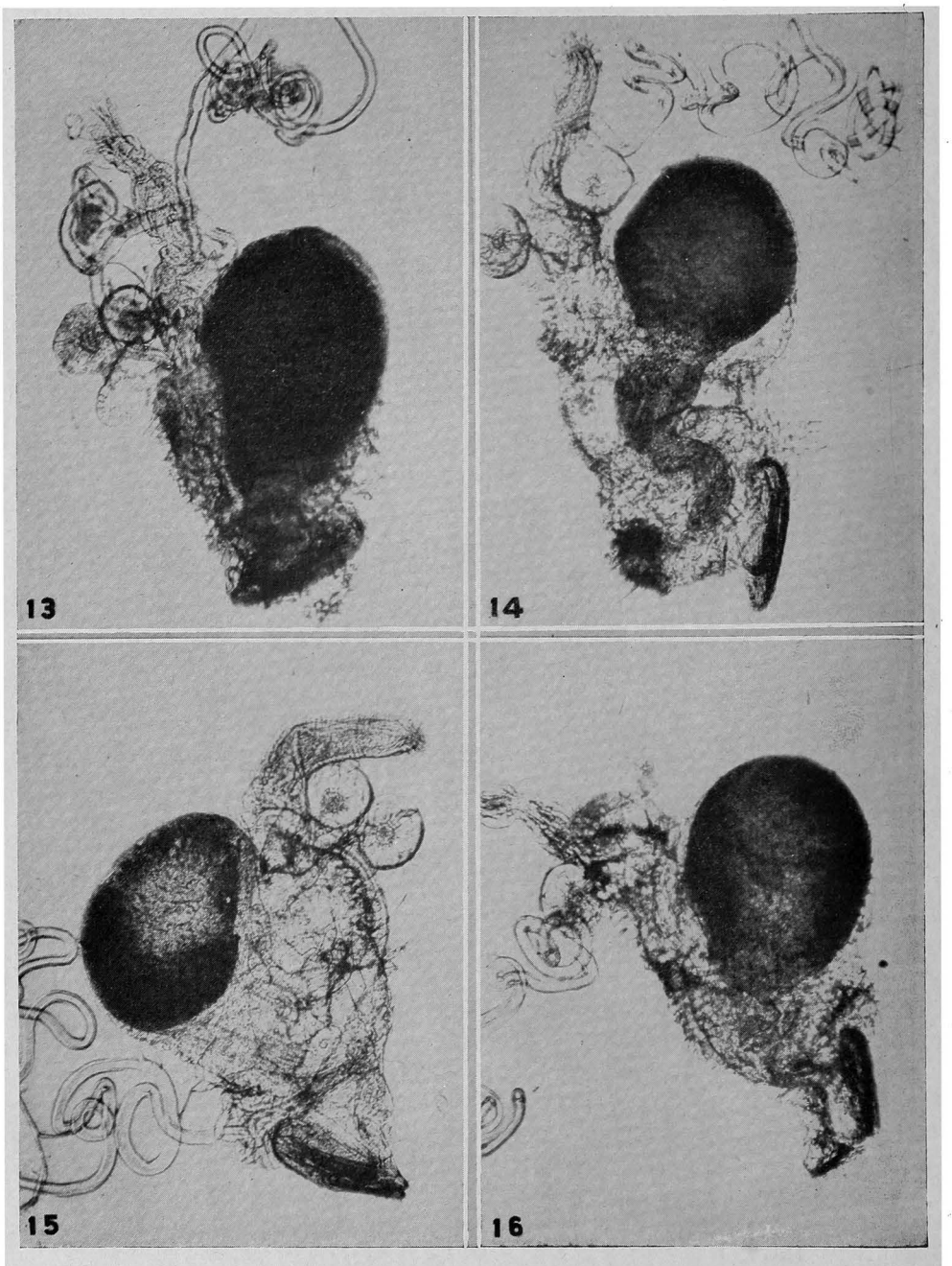


PLATE IV. Four stages of the reaction mass of heterogamic matings of *D. buzzatii* ♀ × *D. arizonensis* ♂. Fig. 13, at twenty-seven hours. Fig. 14, at seventy-two hours. Fig. 15, at ninety-six hours. Fig. 16, at one hundred and twenty hours.

It remains to comment on the fate of the sperm in the ventral receptacle, egg laying, and the possibility of hybrid production in the *buzzatii/arizonensis* cross. As shown in Table 2 (13), ninety-seven of the 156 inseminated females listed still contained motile sperm in their ventral receptacles at the end of ninety-six hours. A few have been found in this receptacle as late as the 168th hour, but eventually all such sperm disappear. In the meantime the females may lay a few abnormal eggs, but examination by the smear technique has failed to reveal a single spermatozoon in such eggs. Sometimes an ovulated egg will remain in the vagina and undergo disintegration. Three such eggs are illustrated in Plate X. In view of the facts just stated, it is not surprising that we have failed to obtain hybrid offspring from this cross, even though many tests have been carried out for this purpose.

In addition to the series from the *buzzatii/arizonensis* cross illustrated in Plate IV, photographs of the inseminated vaginae from twenty other interspecific crosses are shown in Plates V–IX. These are presented in the same order as these crosses are listed in Table 2, with the omission of five combinations (7, 10, 17, 19, 24) in which only one or two per cent of the dissected females were found to have been inseminated.

Females of *D. aldrichi* were inseminated by the males of four other species of the subgroup (Table 2, crosses 1–4). Illustrations of these crosses appear in Plate V. The *aldrichi/arizonensis* cross is shown at the seventy-two hour stage in Figure 17. The reaction mass is still large, but the drainage process is in progress, as is indicated by the broad band of reaction material extending down from the lower side of the mass to the posterior end of the vagina. Thirty-six inseminated females were obtained from this cross (1). Twenty-eight of these had some form of the reaction mass, and in only eight was the vagina entirely clear, although several others probably would have cleared in time. In twenty-four specimens the ventral receptacle contained motile sperm. In two of the females a disintegrating egg was present in the vagina. This cross yields sterile female hybrids.

Fourteen inseminated females were obtained in the *aldrichi/hamatofila* cross (2). A ninety-six hour stage is illustrated in Figure 18. The reaction mass was confined to the vaginal pouch in all fourteen cases, and in the one illustrated the last stages of drainage are indicated by the dark, irregular line extending down from the lower side of the mass. None of the vaginae were clear, and sperm were entirely absent from their ventral receptacles. This cross does not produce offspring.

A seventy-two hour stage of the *aldrichi/mojavensis* cross is shown in Figure 19. Sixty-six inseminated females were obtained from this cross (3), and the reaction mass was present in forty-six specimens. In twenty females the vagina was entirely clear, and forty had motile sperm in their ventral receptacles. In each of two females an abnormal egg was lodged in the vagina. This cross gives sterile females.

In the *aldrichi/mulleri* cross all twenty-four inseminated females had conspicuous reaction masses (Fig. 20), and consequently none of the

vaginae were normal. Only five of the females still had motile sperm in the ventral receptacle. There were five cases of disintegrating eggs in the vagina, and in one of these the debris of several eggs could be

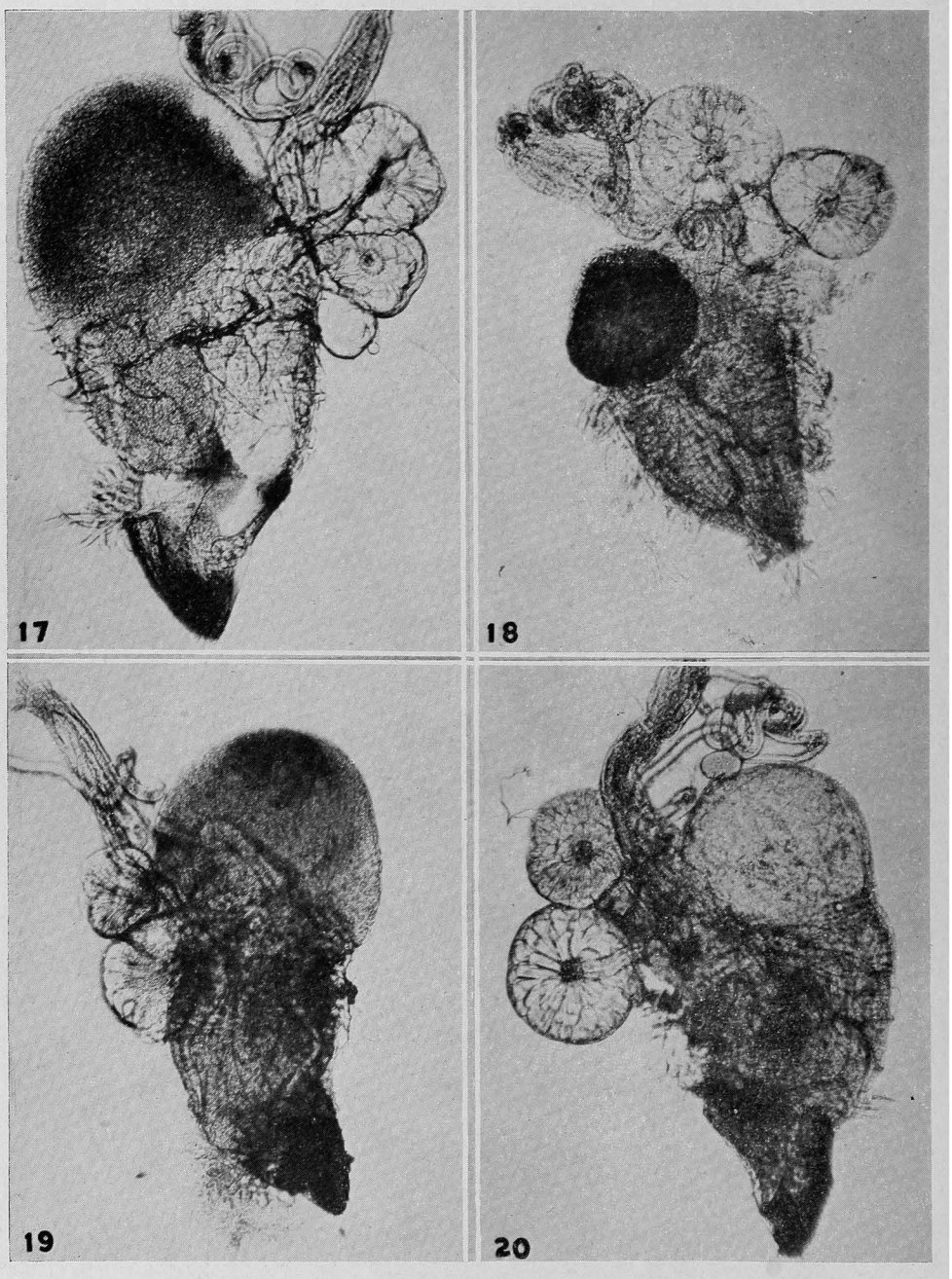


PLATE V. Reaction mass in vagina of *D. aldrichi* from heterogamic matings with four different types of males. Fig. 17, with *D. arizonensis* male at seventy-two hours. Fig. 18, with *D. hamatofila* male at ninety-six hours. Fig. 19, with *D. majovensis* male at seventy-two hours. Fig. 20, with *D. mulleri* male at ninety-six hours.

recognized by the presence of the egg filaments. This cross does not produce offspring.

The females of *D. mulleri* were inseminated by the males of five other species (Table 2, crosses 5–9). The affected vaginae of four of these

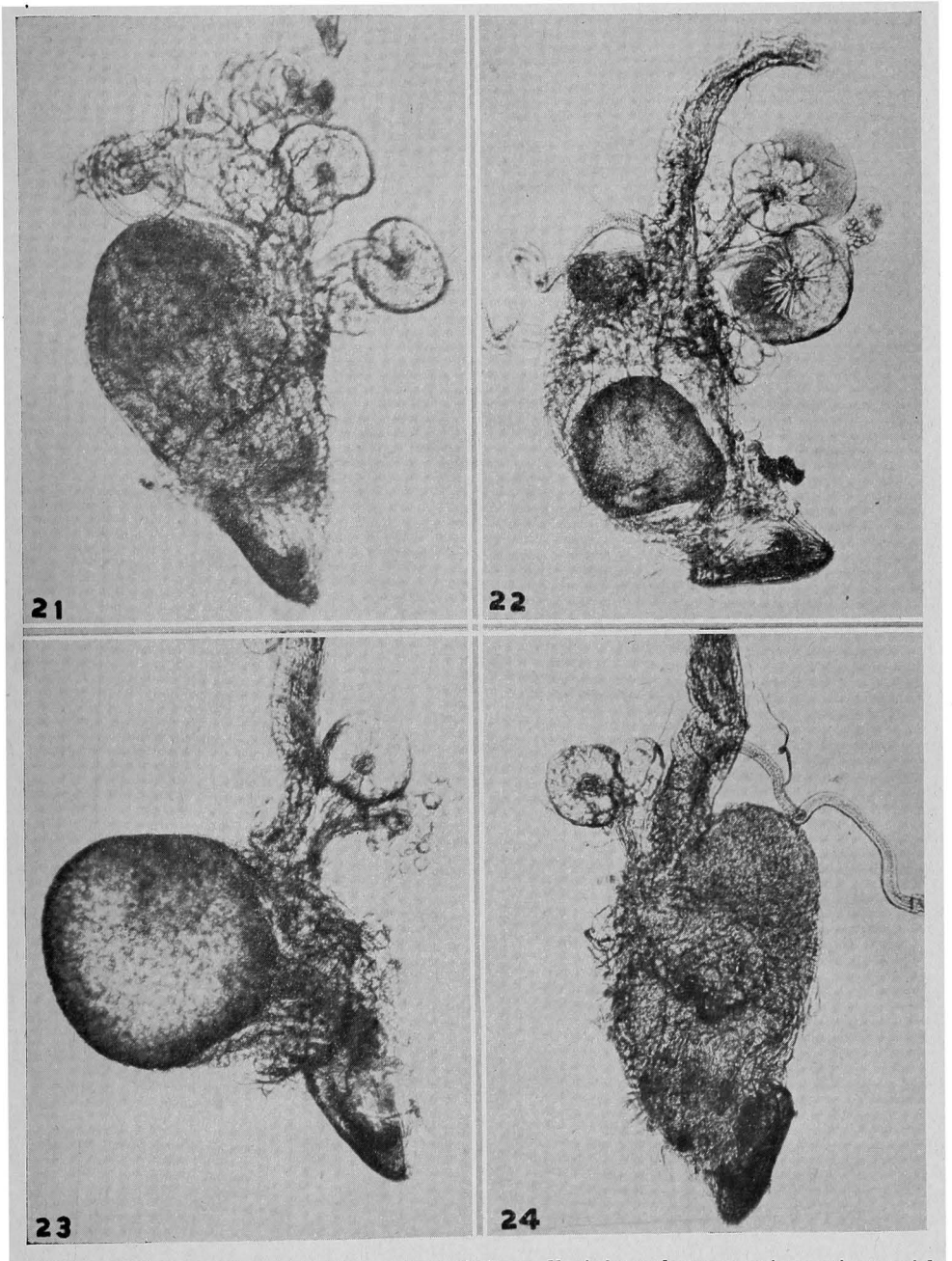


PLATE VI. Reaction mass in vagina of *D. mulleri* from heterogamic matings with four different types of males. Fig. 21, with *D. aldrichi* at seventy-nine hours. Fig. 22, with *D. arizonensis* male at seventy-eight hours. Fig. 23, with *D. hamatofila* male at ninety-six hours. Fig. 24, with *D. mojavensis male* at eighty hours.

are shown in Plate VI. The vaginae of twenty of the thirty-six inseminated females from the *mulleri/aldrichi* cross still showed the reaction mass (Fig. 21), and in the remaining sixteen they had cleared and were practically normal. Motile sperm were present in eighteen specimens. In each of two females with large reaction masses a degenerating egg was present in the vagina. This cross produces sterile males and females.

Only thirteen inseminated females were derived from the *mulleri/arizonensis* cross (6). Nine of these had reaction masses, and in the remaining four the vagina was entirely clear. Eight specimens had motile sperm in the ventral receptacle. This cross produces sterile male hybrids only. Figure 22 illustrates a seventy-eight hour stage.

The *mulleri/buzzatii* cross (7) yielded but two inseminated females in the experimental tests. In one of these the reaction mass was present, but in the other the vagina was clear and its ventral receptacle contained motile sperm. This cross gives a few abnormal flies in mass matings. It is not illustrated in the plates.

The *mulleri/hamatofila* cross (8) gave thirteen inseminated females, and by the end of ninety-six hours, twelve of these still had reaction masses (Fig. 23). In one the vagina was cleared, and in two motile sperm were present in the ventral receptacle. The writer has obtained from this cross, by the use of large mass matings, a few sterile males and females.

The *mulleri/mojavensis* cross (9) gave thirty-two inseminated females, of which twenty-two showed a reaction mass, and ten had the vagina clear. Eighteen females had motile sperm in their ventral receptacles. In each of five females, which still had the reaction mass, a degenerating egg was present in the vagina. This cross gives fertile females and sterile males. An affected vagina at eighty hours is illustrated in Figure 24.

The females of *D. arizonensis* were inseminated by the males of three other species (Table 2, crosses 10–12). The *arizonensis/buzzatii* cross gave but a single inseminated female in the experimental tests, and the vagina was clear by ninety-six hours and the ventral receptacle contained motile sperm. This cross produces hybrid larvae which die during mid-larval stages. In order to obtain such larvae, it was necessary to use large mass matings. This cross is not illustrated in the plates.

The *arizonensis/hamatofila* cross (11) yielded twenty-seven inseminated females, all of which had reaction masses, but only three with motile sperm in their ventral receptacles. Degenerating eggs were present in the vaginae of five females, and in one of these a second egg was present in the median oviduct. This cross never produces offspring. Figure 25 (Plate VII) illustrates the beginning of the drainage process at the seventy-two hour stage.

From the *arizonensis/mojavensis* cross thirty-nine inseminated females were obtained (12), of which sixteen showed the reaction mass and twenty-three had clear vaginae. Motile sperm were present in the ventral receptacle of nineteen females. The vagina clears up rather rapidly in females of this cross, and the reaction mass is not clearly delimited

(Fig. 26) as it is in certain other combinations. Fertile females and sterile males result from this cross.

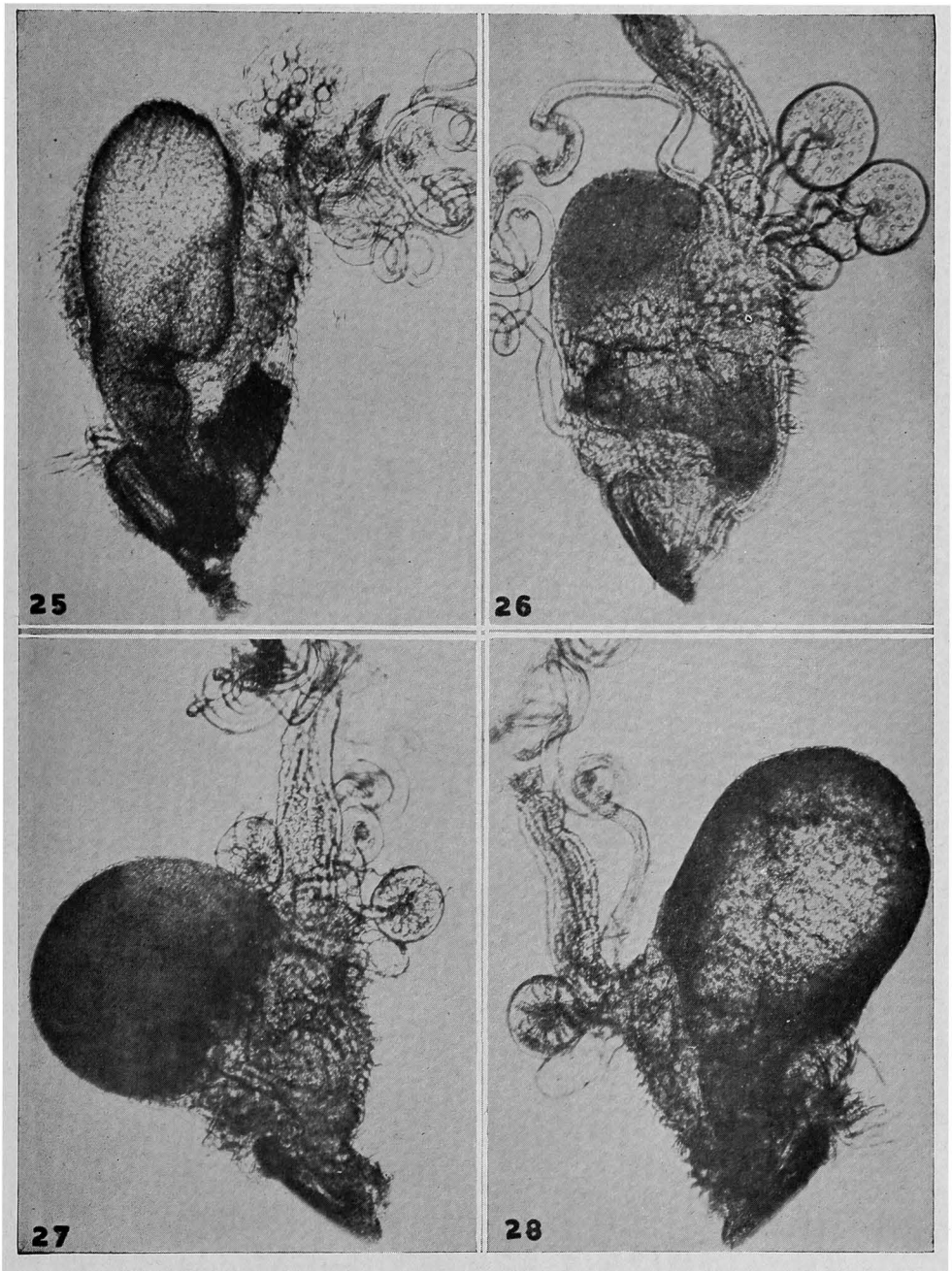


PLATE VII. Reaction mass in vaginae of *D. arizonensis* and *D. buzzatii*, each mated to two different types of males. Fig. 25, *D. arizonensis* ♀ × *D. hamatofila* ♂ at seventy-two hours. Fig. 26, *D. arizonensis* ♀ × *D. mojavensis* ♂ at seventy-two hours. Fig. 27, *D. buzzatii* ♀ × *D. hamatofila* ♂ at sixty-two hours. Fig. 28, *D. buzzatii* ♀ × *D.mojavensis* ♂ at seventy-nine hours.

The females of *D. buzzatii* were inseminated by the males of three other species (Table 2, 13–15). The *buzzatii/arizonensis* cross gave 156 inseminated females (13), all of which had distinct reaction masses at the ninety-six hour stage. This cross has been considered above in connection with the illustrations in Plate IV.

The *buzzatii/hamatofila* cross (14) produced 129 inseminated females, all of which showed a reaction mass. In only two specimens, both recent inseminations, were live sperm present in the ventral receptacle. In all other females the sperm had completely disappeared. The vagina of one female contained an abnormal egg. Figure 27 shows a typical reaction mass of this cross, which never results in the production of hybrids.

Twenty-five females had been inseminated in the *buzzatii/mojavensis* cross (15), and all of these had reaction masses. Fourteen females still had live sperm in the ventral receptacle. A degenerating egg was present in the vagina of four females. Figure 28 illustrates a seventy-nine hour stage. This cross never results in the production of hybrids.

The females of *D. hamatofila* were inseminated by the males of four different species (Table 2, crosses 16–19). In the *hamatofila/arizonensis* cross only seven females were found to have been inseminated (16), and their vaginae all contained a reaction mass. Figure 29 illustrates a seventy-two hour stage. The mass is still very opaque, and the female tract had been severely damaged. Offspring were not produced in this cross.

The *hamatofila/buzzatii* and *hamatofila/ritae* crosses each gave a single inseminated female, with the reaction mass still present (17, 19). Neither cross yielded hybrids, even in mass matings. These two crosses are not illustrated in the plates.

The *hamatofila/mojavensis* cross, as pointed out above, is the only one in which most of the vaginae had become normal, and yet the cross had failed to produce hybrids (18). It should be pointed out that the vagina returns to normal rather early after insemination. For example, the one illustrated in Figure 30 was only forty-eight hours old at the time of dissection, and those dissected between fifty and sixty hours were mostly cleared, and motile sperm were still present in the ventral receptacle. In older specimens the sperm soon become inactivated or killed, as occurs in many interspecific matings.

The females of *D. mojavensis* were inseminated by the males of three other species (Table 2, crosses 20–22). Twenty-nine inseminated females were produced in the *mojavensis/aldrichi* cross (20), and a reaction mass was present in all. By seventy-two hours after insemination, the reaction mass begins to break up and is later eliminated (Fig. 31). No hybrids were obtained from this cross.

The *mojavensis/arizonensis* cross (21) yielded 154 inseminated females, of which ninety-seven contained reaction masses (Fig. 32). The vaginae of fifty-seven were normal, and eighty had motile sperm in their ventral receptacles. This cross is by far the most productive of hybrids of any

combination in the mulleri subgroup, and the F_1 males and females are both fertile.

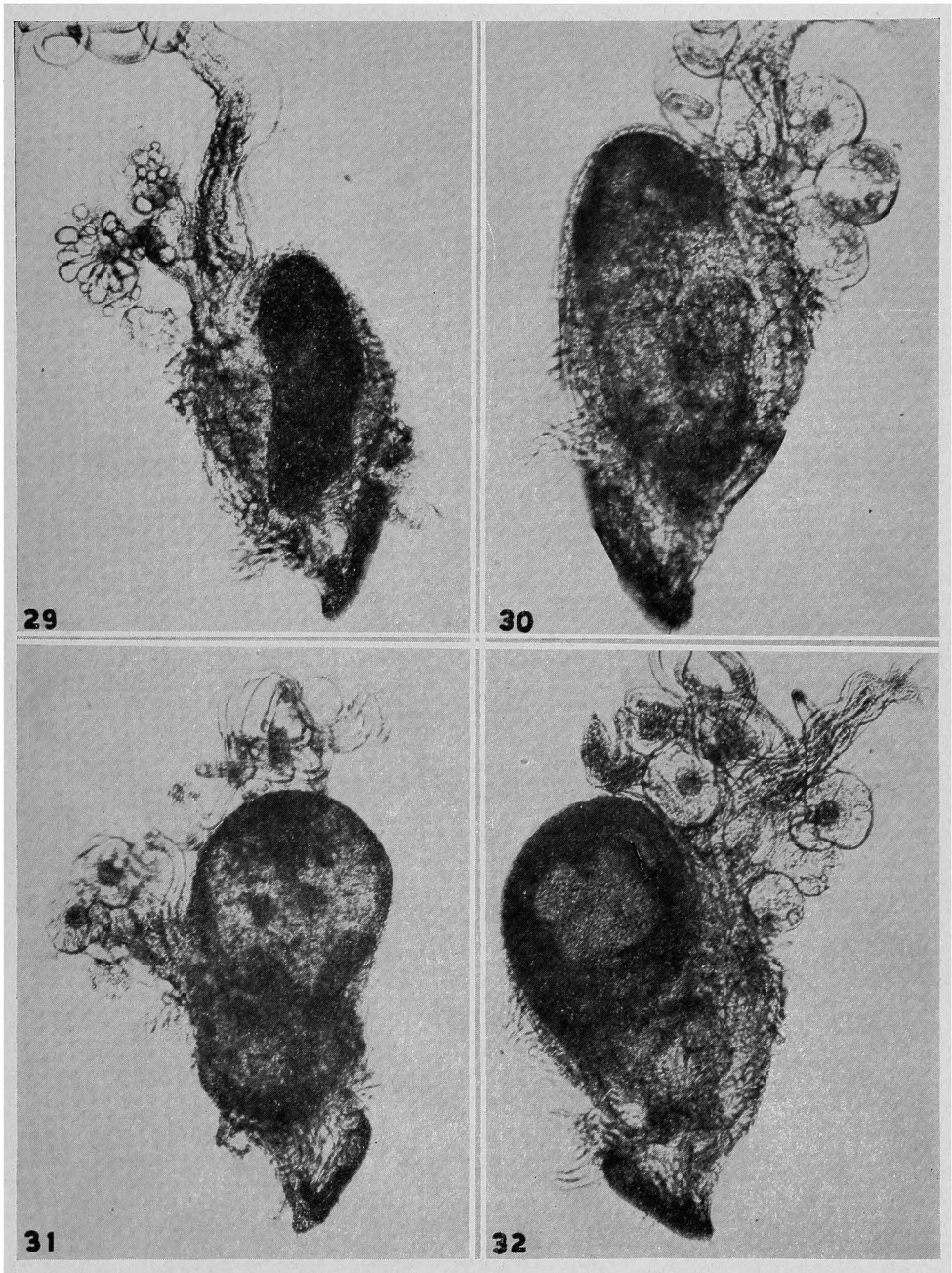


PLATE VIII. Reaction mass in vaginae of *D. hamatofila* and *D. mojavensis*, each mated to two different types of males. Fig. 29, *D. hamatofila* ♀ × *D. arizonensis* ♂ at seventy-two hours. Fig. 30, *D. hamatofila* ♀ × *D. mojavensis* ♂ at forty-eight hours. Fig. 31, *D. mojavensis* ♀ × *D. aldrichi* ♂ at seventy hours. Fig. 32, *D. mojavensis* ♀ × *D. arizonensis* ♂ at seventy-eight hours

Figure 33, Plate IX, illustrates the reaction mass in an inseminated female from the *mojavensis/hamatofila* cross. Forty-six inseminated

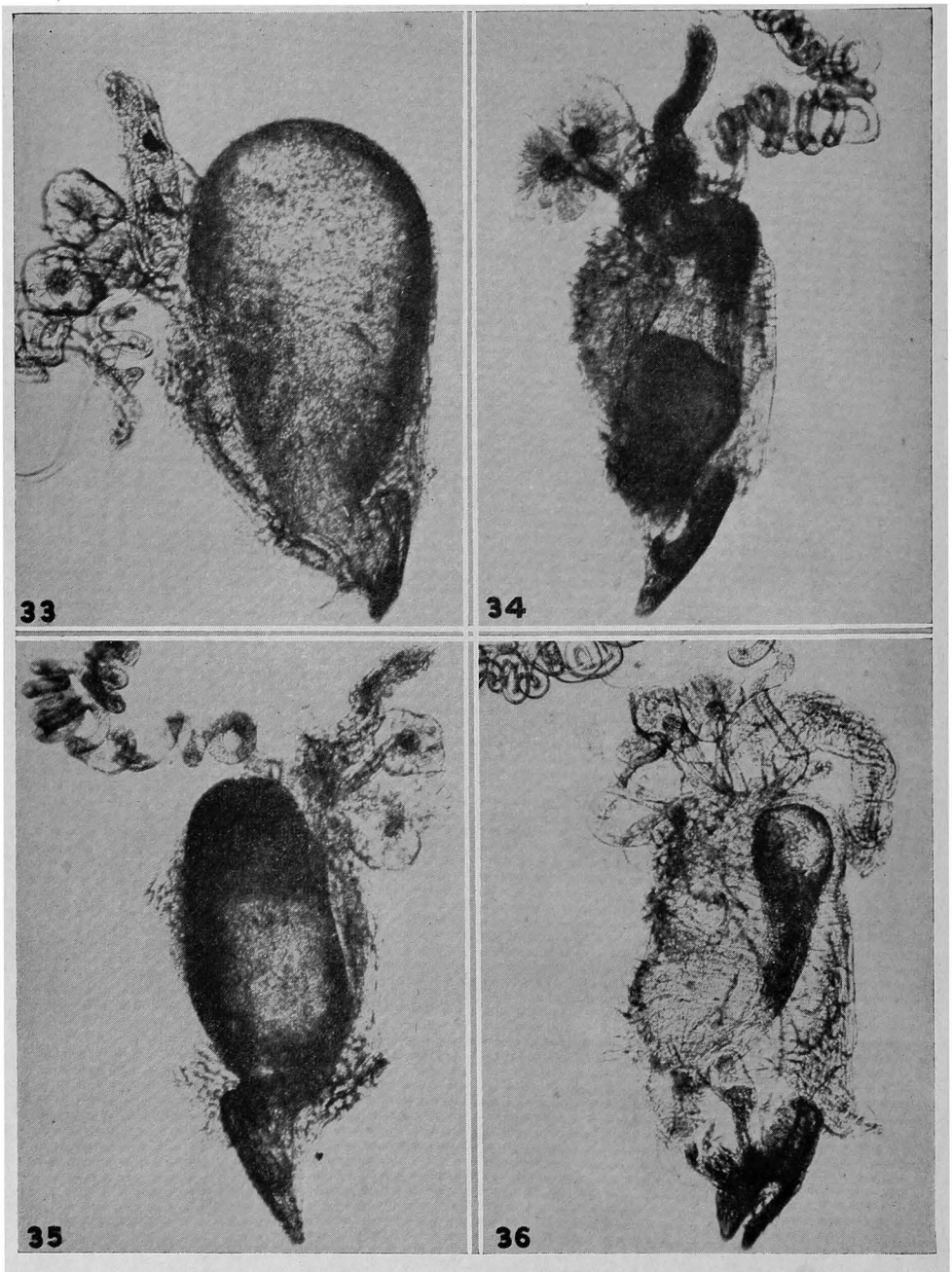


PLATE IX. Reaction masses in vaginae in four different heterogamic crosses. Fig. 33, *D. mojavensis* ♀ × *D. hamatofila* ♂ at forty-eight hours. Fig. 34, *D. peninsularis* ♀ × *D. arizonensis* ♂ at seventy-two hours. Fig. 35, *D. peninsularis* ♀ × *D. hamatofila* ♂ at ninety-six hours. Fig. 36, *D. ritae* ♀ × *D. hamatofila* ♂ at seventy-two hours.

females were obtained and all possessed reaction masses (22). This interspecific cross never produces hybrids.

Females of *D. peninsularis* were inseminated by the males of three other species (Table 2, crosses 23–25). Fifty-two inseminated females were derived from the *peninsularis/arizonensis* cross, and all had reaction masses (23). No hybrids were obtained. A typical specimen at seventy-two hours is shown in Figure 34. A few cases of abnormal eggs in the vaginae were observed, and in one of these there was a normal egg in the median oviduct in addition to the abnormal one in the vagina.

The *peninsularis/buzzatii* cross (24) gave a single inseminated female, which had a reaction mass in the vagina and a few non-motile sperm in the ventral receptacle. No offspring resulted from mass matings of this cross, which is not illustrated in the plates.

The *peninsularis/hamatofila* cross (25) yielded nineteen inseminated females. All of these showed old reaction masses (Fig. 35), and the sperm had completely disappeared from their ventral receptacles. No offspring were obtained from these matings.

The *hamatofila/ritae* cross (26) gave a single inseminated female, which had a reaction mass containing a degenerating egg. Figure 36 was made from another specimen from mass matings. Sperm were absent from the ventral receptacles, and hybrids were not produced.

Figures 37–39, Plate X, represent three vaginae, each with an egg which is undergoing disintegration as a result of the effects of the insemination reaction. The one illustrated in Figure 37 is from the *mulleri/arizonensis* cross, from a specimen dissected 150 hours after insemination. The egg is embedded in the old reaction mass, and has already become abnormal with the cytoplasm condensed into a spherical mass near the center. Figure 38 illustrates a specimen from the *hamatofila/arizonensis* cross from a female dissected seventy-two hours after copulation. The abnormal egg is located in the vaginal pouch of a badly damaged vagina. Figure 39 illustrates a specimen from the *mulleri/mojavensis* cross, dissected seventy-two hours after copulation. The egg is located in the vaginal pouch, and has turned 180 degrees on its long axis with the filament-bearing end directed posteriorly. The content of the egg has largely disappeared and only the partially collapsed shell remains. The scattered material of the reaction mass has been pushed down into the lower end of the vaginal cavity.

Recently discharged eggs from the ovaries of these females, while still in the median oviduct, appear to be entirely normal. It is only after the egg has reached the vaginal cavity with its contained reaction material that the dissolution or "digestive" process begins. If the female is unable to lay the eggs, as usually happens in severe cases, the disintegration may continue to the point where only the hexagonally sculptured chorion and egg filaments are left, and sometimes only the filaments. This made it possible to detect cases in which two or more eggs had been dissolved. The largest number found was in a vagina with the remains of twenty filaments, which would indicate that five eggs had been digested.

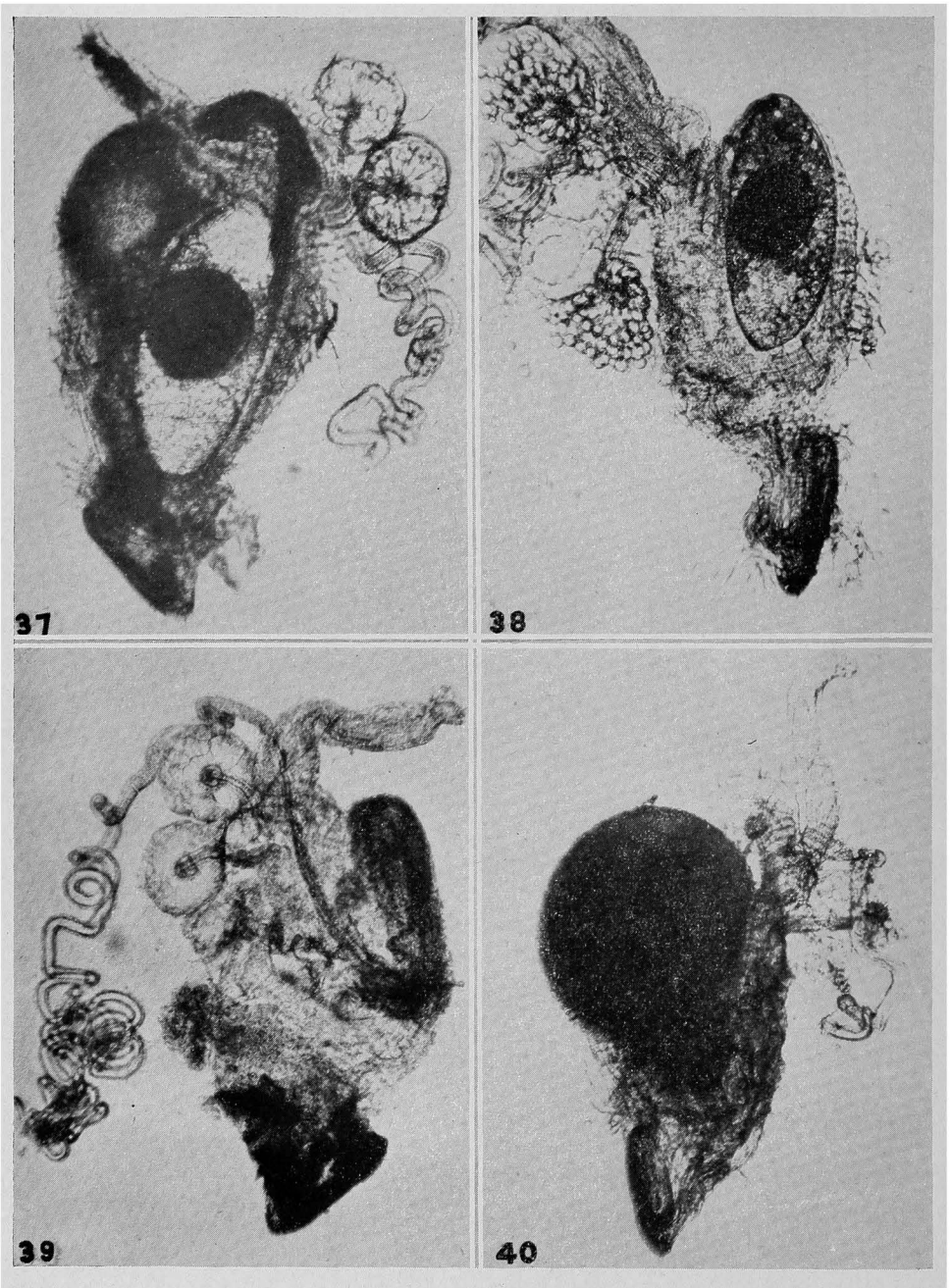


PLATE X. Figures 37 to 39 show abnormal eggs in the vaginae of three females. Fig. 37, *D. mulleri* ♀ × *D. arizonensis* ♂ at 150 hours. Fig. 38, *D. hamatofila* ♀ × *D. arizonensis* ♂ at seventy-two hours. Fig. 39, *D. mulleri* ♀ × *D. mojavensis* ♂ at seventy-two hours. Fig. 40, vagina of *D. mojavensis* ♀ mated to a sterile F_1 male derived from the *arizonensis/mojavensis* cross at three hours. Such males have sperm-free semen.

Some interesting facts concerning the nature of the reaction mass, not emphasized above, should be mentioned at this point. It has already been stated that in homogamic matings, the reaction mass remains soft and is soon eliminated, and consequently does not materially, if at all, affect the production of offspring. In the case of heterogamic matings, not only does the reaction mass persist for a much longer period of time, but it also changes in consistency, frequently becoming extremely hard in texture. The degree of this change depends on the cross. In general, crosses which produce viable hybrids have the softer type of reaction mass and the vagina clears in a shorter time. This includes crosses between *aldrichi, arizonensis, mojavensis,* and *mulleri* (c.f., Figs. 17, 19, 21, 22, 24, 26, 31, 32). On the other hand, crosses involving the other four species usually produce the hard, discrete type of reaction mass, which persists for a long time after copulation (c.f., Figs. 15, 16, 18, 23, 25, 27, 28, 29, 33–36), and hybrids usually do not result from these crosses.

By far the hardest type of reaction mass develops in crosses in which *D. hamatofila* is used as the male parent. The vagina of the female in such crosses, irrespective of the species employed, frequently becomes as hard as a grain of sand, and it is difficult to crush the organ by pressure on the cover glass. If examined under the high power of the microscope, the reaction mass appears to be crystalline in character. This is clearly discernible in such Figures as 23, 25, 33, and 35. This demonstrates that the semen must play a definite rôle in determining the physical character of the reaction mass.

RÔLE OF THE SEMEN IN THE INDUCTION OF THE INSEMINATION REACTION

One of the most important questions concerning the insemination reaction is the possible rôle of the semen and its contained sperm in bringing about the formation of the reaction mass. It seemed clear from the experimental results that the reaction might be induced either by the living spermatozoa, or by the semen alone. In studying the early stages of the formation of this mass, it had been observed that, in a few cases, the reproductive tract of the female contained no detectable sperm, and yet the typical insemination reaction had immediately followed copulation. A single observation will be sufficient to make this point clear. In examining the early stages of the reaction in homogamic matings of *D. aldrichi,* one case was found in which the vagina had already reached its maximum size, although the female was dissected before the termination of coitus and after the pair had been in copula for two minutes and ten seconds. The vagina was large and turgid, and its cavity was filled with a clear fluid throughout which was scattered a grayish reaction material. The most careful examination, under optimum lighting conditions, failed to reveal a single spermatozoon in the distended vagina or the ventral receptacle. From this, and similar observations,

it may be assumed that the sperm are not the active agent in causing the reaction to take place.

Fortunately, an opportunity was present for making a critical test of this assumption. In some of the interspecific crosses sterile F_1 males are produced, and in two such crosses a sufficient number of hybrid males could be obtained for making the test. The two crosses are, *D. arizonensis* ♀ × *D. mojavensis* ♂ and *D. mulleri* ♀ × *D. mojavensis* ♂. In the first of these crosses the F_1 males appear normal, and their testes are nearly as large as those of fertile males, but spermatozoa are never formed in them. Sterile hybrid males from the *mulleri/mojavensis* cross are also rather normal in appearance, except that their testes are small and abnormal. These males likewise fail to produce spermatozoa. Both types of males will sometimes mate in backcross to the parent and other kinds of females. These facts give the needed opportunity for testing the assumption that live spermatozoa are not the active agent in causing the reaction to occur.

The *arizonensis/mojavenses* F_1 males were tested with seven different types of females, and failed to mate with those of *hamatofila, mulleri,* and *peninsularis.* The results obtained with the four other types of females are tabulated in Table 3. In the backcross with *mojavensis* females,

TABLE 3

Sterile F_1 males from the *arizonensis/mojavensis* cross mated to *mojavensis, aldrichi, arizonensis,* and *buzzatii* females

Types of females	Dissected at	Character of reaction mass	Illustrated
D. mojavensis	0 minutes	beginning to enlarge	
D. mojavensis	12 minutes	well-formed, opaque	
D. mojavensis	1 hour	maximum size	
D. mojavensis	2 hours	densely opaque	
D. mojavensis	3 hours	no change	Pl. X, Fig. 40
D. mojavensis	5 hours	no change	
D. mojavensis	10 hours	no change	Pl. XI, Fig. 41
D. mojavensis	16 hours	no change	Pl. XI, Fig. 42
D. mojavensis	22 hours	clearing	Pl. XI, Fig. 43
D. mojavensis	24 hours	mass reduced to ½	
D. mojavensis	26 hours	mass reduced to ¼	
D. mojavensis	30 hours	vagina normal	
D. arizonensis	0 minutes	beginning to form	
D. arizonensis	15 minutes	well-formed	
D. arizonensis	30 minutes	large, opaque	
D. arizonensis	35 minutes	maximum size	
D. arizonensis	23 hours	mass reduced to ⅛	Pl. XI, Fig. 44
D. arizonensis	24 hours	mass reduced to ¼	
D. aldrichi	24 hours	⅔ cleared	Pl. XII, Fig. 45
D. buzzatii	15 minutes	well-formed	
D. buzzatii	26 hours	¾ cleared	Pl. XII, Fig. 46

twenty-three successful copulations were obtained. The females were dissected at regular intervals, and the results obtained from a representative series of twelve of these are listed in the table. From the end of coitus to the twenty-sixth hour, the history of the insemination reaction

closely parallels that of homogamic matings, both in the sequence of events and in the behavior of the reaction mass. The vagina begins to enlarge immediately following copulation and reaches its maximum size in about an hour. Within two hours it has become densely opaque and remains so through the sixteenth hour (Figs. 40–42). Soon after this, the vagina begins to clear and an indistinct reaction mass occupies the vaginal pouch (Fig. 43). Some time between the twenty-sixth and thirtieth hours it becomes clear and normal. In these backcrosses the chief difference in the history of the insemination reaction between pure homogamic matings and those from the sterile males is the time element. It takes about three times as long for the vagina to clear in the latter type of mating.

It was more difficult to obtain backcross matings between the sterile males and *arizonensis* females. Only six successful copulations were secured, and the results from these are listed in Table 3. Four of the females were dissected within thirty-five minutes after mating had occurred. The behavior of the insemination reaction during this time was very similar to that of the matings with *mojavensis* females for the corresponding period. One female was dissected after twenty-three hours, and the vagina had been reduced to about one-half maximum size. Another female was examined after twenty-four hours, and in this one the vagina had decreased to about one-fourth the volume of the maximum size. From this evidence, the vagina would probably have been normal by thirty hours.

In the outcross of the sterile males to *aldrichi* females, only a single successful copulation was obtained. The female was dissected at twenty-four hours, and at this time the vagina contained a reaction mass in the vaginal pouch of the heterogamic type (Fig. 45). Two successful copulations occurred in the outcross matings between the sterile males and females of *buzzatii*. One female was examined after fifteen minutes, and it had a typically enlarged vagina. The other was dissected at twenty-six hours. The reaction mass was located in the vaginal pouch, and was also of the heterogamic type (Fig. 46).

Attempts to backcross the sterile F_1 males from the *mulleri/mojavensis* cross proved to be still more difficult. After many attempts, two matings with *mulleri* females were obtained on the thirteenth day after the emergence of these males. The first successful mating lasted but thirty seconds and the vagina was swollen and slightly opaque when dissected out at twenty minutes. The second mating lasted thirty-seconds, and the female was dissected one hour later. Her vagina was larger than that of the first female, and had a mottled appearance. Every effort to obtain matings with the *mojavensis* females completely failed.

Since the semen of the F_1 hybrid males is sperm-free, the results from the tests with these males demonstrate conclusively that the presence of live spermatozoa are not necessary for the induction of the insemination reaction. This does not exclude the possibility that degenerative products from the abnormal testes may constitute a part of the semen.

Not much is known about the exact composition of the semen in Drosophila. According to Nonidez (1920), the paragonia constitute a pair of accessory glands which secrete into the vas deferens a dense sticky

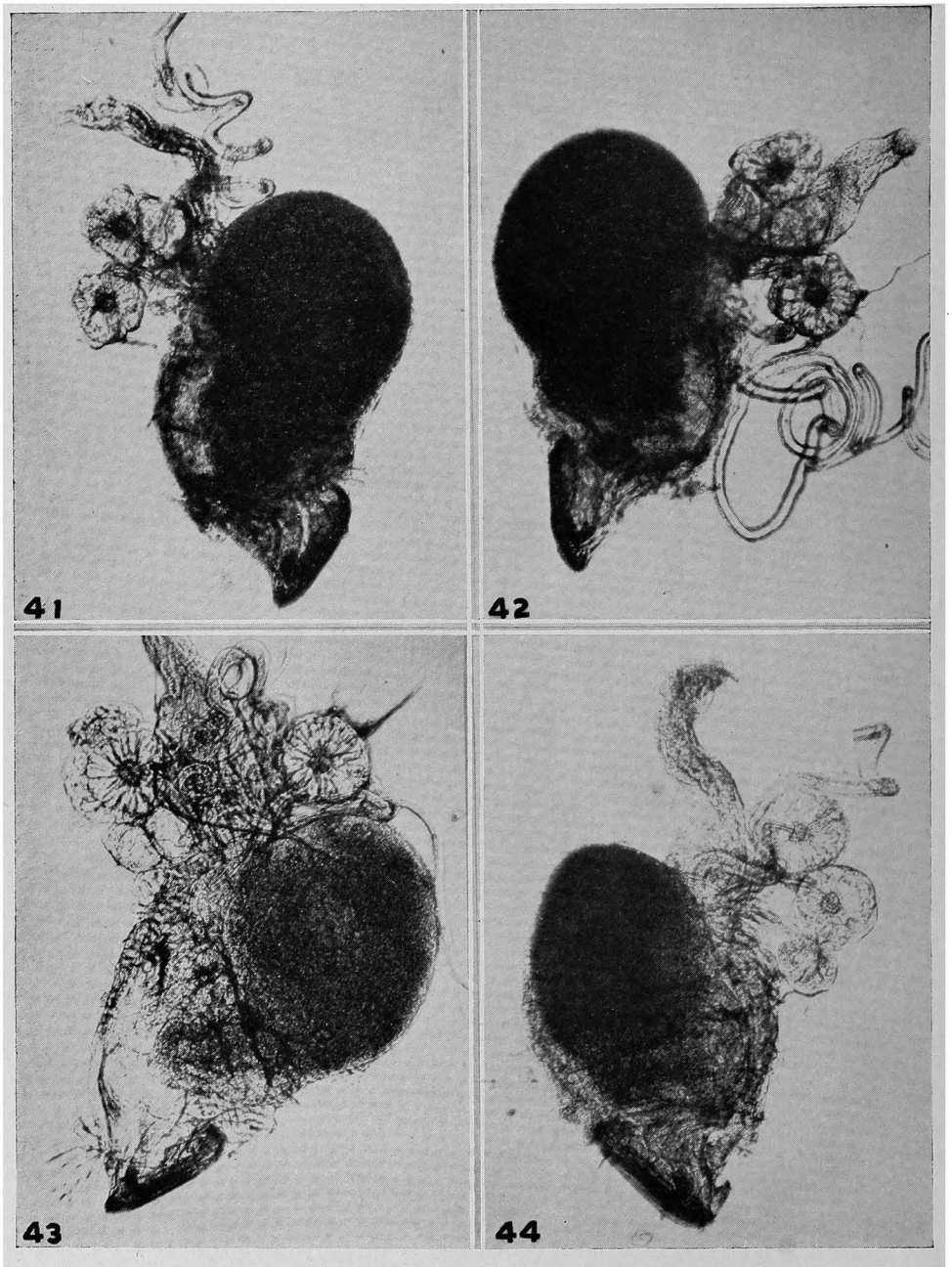


PLATE XI. Figures 41 to 43 belong to the series begun in Fig. 40, and represent different stages of the insemination reaction. Fig. 41, at ten hours. Fig. 42, at sixteen hours. Fig. 43, at twenty-two hours. Fig. 44, from cross of *D. arizonensis* female with one of these sterile males, twenty-three hours after insemination.

fluid with floating refractive granules. This fluid becomes mixed with the spermatozoa from the testes and forms the liquid part of the ejaculate.

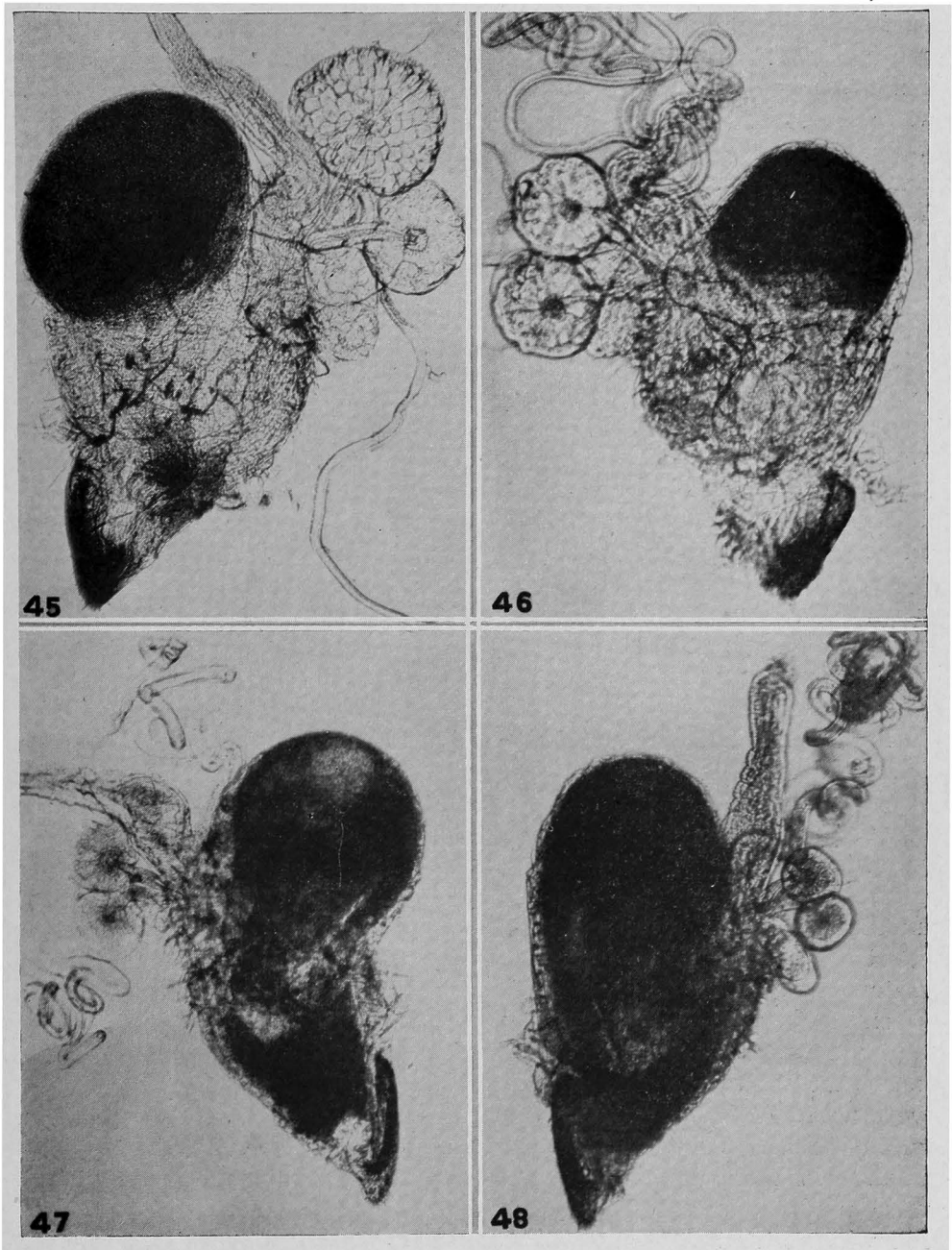


PLATE XII. Fig. 45, from mating of *D. aldrichi* female with sterile F_1 male, dissected at twenty-four hours. Fig. 46, from mating of *D. buzzatii* female with sterile F_1 male, dissected at twenty-six hours. Figures 47–52 are *D. buzzatii* females from the *buzzatii/arizonensis* cross remated at different intervals with *D. buzzatii* males. Fig. 47, female remated at 168 hours and dissected thirty minutes later. Fig. 48, same cross dissected at one hour.

The sexual behavior of the sterile males used in this series of tests was very different from that of the fertile males of the parent species. For ten days after emergence, these males were indifferent to the presence of females, and it was not until the thirteenth day, or later, that the matings listed in the table were obtained. In contrast to this, the fertile males began courting as early as the fifth day, and always carried on a vigorous courtship when placed with females. The duration of coitus was somewhat shorter for the hybrid males, averaging two minutes and sixteen seconds as compared with three minutes and thirteen seconds for the fertile males.

MATING HABITS OF FEMALES WHICH HAD PREVIOUSLY COPULATED

One question which has arisen in connection with these tests on members of the mulleri subgroup, is whether a female which had been inseminated would copulate again, and if so, whether the insemination reaction would occur a second time. There is considerable evidence to show, both from this and other laboratories, that in a species in which the insemination reaction does not occur, the female will mate two or more times, and in some species at frequent intervals. We found, however, that in a species in which a strong reaction occurs, the female usually does not remate for a long time. It was further found, both after homogamic and heterogamic matings, that the females do not immediately respond to the vigorous courtship of the males.

A special test was made of homogamic matings of *D. buzzatii.* A series of ten females which had just copulated for the first time were isolated, and each female was exposed to five males of her own kind at intervals extending from the fourteenth to the 135th hour after the initial copulation. Each exposure lasted from one to two hours at the following periods: 14, 20, 43, 61, 85, 114, and 135 hours. During this entire series of tests the flies were kept under constant observation while exposed, and not a single copulation took place, although the females were courted vigorously by the males. It may not be safe to conclude that the mated females of all members of this group of species never mate a second time. If they do, it must be after a considerable period of time.

We also made tests with females from the heterogamic *buzzatii/arizonensis* cross. Females which had been seen mating with *arizonensis* males were used. A limited series of inseminated females were tested to *arizonensis* males, but we were never able to secure a second mating with this type of male. Much better success was achieved when *buzzatii* males were used. A total of forty-seven females was isolated immediately following coitus, and then tested singly to the *buzzatii* males. The earliest time at which these females would accept the males was about 120 hours after the end of the first mating. Dissections revealed that forty-six of the females still had the old reaction mass present in the vaginal pouch, and one did not, and that thirty-four had been reinseminated. The fail-

ure of the other thirteen specimens to become inseminated is not clear. It is possible that the presence of the old reaction mass adversely affected the insemination act.

The data from a representative sample of twenty-seven specimens are tabulated in Table 4. Female No. 5 is the one which did not have an old reaction mass. Incomplete copulations are not infrequent in Drosophila, especially in heterogamic matings, and this is probably the explanation of this case. The time at which the second mating occurred in the several females is given in hours in the column on the time of mating, while the time of dissection is given in minutes or hours following the second mating. Dissections were begun at fifteen minutes and continued through the eighth hour. They were discontinued at eight hours because by this time the vagina is practically normal, except for the presence of the old reaction mass.

TABLE 4

D. buzzatii females from the *buzzatii/arizonensis* cross remated to *buzzatii* males

Female	Time of remating	Dissected at	Reaction mass old	Reaction mass new	Illustrated
1	144 hours	15 minutes	present	present	----------
2	168 hours	15 minutes	present	present	----------
3	120 hours	20 minutes	present	present	----------
4	120 hours	20 minutes	present	present	----------
5	144 hours	30 minutes	absent	present	----------
6	168 hours	30 minutes	present	present	Pl. XII, Fig. 47
7	144 hours	30 minutes	present	present	----------
8	144 hours	32 minutes	present	present	----------
9	144 hours	35 minutes	present	present	----------
10	124 hours	1 hour	present	present	----------
11	124 hours	1 hour	present	present	Pl. XII, Fig. 48
12	125 hours	1 hour	present	present	----------
13	144 hours	1 hour	present	present	----------
14	144 hours	1 hour	present	present	----------
15	144 hours	1 hour	present	present	----------
16	144 hours	1 hour	present	present	----------
17	160 hours	1 hour	present	present	----------
18	120 hours	2¾ hours	present	present	----------
19	120 hours	3 hours	present	present	----------
20	144 hours	3¾ hours	present	present	Pl. XIII, Fig. 49
21	144 hours	6 hours	present	present	----------
22	168 hours	7 hours	present	present	Pl. XIII, Fig. 50
23	168 hours	7⅓ hours	present	present	----------
24	168 hours	7½ hours	present	present	----------
25	168 hours	7¾ hours	present	present	Pl. XIII, Fig. 51
26	168 hours	7 5/6 hours	present	present	----------
27	168 hours	8 hours	present	cleared	Pl. XIII, Fig. 52

A study of the specimens from this close series of dissections made it possible to follow the history of the insemination reaction which results from a second copulation. In general, the reaction undergoes the same series of changes as occur after the first insemination. Within thirty minutes, the new reaction material is formed and is beginning to surround the old reaction mass (Fig. 47), and by the end of one hour it has practically engulfed this object (Fig. 48). The vagina is densely

opaque and has become greatly enlarged. This condition continues for the next two or three hours, during which time the vagina has become somewhat larger. There then follows the elimination process of the new reaction material. This is evident in the specimen illustrated in Figure 49, which was dissected three and three-fourths hours after copulation. The old reaction mass has again become visible. The new reaction material continues to drain out during the next few hours (Figs. 50, 51). By the end of eight hours, only a small trace of this material is present at the lower end of the vaginal cavity, and the vagina has returned to its original size, while the old reaction mass becomes sharply outlined in the vaginal pouch (Fig. 52).

The point of greatest interest here is that the old reaction mass from the original heterogamic mating does not materially affect the history of the insemination reaction from this second or homogamic mating. The course of the second reaction, both in the sequence of the changes and the time of duration, very closely parallels the corresponding changes and time which follow an original homogamic mating of *D. buzzatii.*

DISCUSSION AND CONCLUSIONS

The main purpose of this article was to present any evidence obtained from a study of the insemination reaction which might have some bearing on the question of speciation in the mulleri subgroup of species. In any attempt to evaluate this evidence, one must keep in mind that evolutionary divergence is rarely, if ever, brought about through the operation of a single *isolating mechanism.* It is usually the total effect of several of these mechanisms which brings about a more or less complete separation of two diverging forms, by reducing, or entirely preventing, the exchange of genes between them. It was, therefore, not surprising to find that at least five such mechanisms were functioning between the several members of this subgroup.

We have shown in the preceding article that sexual isolation is a common mechanism in preventing matings between the eight members of the mulleri subgroup. Of the fifty-six possible crosses, thirty failed to show a single insemination among the one-hundred females examined by dissection for each of these crosses. In addition to this, there were several other crosses which yielded but a few inseminations, some as low as a single specimen. *Sexual isolation* obviously represents one of the most important mechanisms in the evolution of Drosophila.

We have shown in previous publications that the spermatozoa may become inactivated, or even killed, when introduced into the reproductive tract of an alien female. Much evidence of this phenomenon was observed in the present investigation. It was clear from a study of the results from certain crosses that the spermatozoa soon become inactive, and may eventually disappear. For example, in one cross *(hamatofila/mojavensis)* dissections showed that twenty-four hours after copulation there were many motile spermatozoa present in the ventral receptacle,

but later dissections revealed that most of the sperm had become immotile, or had disappeared altogether. *Gametic mortality* constitutes a second type of isolating mechanism.

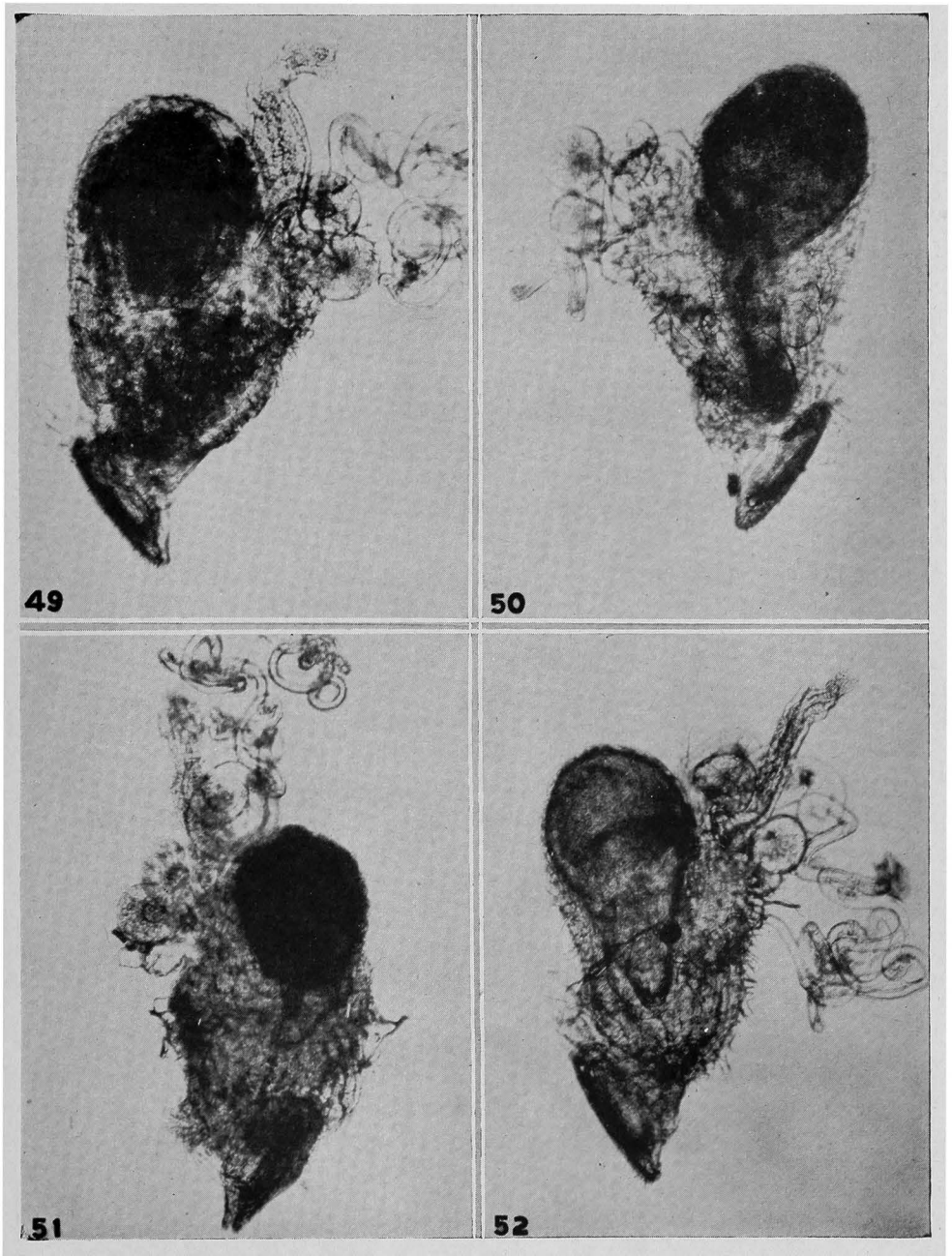


PLATE XIII. Continuation of the series begun with Fig. 47, Plate XII. Fig. 49, same cross, female remated at 144 hours and dissected at three and three-fourths hours. Fig. 50, same cross, female remated at 168 hours and dissected at seven hours. Fig. 51, same cross and same age, dissected at seven and three-fourths hours. Fig. 52, same cross and same age, dissected at eight hours.

In crosses between *arizonensis* ♀ × *buzzatii* ♂ and *mulleri* ♀ × *buzzatti* ♂ the larvae frequently perish. In the first cross they all die during mid-larval stages, and in the second a majority of the zygotes die in the pupal stage, with only a few of the adults emerging (Patterson 1942). *Zygotic mortality,* therefore, represents a third type of isolating mechanism in the mulleri subgroup.

Reference to Table 1 of the preceding article shows that only ten of the fifty-six possible interspecific crosses gave hybrids of any kind. Moreover, most of these hybrids were found to be completely sterile. Only the following combinations yield fertile offspring: 1, the *mulleri* ♀ × *mojavensis* ♂ cross gives fertile females and sterile males; 2, the *arizonensis* ♀ × *mojavensis* ♂ cross likewise gives fertile females and sterile males; 3, the reciprocal cross, *mojavensis* ♀ × *arizonensis* ♂, gives fertile males and females. *Hybrid sterility* represents a fourth type of isolating mechanism.

With this array of isolating mechanisms functioning in the mulleri subgroup (and there are probably others), it is somewhat difficult to determine precisely just what proportion of the sum total of isolation in this group should be assigned to the effects of the insemination reaction. It is clear from the experimental results that this reaction constitutes a potent isolating mechanism in the interspecific crosses of the mulleri-like forms. Among thirteen such crosses which did not yield hybrids, 557 inseminated females were found in 2,600 dissected specimens (21%). In one cross *(buzzatii/arizonensis),* a total of 159 of the 200 dissected females had been inseminated (78%). It is safe to assume that, in the absence of a reaction mass from the vagina, many of these females would have produced offspring. All of these females did have a reaction mass in the vagina, and motile sperm were present in the ventral receptacles of sixty-two per cent of them (Table 2). Their failure to produce offspring was not due to the absence of motile sperm in all specimens, but rather to their inability to lay fertile eggs in the presence of the reaction mass. The insemination reaction, in addition, adversely affects reproduction in fertile crosses, by reducing the number of hybrids. Thus, in the *mojavensis/arizonensis* cross, which is the most fertile one of the entire series, only seventy-five per cent of the females produced hybrids, although ninety-three per cent of them had been inseminated.

In the absence of workable paleontological material of Drosophila, it is not possible to work out a satisfactory phylogeny for the different forms. However, the several species belonging to the mulleri subgroup must have evolved from a common stem, and their morphological features, together with the experimental results now available, do make it possible to indicate some of their relationships to one another. From their taxonomic features alone, *mulleri, arizonensis, aldrichi,* and *mojavensis* are more closely related to one another than to the other four species. This conclusion is supported by the results obtained in the cross-fertility tests. Seven of the ten crosses producing hybrids were

restricted to members of these four species, and one member of each of the other fertile crosses also belonged to this group (Table 2).

The two most closely related of these four species are *arizonensis* and *mojavensis,* for their reciprocal crosses yield by far the largest number of fertile hybrids of any combination. In addition, *arizonensis* females are sometimes inseminated by *buzzatii* males, but the larvae die. Next in closeness of relationship would be *mulleri* and *aldrichi.* These two species are very similar morphologically, but have developed complete reproductive isolation, and for this reason are able to maintain their identities in the large area of their overlapping distribution ranges. Fertile crosses occur between *mulleri* females and the males of both *arizonensis* and *mojavensis,* and in addition, this species shows its relationship to two other members of the subgroup by virtue of hybridization. Its females are sometimes inseminated by *buzzatii* males, and although most of the zygotes die in the pupal stage, yet a few adult flies emerge. Its females are also occasionally inseminated by *hamatofila* males, producing a few sterile hybrids.

In all nonfertile crosses the duration of the effects of the insemination reaction is longer than in fertile crosses. This is due to the deleterious effects caused by the prolonged retention of the reaction material. Or to express it in another way, the sooner this material is eliminated from the vagina, the better are the chances that hybridization will follow. The only observed exception to this rule was the *mojavensis/hamatofila* cross, but we have already commented on that case.

The remaining crosses listed in Table 2 represent nonfertile combinations, that is, crosses in which the females have been inseminated but do not produce offspring. As to what value should be placed on these data in attempting to estimate a degree of relationship is difficult to determine. There is very little reliable experimental evidence to show whether hybridization occurs between two forms belonging to different species groups, and there is still less relating to the question of insemination. Nevertheless, it seems reasonable to assume that any two species in which a large number of females had been inseminated in nonfertile heterogamic matings would be more closely related than two other forms in which only one or a few females had been impregnated. The following attempt to indicate possible relationships from the data on nonfertile crosses is based on this assumption.

The nonfertile crosses involve, in the main, members of the second group of species, namely, *buzzatii, hamatofila, peninsularis,* and *ritae.* Of these four species, *buzzatii* is probably the most closely related to members of the first group. It is morphologically more similar to them and has the same metaphase chromosome pattern (Wharton 1943). Females of this species were inseminated in nonfertile crosses by the males of three other species *(arizonensis, hamatofila, mojavensis),* and gave a total of 310 impregnations in 600 dissected females (51.6%). The males of this species inseminated females of four other species, two representing fertile crosses *(mulleri, arizonensis),* and two nonfertile crosses

(hamatofila, peninsularis). The total number of alien females inseminated in these four crosses was only five in 1,000 dissected specimens (0.5%).

On some grounds *D. hamatofila* might be regarded to be more closely related to the first group of species than *buzzatii*. But it is not as similar morphologically as *buzzatii* and has a different metaphase chromosome pattern (Wharton). Females of this species were inseminated in nonfertile crosses by males of four other species *(arizonensis, buzzatii, mojavensis, ritae)*. The total number of inseminations was thirty-three in 800 dissected females (4.1%). The males of this species inseminated females of the other seven species, with 258 inseminations in 1,400 dissected specimens (18.4%). Only one of the crosses was fertile.

Of the two remaining species, *D. peninsularis* has the same metaphase chromosome pattern as *hamatofila*, but otherwise does not resemble that species. Its females were inseminated by males of three other species *(arizonensis, buzzatii, hamatofila)*, with a total of seventy-two inseminations in 600 dissected specimens (12%). All three crosses were nonfertile. The males of this species failed to inseminate females of any of the other seven species. The inseminations with *ritae* occurred only in the reciprocal crosses with *hamatofila*. Ten *ritae* females were inseminated by *hamatofila* males (5%), and a single *hamatofila* female was inseminated by a *ritae* male (0.5%). Both crosses were nonfertile. These two species have reached the highest degree of divergence of any members of the mulleri subgroup, and both have developed complete reproductive isolation. The species *ritae* is further separated from all other members of the subgroup by morphological differences, and by a different type of metaphase chromosome pattern (Wharton 1943).

The nature of the insemination reaction is a question which remains to be discussed. It was pointed out in the preliminary paper that this reaction follows almost immediately after the introduction of the semen into the cavity of the vagina, and is visibly expressed in the form of a rapid swelling of that organ. This increase in size is due to a secretion of fluid by the cells of the lining membrane of the vagina. Evidently this is caused by a response of this membrane to the presence of foreign material, presumably proteins. This would imply that the membrane is hypersensitive to the proteins. Moreover, its hypersensitiveness must be inherited, since the reaction occurs after the first copulation and without any previous sensitization. In any event, the insemination reaction in its manifestations does resemble certain features of some immunological reactions. Unfortunately there is very little known about hypersensitivity and anaphylaxis in insects, and any further speculations must await future investigations.

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IV. THE INSEMINATION REACTION IN INTRASPECIFIC MATINGS OF DROSOPHILA

MARSHALL R. WHEELER*

INTRODUCTION

The insemination reaction, first described by Patterson (1946) and discussed more fully by him in the preceding article of this bulletin, provides one of the most striking examples of an isolating mechanism operating to reduce or prohibit the production of hybrids between species which are not deterred from mating by sexual or psychological isolation. Patterson has amply demonstrated, in the papers cited, the effectiveness of this barrier to gene exchange in heterogamic matings, i.e., between males and females of different species. An unusual aspect of the reaction phenomenon, however, is its occurrence in homogamic matings, i.e., between individuals of the same species. The presence of such a reaction in intraspecific matings forces one to the conclusion that it is a normal consequence of insemination and may have some useful function in insemination, fertilization, or oviposition. The extreme form of many heterogamic reactions, then, can best be interpreted as an aberrant condition caused by the deposition of semen in the reproductive tract of an alien female.

Early in the insemination studies it was noticed that the extent and appearance of the reaction in homogamic matings varied widely among the different species. In some forms the development of the reaction mass followed the pattern described by Patterson for members of the mulleri subgroup of species. In these forms the vagina of the female swells to three or four times its normal size and is filled for many hours with a dense, opaque reaction mass. In other forms, however, it is much less extreme, while in still others it appears to be weak or entirely absent. As will be pointed out later, it is now believed likely that a reaction is present in all Drosophila species, but, due to its varied expression, is not visibly apparent in some forms.

A preliminary report on this phase of the insemination studies was presented by Patterson (1946) in which he described briefly the course of the reaction in homogamic matings of one of the members of the mulleri subgroup of species and indicated the presence or absence of a reaction in the thirty-five species which had been investigated up to that time. It is the purpose of the present paper to present the results of a more thorough study of the insemination reaction in homogamic matings in the seventy-eight species available for study. Descriptions of the appearance of the vaginae following insemination will be given for the various species, including selected plates of photomicrographs of typical preparations. Finally, possible functions of the reaction in intraspecific matings will be discussed.

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MATERIALS AND METHODS

Most of the species used in this investigation were maintained as stocks in this laboratory. The writer wishes to express his appreciation to the following persons for additional stocks which they sent to us for use in this and other experiments: to Dr. A. H. Sturtevant, for stocks of *D. duncani, ritae, cordata, elliptica, emarginata, rectangularis, micromelanica,* and *nigromelanica;* to Dr. Th. Dobzhansky, for a stock of *D. persimilis;* and to Dr. H. T. Spieth, for stocks of *D. sucinea* and *fumipennis.*

To determine the character of the insemination reaction, females were dissected and examined at varying intervals after copulation. The ideal has been to place a single virgin female in a vial with about five males, all of known age, and observe mating so that a timed series of dissections could be secured. This procedure was relatively easy with many species once the proper age for mating had been determined.

In certain instances where it seemed too difficult to obtain matings in the manner outlined above, the stock culture bottles were observed from time to time in an effort to detect a pair in copula. When such a pair was found it was isolated by inserting a test tube into the culture bottle, thus imprisoning the pair. After completion of mating the flies were removed by inverting the apparatus and shaking the mated pair down into the tube. This method had an obvious disadvantage in that one could not know whether the female so obtained was virgin prior to mating. A variation of this technique consisted of placing virgin flies of assorted ages together in a bottle and removing mated pairs by the test tube method.

Finally, we have been unable to devise any method of obtaining matings with a few species. In order to secure any sort of result with these forms we have been forced to dissect females from the stock culture tubes at various times in the hope of finding one inseminated recently enough to afford some indication of its reaction tendencies. At the risk of stating the obvious, it might be pointed out that positive results obtained in this way are fairly reliable, while negative results are inconclusive.

The techniques employed in dissecting and photographing the reproductive organs have been discussed in detail in the preceding article by Dr. Patterson. The plates accompanying this paper, numbered consecutively in the two papers, will be discussed in the following section dealing with the individual species.

DESCRIPTIONS OF THE INSEMINATION REACTIONS

In the following section the nature of the insemination reaction will be given for two species of the genus *Chymomyza,* one species of *Scaptomyza,* and sixty-seven species of *Drosophila.* The eight members of the mulleri subgroup of species, namely, *D. mulleri, aldrichi, arizonensis, mojavensis, buzzatii, hamatofila, ritae,* and *peninsularis,* have been discussed by Dr. Patterson in the preceding article. The various species will be treated in the order outlined by Sturtevant (1942).

The age of the flies when mated and the approximate average length of copulation will be given for each species, as well as descriptions of the female reproductive tracts at various intervals after copulation. Unless otherwise stated the time of dissection is recorded as the elapsed time from the completion of mating. Discussion of the type of reaction concerned in each case will be deferred to the following section of the paper.

GENUS CHYMOMYZA Czerny

Chymomyza amoena Loew.

Matings were secured with flies from 1 to 5 days old. The average length of copulation was about 14 minutes. Dissection at 30 minutes revealed a large sperm mass in the cavity of the vagina which was not enlarged except at the postero-ventral angle, the latter swollen in the form of a small pouch. The ventral receptacle contained a moderate number of sperm while the spermathecae were so filled as to appear solid. At one hour the mass in the pouch was smaller and surrounded by motile sperm while the main cavity of the vagina was empty. At one and one-half hours there still remained a moderate sized mass in the pouch and there appeared to be a small mass stringing posteriorly to the ovipositor. This is indicative of the manner in which the females are believed to expel the excess sperm and fluids. In this specimen a small number of motile sperm was still visible around the mass remaining in the pouch.

Chymomyza procnemis Williston.

Matings were secured with flies 6 days old. The average length of copulation was about 28 minutes. The vagina of a female dissected 15 minutes after mating appeared normal in all respects, being free of semen and sperm. The ventral receptacle contained a moderate number of sperm and the spermathecae were quite dense with them. A female dissected at 30 minutes extruded a small "wad" of non-motile sperm from the ovipositor upon dissection. This specimen was similar in all respects to the previous one.

GENUS SCAPTOMYZA Hardy

Scaptomyza graminum Fallén.

One mating was secured with flies 2 and 3 days old. The length of copulation was one minute and 55 seconds. A dissection of the female 30 minutes after mating revealed motile sperm in the ventral receptacle but none in the vagina which was of normal size. The spermathecae were torn off and lost in dissection.

GENUS DROSOPHILA Fallén

Subgenus Hirtodrosophila Duda.

Drosophila duncani Sturtevant.

Matings were secured with flies 8, 9, 10, and 13 days old. The average length of copulation was about 15 minutes. The earliest dissection performed was of a female in copula, exact duration unknown. The vagina was full of semen and was already beginning to exhibit a posterior pouch. The ventral receptacle and spermathecae contained many sperm. In a dissection at 20 minutes the vagina appeared still further enlarged and the pouch was more pronounced. The entire lumen of the vagina was filled with the mass. The typical appearance of a specimen dissected at 30 minutes is shown in Fig. 53 (Pl. XIV). The reaction mass was rather soft and was easily forced through the broken end of the oviduct. Specimens dissected at one and one-half hours after mating showed little change from the earlier condition. The mass, however, was more firm and was not expelled under pressure. The latest dissection observed was of a female from the stock culture. In this specimen the vagina and pouch were much smaller and were only about half filled with the reaction mass, revealing a moderate number of motile sperm remaining in the cleared portion of the lumen. Motile sperm were observed in the stalk and lumen of the parovaria in this female—a situation not normally occurring in members of the genus.

Subgenus Pholadoris Sturtevant.

Drosophila victoria Sturtevant.

Matings were secured with flies 6, 7, and 8 days old. The average length of copulation was about 43 seconds. The earliest specimen examined was dissected 3 minutes after copulation. A large part of the semen was expelled from the ovipositor upon dissection. The vagina contained a large reaction mass, granular in appearance and containing many motile sperm. No sperm were visible, however, in the receptacles. The vagina of an individual dissected at 30 minutes appeared quite enlarged and was filled with a dense, opaque mass containing motile sperm. No sperm were seen in the spermathecae at this time although the ventral receptacle appeared to be filled with these cells. In a specimen dissected at one hour the sperm around the edges of the mass were still motile while those within the mass seemed to have ceased all activity. Two specimens dissected 3 hours after mating revealed but little change. The mass was a little smaller, leaving a cleared area in the dorsal region. Motile sperm were still evident in this portion. In one specimen the spermathecae contained motile sperm while these receptacles were still empty in the other. The individual illustrated in Fig. 55 (Pl. XIV) was dissected at about 5 hours after mating. By this time the mass had developed a rather definite border and was clearly outlined as a dense, opaque mass.

Motile sperm were still present, principally in a pocket dorsal to the mass. Dissections at 6 and 7 hours showed no appreciable change but at 8 hours the mass was smaller and was beginning to lose its opaque quality, although still rather sharply defined. At 10 hours the size of the

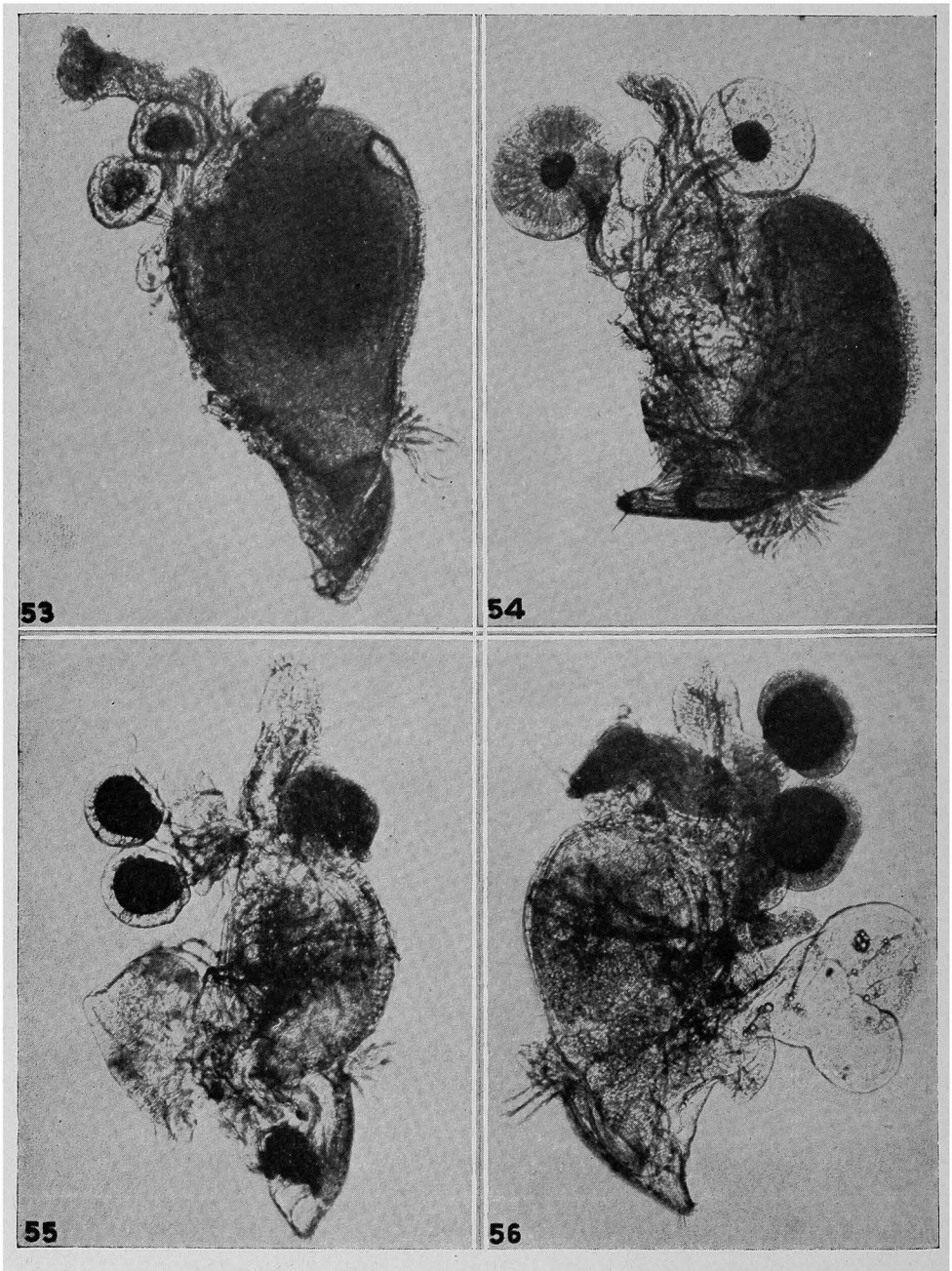

PLATE XIV. Vaginae from homogamic matings of four species of Drosophila. Fig. 53, *D. duncani* at thirty minutes. Fig. 54. *D. victoria* at five hours. Fig. 55, *D. sucinea* at thirty minutes. Fig. 56, *D. fumipennis* at one hour.

reaction mass seemed unchanged but the loss of opacity was becoming pronounced. By 12 hours this clearing had proceeded still further so that the mass was becoming crystalline in appearance. In this specimen some of the reaction material seemed to be passing from the main body of the mass to the ovipositor. Motile sperm were still present in the vaginal cavity dorsal to the mass. The latest dissection performed was at 15 hours and 15 minutes after mating. The reaction mass was practically clear by this time but was still in evidence due to its discrete outline. Neither motile nor non-motile sperm were visible in the vaginal cavity at this time.

Subgenus Dorsilopha Sturtevant.

Drosophila busckii Coquillet.

Matings were secured with flies 1 and 2 days old. The average length of copulation was about 3 minutes. In a specimen dissected at 20 minutes both the ventral receptacle and spermathecae were teeming with sperm. The vagina was filled with an emulsion-like mass containing many motile sperm. There was a slight enlargement of this organ when compared with that of a virgin female. Dissections at 30 minutes and at one hour showed no obvious change, the vagina in both instances containing a fair amount of the emulsion-like material with many motile sperm. In a specimen dissected at 2 hours after mating the mass appeared to have consolidated into an irregular, indefinitely defined mass in the central area of the vagina, leaving a large cleared area around its edges in which motile sperm still persisted.

Subgenus Sophophora Sturtevant.

1. *saltans group*

Drosophila sturtevanti Duda.

A pair of flies observed copulating in the stock bottle was isolated and the female dissected 30 minutes after the completion of mating. The vagina appeared normal in size and did not contain any sperm or semen. The ventral receptacle was teeming with sperm.

Drosophila rectangularis Sturtevant.

A pair of flies observed mating in the stock bottle was isolated and the female dissected 30 minutes after the completion of copulation. The vagina was not enlarged but was filled with an emulsion-like mass containing many sperm, most of which appeared non-motile. The ventral receptacle and spermathecae contained a moderate number of sperm. Pressure on the cover slip forced most of the sperm mass out through the broken end of the median oviduct revealing a large number of spermatozoa which were quite active in the saline solution. Attempted matings with flies 12, 13, and 14 days old were unsuccessful.

Drosophila prosaltans Duda.

Matings were secured with flies 5, 6, and 9 days old. The average length of copulation was 18 minutes. Two specimens were dissected one hour after the completion of mating. The vagina appeared normal in size and was not dense and opaque although it was filled with an emulsion-like material. No sperm were observed in the vagina of either specimen, but in both instances a large mass of motile sperm was observed moving through the median oviduct and emerging into the saline mounting medium. The ventral receptacles and spermathecae were filled with sperm.

Drosophila cordata Sturtevant.

A pair of flies observed copulating in the stock bottle was isolated and the female dissected 30 minutes after the completion of mating. The vagina appeared swollen and rounded, not particularly elongate, and was filled with an emulsion-like mass. Near the anterior end of the vaginal cavity was a dense brown mass with motile sperm around its edges. The ventral receptacle and spermathecae were both filled with active spermatozoa.

Drosophila elliptica Sturtevant.

A pair of flies observed mating in the stock bottle was isolated and the female dissected 30 minutes later. The vagina was somewhat enlarged and elongated, the entire cavity filled with a dense, opaque, granular mass. A large pocket of motile sperm was visible dorsally near the base of the oviduct. The ventral receptacle was teeming with sperm but none were visible in either spermatheca.

Drosophila emarginata Sturtevant.

Attempted matings with flies 10 to 14 days old were unsuccessful. A pair of flies observed in copula in the stock bottle was isolated and the female dissected 30 minutes after the completion of mating. The vagina was not swollen but was rather elongate and contained two rather dense masses separated by emulsion-like material. Each of these masses was surrounded by small numbers of sperm. It is likely that copulation was interrupted by the isolation procedure so that insemination was disturbed midway in the process. This would account for the presence of two masses in the vaginal cavity. The ventral receptacle and spermathecae contained a moderate number of sperm.

2. *willistoni group*

Drosophila willistoni Sturtevant.

A pair of flies less than 24 hours old was observed in copula. The female was isolated and dissected 30 minutes after copulation. The vagina did not appear to be enlarged and, although the sperm mass filled about half the vaginal cavity, it did not appear dense or opaque. The sperm mass was almost clear, having a slightly granular appearance and contained

some motile sperm. No spermatozoa were observed in the ventral receptacle or spermathecae.

Drosophila equinoxialis Dobzhansky.

Attempted matings with flies one day old were unsuccessful. These flies were then left together overnight and the females dissected early the following morning. Two inseminations were secured. In both specimens the ventral receptacle was teeming with sperm while the spermathecae contained only a few. The vagina was filled with semen, partly consolidated into a granular, semi-formed mass containing a few sperm. The remainder of the lumen contained relatively clear fluid with many motile sperm. The vaginae of these specimens were not noticeably enlarged.

Drosophila nebulosa Sturtevant.

Matings were secured with flies 7 and 8 days old. The average length of copulation was about one minute and 30 seconds. In a specimen dissected 15 minutes after copulation the ventral receptacle and spermathecae were full of sperm while the vagina contained no sperm or semen. In a specimen dissected at 30 minutes, however, a mass of motile sperm was observed in the median oviduct and motile sperm were emerging from the broken end of this organ. The vagina was likewise free of semen in a specimen dissected at one hour, but a specimen dissected at one and one-half hours revealed a few sperm along the dorsal and ventral edges of the vagina as well as a pocket of motile sperm in the oviduct. A specimen dissected at two hours appeared normal but had an egg in the vaginal cavity. In none of these individuals did the vagina seem to be enlarged.

Drosophila sucinea Patterson and Mainland.

One mating was observed in a bottle of previously unmated flies of mixed ages. The female was isolated and dissected at 30 minutes. The vagina was not noticeably enlarged but contained a moderate amount of granular material in the ventral half. This material did not appear dense or opaque and contained no visible spermatozoa. Both the spermathecae and ventral receptacle were teeming with sperm. This individual is illustrated in Fig. 55 (Pl. XIV).

Drosophila fumipennis Duda.

Two matings were observed in the stock including one complete copulation which lasted 5 minutes and 30 seconds. One female was dissected with the ovaries attached 30 minutes after mating. The vagina was slightly enlarged and was completely filled with the sperm mass, part of which had moved into the median oviduct. A number of sperm were seen at the base of the eggs in the lateral oviduct. The ventral receptacle was filled with sperm while only one of the spermathecae contained sperm. The second female was dissected at one hour. The vagina was a little enlarged and was filled with a rather clear, granular material, motile

sperm being seen only near the anterior end at the base of the oviduct, within which a small mass of sperm was visible. The ventral receptacle and both spermathecae were teeming with motile sperm. This specimen is shown in Fig. 56 (Pl. XIV).

In this and the previous figure (Fig. 55, *D. sucinea*) an additional structure has been included in the dissections. This organ, whose function is completely unknown at present, has been observed only in members of the willistoni species group. It is typically a clear-walled, bladder-like sac composed of two lobes and a narrowing neck which seems to be attached to the base of the ovipositor. Since it invariably remains in position when the intestine is removed from the specimen it must have no connection with the digestive tract, and, conversely, since it remains fastened to the base of the ovipositor it is quite possible that it is related in some way to the reproductive tract.

3. *melanogaster group*

Drosophila melanogaster Meigen.

Matings were secured with flies of various ages. The average length of copulation was about 10 minutes. The earliest dissection performed was 15 minutes after the completion of mating. The vagina was not enlarged but was filled with a sperm mass, large numbers of motile sperm passing through the oviduct and emerging from the broken end of this organ. The ventral receptacle and spermathecae contained many sperm. In a specimen dissected at 30 minutes the sperm remaining in the vagina were largely non-motile. At one hour the vagina was free of sperm and semen in one specimen, while at one and one-half hours another specimen still retained a moderate number of sperm in the vaginal cavity. The vagina of a specimen dissected at 3 hours appeared normal in all respects. A specimen dissected 6 and one-half hours after mating contained an egg in the vagina. Since there were no eggs on the food of the vial in which she had been kept, this would represent the first egg laid by this female after mating. A small droplet on the surface of the food was mounted in saline and observed under the microscope. It was found to be a mass of non-motile sperm, thus affording direct evidence that these elements are expelled by the female in some homogamic matings.

Drosophila simulans Sturtevant.

A pair of flies observed in copula in the stock was isolated and the female dissected 15 minutes after the completion of mating. The vagina was not enlarged and was free of semen and sperm, although the ventral receptacle and spermathecae were filled with these elements. It might be of interest to record here the results of dissections of two *melanogaster* females inseminated by *simulans* males. Neither specimen showed an enlarged vagina or a reaction mass present after 24 hours exposure.

Drosophila ananassae Doleschall.

Matings were secured with flies 2, 3, and 4 days old. The average length of copulation was about 4 minutes. The only accurately timed dissection was made 30 minutes after the completion of mating. All the semen appeared to be in the cavity of the vagina, no sperm being visible as yet in the ventral receptacle or spermathecae. The sperm in the vagina were mostly motile. Near the posterior end, in the region of the ovipositor, a part of the semen was formed into an irregular, crystalline-appearing mass surrounded by granular material. This posterior "plug" was later seen in several other specimens. It seems likely that the first portion of the ejaculate contains the motile sperm in its carrier fluid while the portion ejaculated near the conclusion of copulation is sperm free and soon forms a gel-like mass which forces retention of the semen for some time, thus affording the spermatozoa ample time to reach the receptacles. This plug is probably softened in time by the secretions of the vagina and is expelled, along with the excess semen, or it is possible that muscle contractions eventually force the plug out through the posterior orifice and expulsion of the semen follows.

4. *obscura group*

Drosophila pseudoobscura Frolova.

Matings were secured with flies 2, 3, 4, 5, and 6 days old. The average length of copulation was about 5 minutes. Mayr (1946) gives the median duration as 6 minutes and 15 seconds. Several specimens dissected 15 minutes after copulation did not have enlarged vaginae, but were filled with semen containing motile sperm. No sperm were visible, however, in the ventral receptacles or spermathecae. The pressure of the cover glass forced most of the sperm mass into the oviduct. Additional specimens were dissected at 30 minutes, one, one and one-half, and two hours. In each case a mass of sperm was forced into the oviduct upon dissection or shortly thereafter. The spermatozoa seem to move very slowly into the receptacles in this species. Only 10 to 15 sperm were visible in the ventral receptacle of one individual dissected at one hour, and about two dozen were seen in this organ in the one and one-half hour specimen. The two-hour specimen revealed a large number of sperm in the ventral receptacle but only a moderate number in the spermathecae where they were observed mainly in the stalks.

Drosophila persimilis Dobzhansky and Epling.

A pair of flies observed mating in the stock bottle was isolated and the female dissected 30 minutes later. The vagina was very slightly enlarged and was filled with granular material which was neither dense nor opaque. No sperm were visible in the mass or in the receptacles. Pressure was placed on the cover glass to expel the contents of the vagina revealing a large number of motile sperm in the saline mounting fluid. It seems likely that at this time the secretions from the female tract necessary to

stimulate the spermatozoa into motility were insufficient. This theory would similarly explain the long time required for the sperm to enter the receptacles in the closely related *D. pseudoobscura.*

Drosophila affinis Sturtevant.

Matings were secured with previously unmated flies of various ages mixed together in a stock bottle. The vagina of a specimen dissected at 30 minutes was torn in dissection allowing the contents to exude into the saline solution. A large number of motile sperm were observed in an extremely small quantity of granular material. Very few sperm were visible in the ventral receptacle, none in the spermathecae. In a second specimen, dissected at one hour, the vagina was clear and normal except for a small pocket of motile sperm in a slight pouch. No sperm were visible as yet in the spermathecae.

Drosophila azteca Sturtevant and Dobzhansky.

One mating was secured with previously unmated flies of mixed ages, and the female was dissected 30 minutes later. The vagina was not enlarged but was filled with a granular material. A large sperm mass was expelled through the oviduct immediately following dissection. No sperm were seen in the vaginal cavity although both the ventral receptacle and spermathecae were teeming with spermatozoa.

Drosophila tolteca Patterson and Mainland.

One mating was obtained by mixing previously unmated flies of various ages in a culture bottle. The dissection was made at one hour. The vagina was not noticeably enlarged but was filled with the sperm mass. The contents were granular and relatively clear and contained motile sperm throughout. Part of this mass had migrated into the oviduct. The ventral receptacle contained a moderate number of sperm while none were visible in the spermathecae.

Subgenus Drosophila. Fallen.

1. *quinaria group*

Drosophila transversa Fallén.

Matings were secured with flies 5 and 7 days old. The average length of copulation was about 9 minutes and 30 seconds. The earliest dissection was performed 15 minutes after mating. The vagina was about three times normal size and was quite dense and opaque. Motile sperm were observed around the edges of the reaction mass but only non-motile sperm were visible within the mass. Both the ventral receptacle and spermathecae were teeming with sperm. A specimen dissected 30 minutes after mating was similar in most respects to the previous one, and is illustrated in Fig. 58 (Pl. XV).

Drosophila munda Spencer.

A series of matings was obtained with stock flies by transferring them to fresh food and placing them before a bright light. Copulation times

were recorded for two of these matings, the average time being about 3 minutes and 40 seconds. Several specimens dissected 30 minutes after mating were alike in having a very large vagina filled with the dense, opaque mass. A few motile sperm were visible around the edges of the mass. The ventral receptacles contained a large number of sperm but

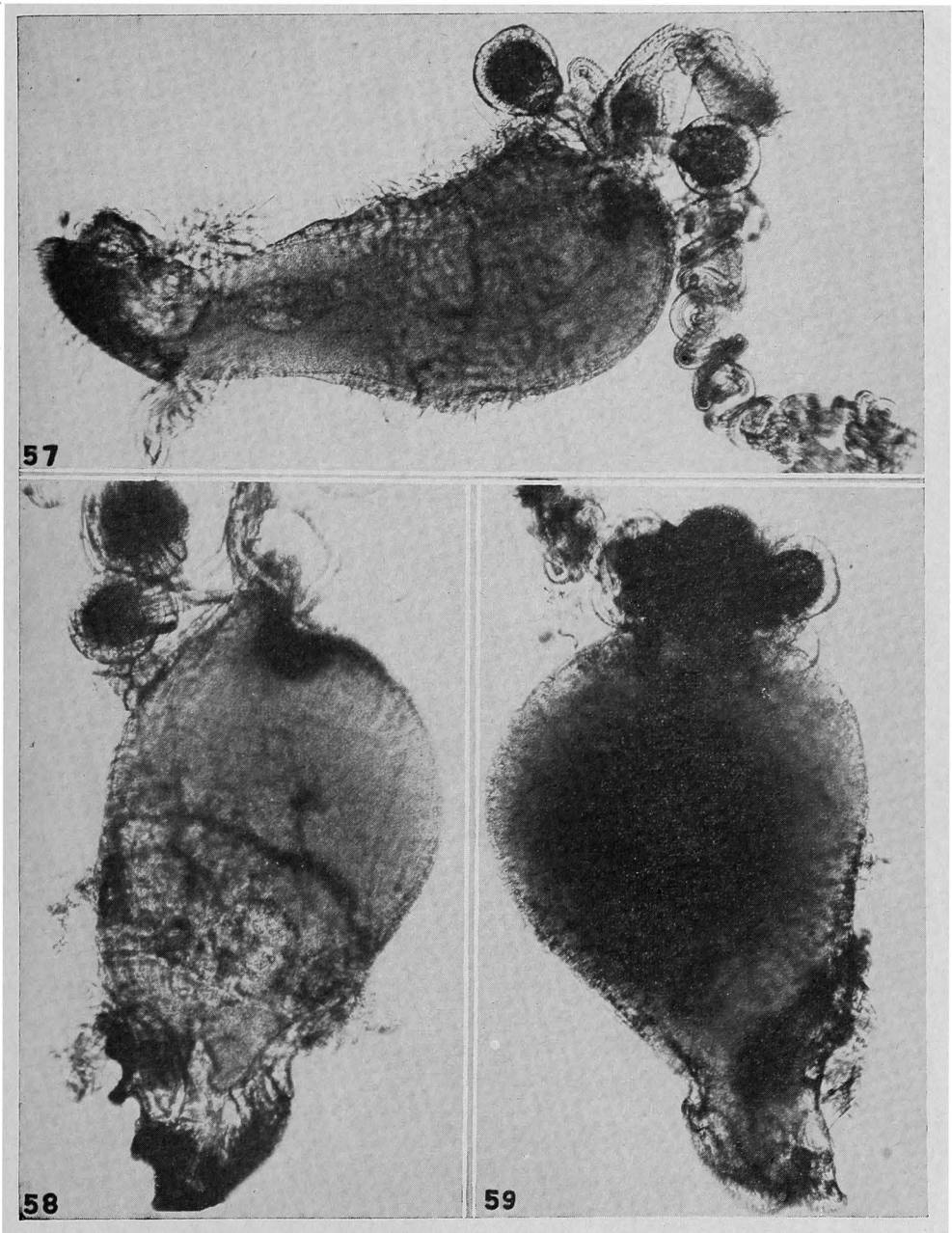

PLATE XV. Vaginae from homogamic matings of three species of Drosophila. Fig. 57, *D. munda* at thirty minutes. Fig. 58, *D. transversa* at thirty minutes. Fig. 59, *D. subquinaria* at one hour.

only a moderate number were seen in the spermathecae. A specimen dissected 30 minutes after mating is shown in Fig. 57 (Pl. XV). A peculiarity of this species is the tendency for the tissues of the vagina, just anterior to the ovipositor, to stretch when the dissection is being made. This is clearly shown in the illustration. A specimen dissected at one hour revealed that the contents of the vagina had cleared somewhat, having lost much of the opacity present in earlier specimens. By this time the spermathecae seemed quite solid with sperm.

Drosophila quinaria Loew.

Two pairs of flies were observed mating and were isolated. The complete copulation time, obtained for one of them, was 7 minutes and 30 seconds. The first specimen was dissected 30 minutes after mating. The vagina was greatly enlarged and filled with a very dense, opaque reaction mass. A few motile sperm were visible around the edges of the mass, while the ventral receptacle and spermathecae contained many sperm. The second specimen was dissected one hour after mating. The vagina appeared about normal in size and contained but a small amount of the granular mass near the base of the ovipositor. A few motile sperm were seen in a pocket along the edge. Both the ventral receptacle and spermathecae were teeming with sperm. The position of the remainder of the mass near the ovipositor suggests that females of this species expel the semen and excess sperm as has been previously suggested for several other species.

Drosophila subquinaria Spencer.

Matings were secured with flies 7 and 8 days old. The average length of copulation was about 8 minutes and 45 seconds. The vagina of a specimen dissected 30 minutes after copulation was extremely enlarged and filled with a very dense, opaque reaction mass. Motile sperm were observed around the edges of the mass and many non-motile sperm could be seen within the mass. The ventral receptacle was filled with sperm while the spermathecae contained only a moderate number of these cells. In another specimen dissected at 30 minutes a part of the mass was moving into the oviduct and motile sperm were seen emerging from the broken end of this organ. A specimen dissected one hour after mating burst when the cover slip was added. The vaginal contents were granular and quite dense. No motile sperm were seen. The last dissection was performed one and one-half hours after mating. The following notes, made at the time, adequately describe this preparation: "Vagina exceedingly enlarged, perfectly round and too dense and opaque to see through. The ventral receptacle and spermathecae are teeming with sperm and many sperm are emerging from the oviduct, the latter also somewhat opaque. The most extreme reaction yet seen." A specimen dissected at one hour is shown in Fig. 59 (Pl. XV).

Drosophila suboccidentalis Spencer.

Matings were secured with flies 7, 8, and 9 days old. The average length of copulation was about 10 minutes. The first specimen, dissected at 30 minutes, was peculiar in several respects. Copulation lasted only two minutes and the female appeared quite agitated during the entire time. The vagina was somewhat enlarged and rather opaque. Its surface had an unusual appearance, being extremely rough and knobby looking, and possessed a pouch at the distal ventral end. No sperm were visible in the preparation. It is probable that this was a malformed individual and that complete copulation was prevented by its abnormalities. A second specimen was dissected one hour after mating. The vagina was quite large, rounded, and filled with a dense, opaque mass which slowly oozed through the ovipositor while under observation. Although only non-motile sperm could be detected within the mass in the vaginal cavity, many motile sperm were seen in the saline mounting fluid as this mass was extruded. Both the ventral receptacle and spermathecae were teeming with sperm. A last specimen, dissected 3 hours after mating, appeared normal in all respects. The only sperm seen were in the receptacles.

Drosophila innubila Spencer.

Matings were secured with flies 8 and 9 days old. The only complete copulation recorded lasted 3 minutes. The vagina of a female dissected 30 minutes after mating was quite large, dense, and opaque. Motile sperm were visible along the edges of the mass while the sperm seen within the mass appeared completely non-motile. Both the ventral receptacle and spermathecae contained sperm. The reaction mass of a specimen dissected one hour after mating was so dense that one could not see through it. A part of the mass had moved into the median oviduct.

Drosophila subpalustris Spencer.

A pair of flies observed copulating in the stock bottle was isolated and the female dissected 30 minutes after the completion of mating. The vagina was greatly enlarged and filled with the dense, granular mass. Sperm were visible in the ventral receptacle and spermathecae.

2. *guttifera group*

Drosophila guttifera Walker.

Matings were secured with flies 4 and 5 days old. The average length of copulation was about 9 minutes. In a specimen dissected at 30 minutes the vagina was slightly enlarged and contained some granular material but was not noticeably opaque. Motile sperm were present in this organ as well as in the ventral receptacle and spermathecae. In one specimen dissected at one hour the vagina was not particularly enlarged or opaque. A few motile sperm were present as scattered bunches in the cavity. In another specimen, also dissected at one hour, the vagina was definitely enlarged and showed a slight tendency toward pouch formation. This

preparation is shown in Fig. 60 (Pl. XVI). The granular nature of the reaction mass is easily seen. The vagina of a specimen dissected at one and one-half hours was noticeably smaller but still enlarged. The opaque mass still filled the lumen and contained non-motile sperm.

3. *pinicola group*

Drosophila pinicola Sturtevant.

This species, considered by Sturtevant (1942) as the most primitive species in the genus, was unavailable for study. This is indeed unfortunate since it would have been most interesting, from an evolutionary viewpoint, to compare the effects of insemination in this species with the results observed in the more highly evolved forms.

4. *virilis group*

Drosophila virilis Sturtevant.

Matings were secured with flies of various ages, the youngest being 7 days old and the oldest 31 days old. The average length of copulation was about 2 minutes and 55 seconds. It is very difficult to describe the sequence of events following insemination in this species because the contents of the vagina escaped through the ovipositor or the broken end of the oviduct in nearly every dissection performed. Dissections have been made at 8, 20, 25, 30, 35, 40, and 50 minutes after mating and in every case the vagina appeared to have been enlarged and filled with an emulsion-like mass containing either motile or non-motile sperm. By 20 minutes both the ventral receptacle and spermathecae contained large numbers of sperm. In some of the individuals the expulsion of the mass took place when the flies were etherized. In a few cases the mass extruded from the ovipositor retained its shape, indicating that it was not completely fluid.

Because of the indeterminate nature of the reaction in this species and since matings were very easy to secure, it was decided to try remating in order to determine if later inseminations called forth a reaction more severe than that of the first mating. Accordingly females were dissected 30 minutes after a second mating and 30 minutes after a fifth mating. The earliest time at which remating could be accomplished was 5 hours and 15 minutes after the first. Rematings at 24-hour intervals were, in general, not difficult to secure. The results of the dissections of females who had mated a second time were definitely inconclusive. No difference in the severity of the reaction could be detected. Similarly, dissections after the fifth mating in as many days resulted in a reaction not noticeably more severe than the first.

Finally, an attempt was made to determine if the age of the flies used would influence the severity of the reaction. When 31-day-old females were placed with 4-day-old males no matings were obtained. When 4-day-old females were placed with 25-day-old males some courting ensued but, once again, no matings were obtained. One mating was secured when a

female 7 days old was placed with males 25 days old. Dissection, however, revealed that the female had not been inseminated. Two matings were secured with 31-day-old females and 7-day-old males. The first of these was dissected 30 minutes after mating. Early in dissection the

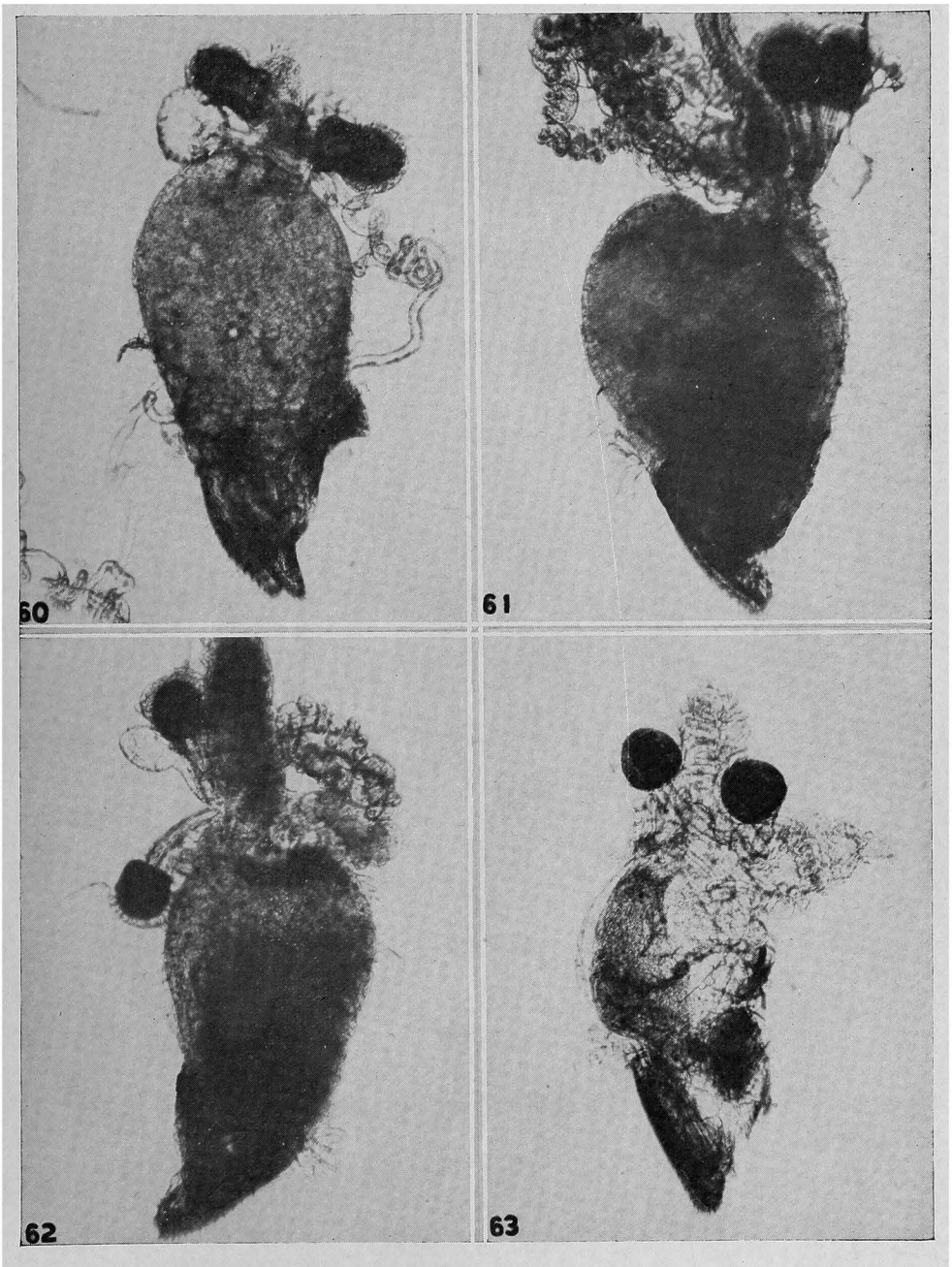

PLATE XVI. Vaginae from homogamic matings of four species of Drosophila. Fig. 60, *D. guttifera* at one hour. Fig. 61, *D. americana* at thirty minutes. Fig. 62, *D. montana* at thirty minutes. Fig. 63, *D. lacicola* at thirty minutes.

sperm mass was expelled through the oviduct and, although irregular in shape, retained its form when in the saline solution. The second mating was dissected one hour later. The notes recorded at the time are as follows: "Vagina very large, rounded, and filled with an opaque mass but containing only a few sperm. The ventral receptacle and spermathecae have a moderate number of sperm and there are a few pockets of sperm in the vagina near their openings. Generally speaking, the reaction mass is too dense to see through." Although the evidence is slight, it is quite possible that the reaction in older females is more severe than in younger females.

Drosophila americana Spencer.

A pair of flies observed mating in the stock bottle was isolated and the female dissected 30 minutes after the completion of copulation. The vagina was enlarged about three times its normal size, and, as may be seen in Fig. 61 (Pl. XVI), showed a moderate-sized pouch. The entire cavity of the vagina was filled with a granular, dense, opaque mass with a few motile sperm visible along its edges. Part of the mass had moved into the oviduct and motile sperm were emerging from the broken end of this organ. The ventral receptacle and spermathecae contained many spermatozoa.

Drosophila americana texana Patterson, Stone, and Griffen.

Two pairs of flies observed mating in a stock bottle were isolated and the females were dissected at 30 minutes and at one hour, respectively. In the specimen dissected at 30 minutes the vagina was seen to be noticeably enlarged while dissecting but before the operation was completed a large mass of sperm and granular material escaped through the oviduct, leaving the vagina about twice its normal size. It was still filled, however, with granular material but did not appear particularly dense. A small pocket of motile sperm was visible near the base of the ventral receptacle which was filled with sperm. Both the stalks and the bodies of the spermathecae contained sperm. As the first dissecting needle was placed in the thorax of the specimen dissected at one hour a large dense mass was extruded from the ovipositor. It retained its shape in the saline solution in which the dissection was being made. The vagina appeared only slightly enlarged and was quite clear except for a small mass of non-motile sperm near the ovipositor and a few motile sperm along the ventral side of the vaginal cavity. A few non-motile and a single motile sperm were seen around the mass which had been expelled.

Drosophila montana Patterson and Wheeler.

Two pairs of flies observed mating in the stock bottles were isolated and the females dissected at 30 minutes and at one hour after mating, respectively. The vagina of the specimen dissected at 30 minutes was about twice the normal size of this organ but was probably larger since a part of the sperm mass was expelled through the oviduct. The vagina

was filled with a granular, dense, opaque material except near the oviduct where some motile sperm were visible. There appeared to be slight tendency for pouch formation although it was not as pronounced as in *D. americana*. Both the ventral receptacle and spermathecae were teeming with sperm. This specimen is illustrated in Fig. 62 (Pl. XVI). The second specimen was dissected at one hour after mating and showed no obvious changes from the earlier dissection.

Drosophila lacicola Patterson.

Matings were secured with previously unmated flies of various ages placed together in a stock bottle. A specimen dissected 30 minutes after mating is shown in Fig. 63 (Pl. XVI). The vagina was slightly enlarged but showed no tendency for pouch formation. There was a moderate amount of granular material in the cavity of the vagina. Many non-motile sperm were visible in the mass while motile sperm were seen along the ventral edge. A fair number of sperm were present in the ventral receptacle but none were seen in the spermathecae. A specimen dissected at one hour had practically returned to normal. The vagina contained a small amount of granular material and many non-motile sperm. No sperm were seen in either the ventral receptacle or the spermathecae of this individual.

5. *testacea group*

Drosophila putrida Sturtevant.

Matings were secured with flies 4 days old. The average length of copulation was about 29 minutes. The vagina of a specimen dissected at 30 minutes was greatly enlarged, being at least three times normal size, and was completely filled with a very dense, opaque reaction mass. No motile sperm were visible in the mass, possibly because it was too dense to see through clearly. The ventral receptacle was teeming with sperm and the spermathecae contained a moderate number. While under observation the pressure of the cover glass forced most of the mass out through the oviduct liberating a small number of motile sperm. The appearance of the extruded mass was peculiar. This material retained its linear order as it emerged, forming a coiled and twisted ribbon in the saline solution. The specimen illustrated in Fig. 64 (Pl. XVII) was dissected at one hour after mating. The exceedingly dense, balloon-like vagina, filled with opaque material, was likewise typical of specimens dissected 2 and 3 hours after mating. A dissection at 4 hours revealed a normal vagina in most respects, no mass or sperm being visible in this organ.

6. *tripunctata group*

Drosophila tripunctata Loew.

Matings were secured with flies 6 to 11 days old. The average length of copulation was about 48 minutes, the longest successful copulation recorded being 82 minutes. The earliest dissection performed was imme-

diately following a copulation of 65 minutes. The vagina appeared normal in all respects, containing no sperm or mass. Both the ventral receptacle and spermathecae contained large numbers of spermatozoa. Additional dissections were made at the following intervals after mating:

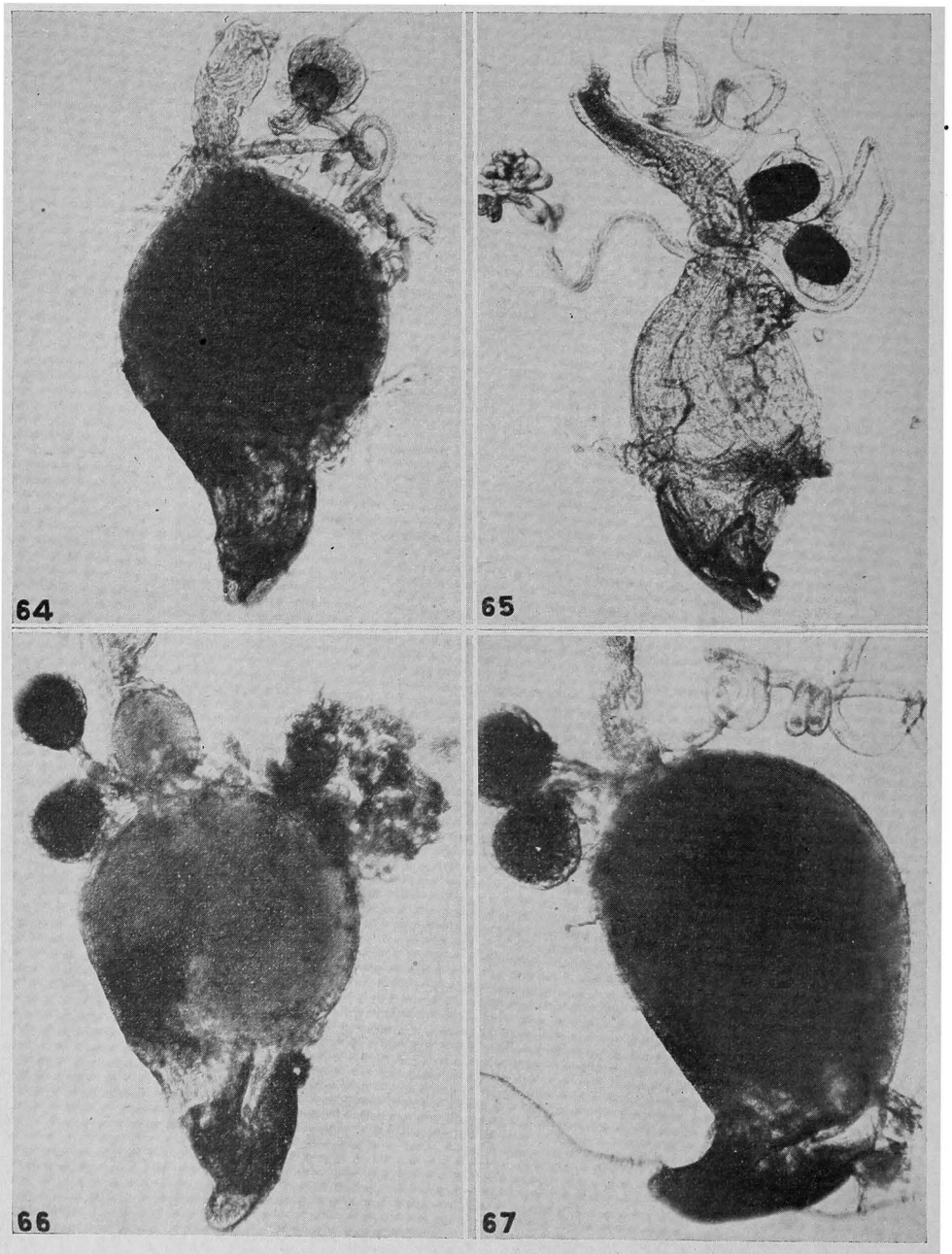

PLATE XVII. Vaginae from homogamic matings of four species of Drosophila. Fig. 64, *D. putrida* at one hour. Fig. 65, *D. tripunctata* at thirty minutes. Fig. 66, *D. subfunebris* at one hour. Fig. 67, *D. macrospina* at thirty minutes.

5, 10, 15, 20, 25, 30, and 45 minutes, and at one, one and one-half, and 4 hours. In none of these specimens was there any sign of vaginal enlargement. The appearance of a specimen dissected at 30 minutes after mating is shown in Fig. 65 (Pl. XVII).

Eighteen eggs were observed in the vial in which the 4-hour specimen had been kept. Only three of them failed to hatch. This is of particular interest since attempts to get rematings with this species were unsuccessful until at least six days after the first mating. It is also of interest to compare the copulation times of these two matings. The length of the first mating was 47 minutes, that of the second, 82 minutes. This is apparently the reverse of the situation found by Mayr (1946) for *D. pseudoobscura,* in which later matings were usually shorter than the first.

Drosophila crocina Patterson and Mainland.

One mating was secured with flies 9 days old. The vagina of the female, dissected 30 minutes after mating, appeared normal in all respects. Both the ventral receptacle and spermathecae were teeming with spermatozoa.

7. *funebris group*

Drosophila funebris (Fabricius).

Matings were secured with flies 6, 7, 8, and 10 days old. The average length of copulation was 16 minutes and 33 seconds. Several dissections were made 30 minutes after mating. The vagina was two to three times its normal size, rounded, and filled with a granular, opaque mass. A few motile sperm were visible around the edges of the mass and a large pocket of them was invariably observed in the median oviduct. Both the ventral receptacle and spermathecae were teeming with sperm by this time. It might be of interest to record here the observation of a successful copulation of a pair of flies of which the male had been thoroughly etherized less than two hours previously.

Drosophila subfunebris Stalker and Spencer.

Matings were secured with flies 9 days old. The average length of copulation was about 23 minutes and 30 seconds. The vagina of a specimen dissected 30 minutes after mating was definitely enlarged but showed no tendency for pouch formation. The entire cavity was filled with a large opaque, emulsion-like mass. No sperm were visible, however, until the contents had been expelled into the saline solution by pressure on the cover slip. A few active sperm were then seen in the mounting fluid. Specimens dissected at one hour after mating showed no apparent change from the previous one. Such a dissection is shown in Fig. 66 (Pl. XVII).

Drosophila macrospina Stalker and Spencer.

Matings were secured with flies 10 days old. The average length of copulation was about 35 minutes. A specimen dissected at 20 minutes appeared to have had an enlarged vagina but nearly all the mass passed out through the oviduct upon dissection. One specimen dissected at 30

minutes had expelled the entire contents of the vagina before observation. Another dissection made at 30 minutes is shown in Fig. 67 (Pl. XVII). The vagina was definitely enlarged and filled with a dense reaction mass. A few motile sperm were visible along the edges of the mass. A last specimen, dissected at 40 minutes, had cleared and the vagina appeared normal.

8. *repleta group*

Drosophila repleta Wollaston.

Several matings were observed in a stock bottle in which had been mixed previously unmated flies of various ages. The males of five of the seven copulations observed failed to inseminate the females. The appearance of a specimen dissected at 30 minutes is shown in Fig. 68 (Pl. XVIII). The vagina was but slightly larger than that of a virgin and contained a moderate amount of granular material. This mass was relatively clear, not dense and opaque, and contained motile sperm in clumps scattered throughout the vaginal cavity. The ventral receptacle and spermathecae contained many spermatozoa. A specimen dissected one hour after mating was similar in most respects to the former, the only observable change being the presence of greater numbers of sperm in the receptacles.

Drosophila neorepleta Patterson and Wheeler.

One mating was observed in a stock bottle of 12-day-old flies. Additional dissections were made of females selected at random from this bottle. A specimen dissected 30 minutes after mating is shown in Fig. 69 (Pl. XVIII). The vagina was somewhat enlarged and showed a definite tendency for pouch formation. The vaginal cavity was nearly filled with a granular mass in which many sperm were visible. Part of the sperm mass moved into the oviduct while under observation. Both the ventral receptacle and spermathecae were teeming with sperm. Several dissections of the females selected at random appeared to have been made at a time greater than 30 minutes after mating. Most of the vaginal sperm mass was concentrated in the pouch where their motility was easily observed.

Drosophila species (= *limensis* Pavan in press).

Females from the stock culture were dissected at random since no observed matings were secured. Several of these specimens appeared to have been inseminated fairly recently. The vaginae were slightly enlarged and filled with a relatively clear granular material. Many sperm were present in the vaginal cavity, those along the ventral side being highly motile.

Drosophila canapalpa Patterson and Mainland.

Two pairs of flies were observed mating in the stock bottle and isolated. A specimen dissected 30 minutes after mating had a slightly enlarged vagina with no evidence of pouch formation. The sperm mass, which

filled the entire vaginal cavity and part of which had moved into the median oviduct, was granular and dense and contained many sperm which appeared only slightly motile. The ventral receptacle and spermathecae contained a moderate number of sperm. The vagina of a specimen dissected at one hour had returned to normal and was cleared, except for a small pocket of non-motile sperm near the ovipositor.

Drosophila melanopalpa Patterson and Wheeler.

Two matings were observed in the stock culture bottle. Additional dissections were made of females chosen at random from this bottle. A specimen dissected at 30 minutes did not appear to have been inseminated. The vagina of a specimen dissected at 40 minutes was of normal size and contained no visible mass of any kind. The only evidence of a recent insemination was the presence of motile sperm in the basal section of the ventral receptacle. There were many sperm in the mid-section of this organ but none were visible in the spermathecae. The only information obtained from the females taken at random from the culture bottle was the observation that most inseminated females contained but a small number of visible sperm in their receptacles.

Drosophila hydei Sturtevant.

Matings were secured with flies 11 and 13 days old. The average length of copulation was about 4 minutes. The vagina of a specimen dissected 15 minutes after mating was about of normal size with a small opaque area at the anterior end. There were a few motile and many non-motile sperm in the vaginal cavity and both the ventral receptacle and spermathecae were teeming with them. Dissections 30 minutes and one hour after mating revealed no signs of enlargement and no tendency for reaction mass formation.

Drosophila mulleri Sturtevant.
Drosophila aldrichi Patterson and Crow.
Drosophila arizonensis Patterson and Wheeler.
Drosophila mojavensis Patterson and Crow.
Drosophila buzzatii Patterson and Wheeler.
Drosophila hamatofila Patterson and Wheeler.
Drosophila ritae Patterson and Wheeler.
Drosophila peninsularis Patterson and Wheeler.

The eight species listed above, all members of the mulleri subgroup of species, have been discussed in detail by Dr. Patterson in the preceding article of this publication. May it suffice here to point out that homogamic inseminations in the first five members of this group are followed almost immediately by a striking enlargement of the vagina, followed by the development of a very dense, opaque reaction mass which persists for several hours. Each of these species shows a definite pouch when enlarged. *D. hamatofila* and *D. ritae,* however, are characterized by an elongated swelling of the vagina, the latter similarly filled with a dense reaction mass. The last species, *D. peninsularis,* develops the least extreme reac-

tion mass of the group, the vagina exhibiting only a small amount of swelling and the mass never becoming as dense and opaque as in the other members of this subgroup.

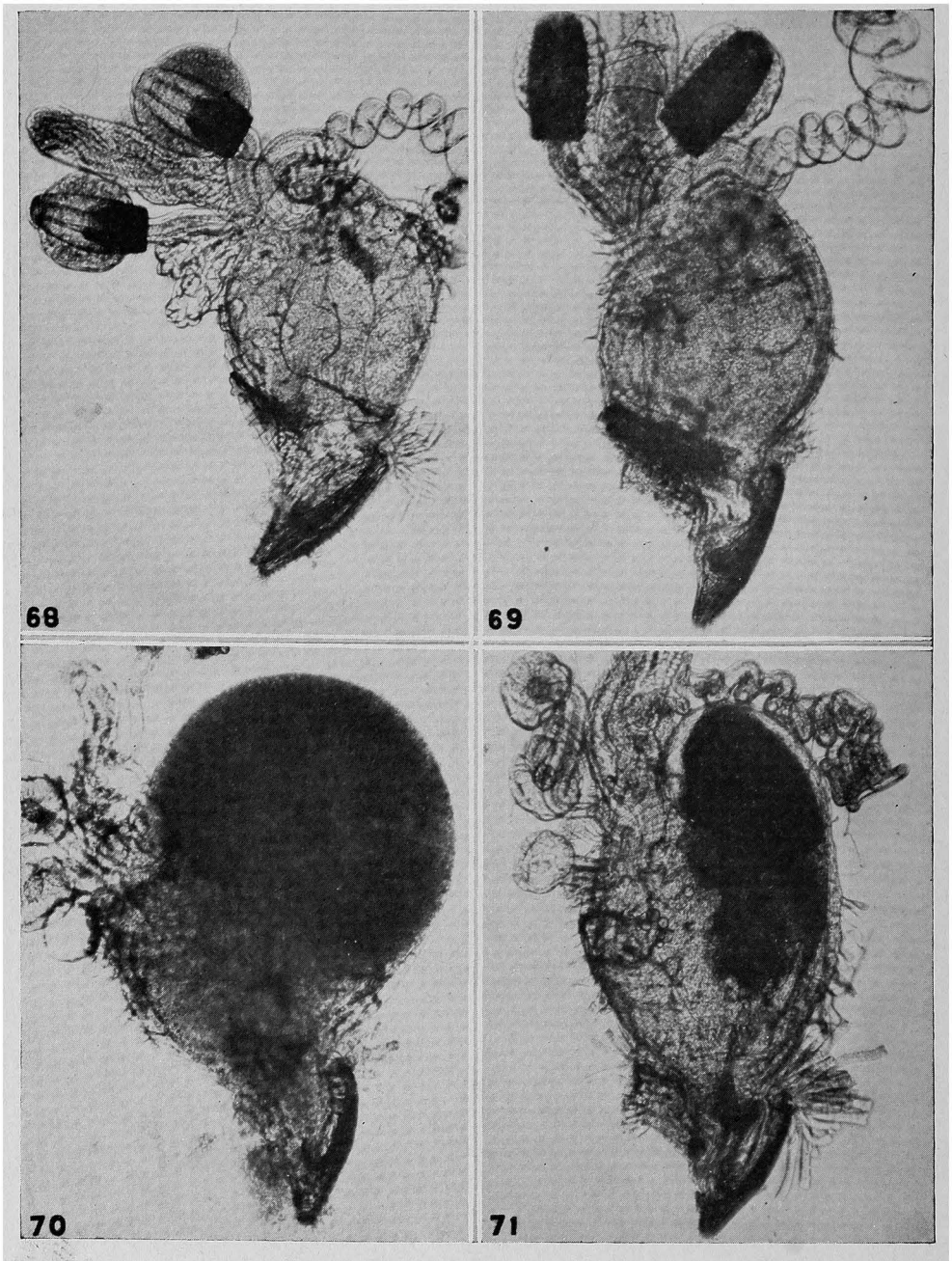

PLATE XVIII. Vaginae from homogamic matings of four species of Drosophila. Fig. 68, *D. repleta* at thirty minutes. Fig. 69, *D. neorepleta* at thirty minutes. Fig. 70, *D. mercatorum* at one hour and thirty minutes. Fig. 71, *D. hexastigma* at one hour and fifteen minutes.

Drosophila mercatorum Patterson and Wheeler.

Matings were secured with flies 5 days old. The average length of copulation was about 3 minutes. A specimen dissected 30 minutes after mating had a greatly enlarged vagina consisting mainly of an immense pouch, the entire cavity being filled with an emulsion-like mass. No sperm were visible in the mass or in the spermathecae although the ventral receptacle was filled with them. Dissections at one, one and one-half, and two hours after mating revealed no observable change from the condition described above. A specimen dissected at one and one-half hours after mating is illustrated in Fig. 70 (Pl. XVIII).

Drosophila mercatorum pararepleta Dobzhansky and Pavan.

One mating was observed in the stock culture bottle. Additional females from this bottle were selected at random for dissection. The mated female was dissected 30 minutes after the completion of copulation. The vagina was quite enlarged with a rounded pouch, the entire cavity being filled with an emulsion-like material. The ventral receptacle was teeming with sperm but none were visible in the spermathecae. Several of the stock females which were dissected were quite similar to that described above while others were apparently very late stages in which the vagina had practically cleared.

Drosophila meridiana Patterson and Wheeler.

Matings were secured with flies 10 days old. The average length of copulation was about 2 minutes. The vagina of a specimen dissected 10 minutes after mating was moderately enlarged, granular, and slightly opaque, and exhibited a small pouch. A few motile sperm were seen in the pouch area. A part of the granular mass was observed in the oviduct. The ventral receptacle was teeming with sperm while only the stalks of the spermathecae contained sperm. A specimen dissected at 30 minutes was much like the previous one, but the entire contents of the vagina were expelled through the oviduct before observation under the microscope. The vagina of a last specimen, dissected at one hour, was practically cleared, no mass remaining in the vagina or pouch.

Drosophila anceps Patterson and Mainland.

Matings were secured with flies 7, 10, and 11 days old. The average length of copulation was about 5 minutes and 30 seconds. The appearance of a specimen dissected at 30 minutes is shown in Fig. 73 (Pl. XIX). The vagina was somewhat enlarged with a slight pouch. The contents were fairly clear near the ovipositor but were darker and more dense in the pouch where a few motile and many non-motile sperm could be seen. A dissection at one hour revealed very little change. The vagina of a specimen dissected at 3 hours was much smaller, however, and the pouch was greatly reduced in size. The dark area of the mass was likewise smaller, the lighter material remaining as before. As a 4-hour dissection was being performed a semisolid mass was extruded from the ovipositor.

On a slide in saline this material was seen to consist largely on non-motile sperm with a few motile sperm around the edges. The size of the vagina of this specimen was normal when examined.

Drosophila hexastigma Patterson and Mainland.

Two matings were secured by mixing previously unmated flies of various ages in a culture bottle. The length of copulation was 6 minutes and 30 seconds for one timed mating. Additional females were selected at random from the stock and dissected. In a specimen dissected at one hour the sperm had not yet entered the ventral receptacle but were quite active along the ventral side of the vagina, the dorsal side being filled with a very dark reaction mass which extended into a rather elongate pouch. A specimen similar to this, dissected at one hour and 15 minutes after mating, is shown in Fig. 71 (Pl. XVIII). The vagina proper was slightly enlarged and contained a small amount of the reaction material but the greatly enlarged pouch was filled with this exceedingly dense, elongate mass. A small "wad" of this material was observed in the ovipositor indicating that the female was in the act of expelling this material.

Drosophila gibberosa Patterson and Mainland.

Matings were secured with flies from 10 to 14 days old. The average length of copulation was about 8 minutes and 24 seconds. The vagina of a specimen dissected 30 minutes after mating was much enlarged, forming a pouch, and the entire cavity was filled with an extremely dense, opaque, granular mass teeming with motile sperm. A few sperm were visible in the basal part of the ventral receptacle and, although one spermatheca contained no sperm, the other was about half filled with these cells. The vagina was still more enlarged at one hour, but at two hours it had begun clearing a little and was definitely smaller. The appearance of this dissection is shown in Fig. 72 (Pl. XIX). No motile sperm were seen in or around the mass but a moderate number were visible in the ventral receptacle. Specimens dissected at 5 and at 23 hours after mating appeared normal. Several matings were secured in which the males failed to inseminate. In one instance a copulation lasting 6 minutes was followed one minute later by a second copulation with another male lasting 2 minutes. Dissection of the female revealed that she had not been inseminated, suggesting that the failure of mating was due somehow to the female and not to the males concerned.

9. *robusta group*

Drosophila robusta Sturtevant.

Matings were secured with flies 20 and 21 days old, although, in mass matings, insemination occurred with flies 11 and 13 days old. The average length of copulation was about one minute. The vagina of a specimen dissected 30 minutes after mating did not appear to be enlarged but contained a rather small granular mass in the lumen in which some non-motile sperm were visible along the edges. Both the ventral receptacle and

spermathecae were teeming with sperm. Dissection at one hour revealed very little change, the granular mass being relatively clear. No sperm were visible in the cavity of the vagina. By one and one-half hours the vagina appeared to be entirely normal.

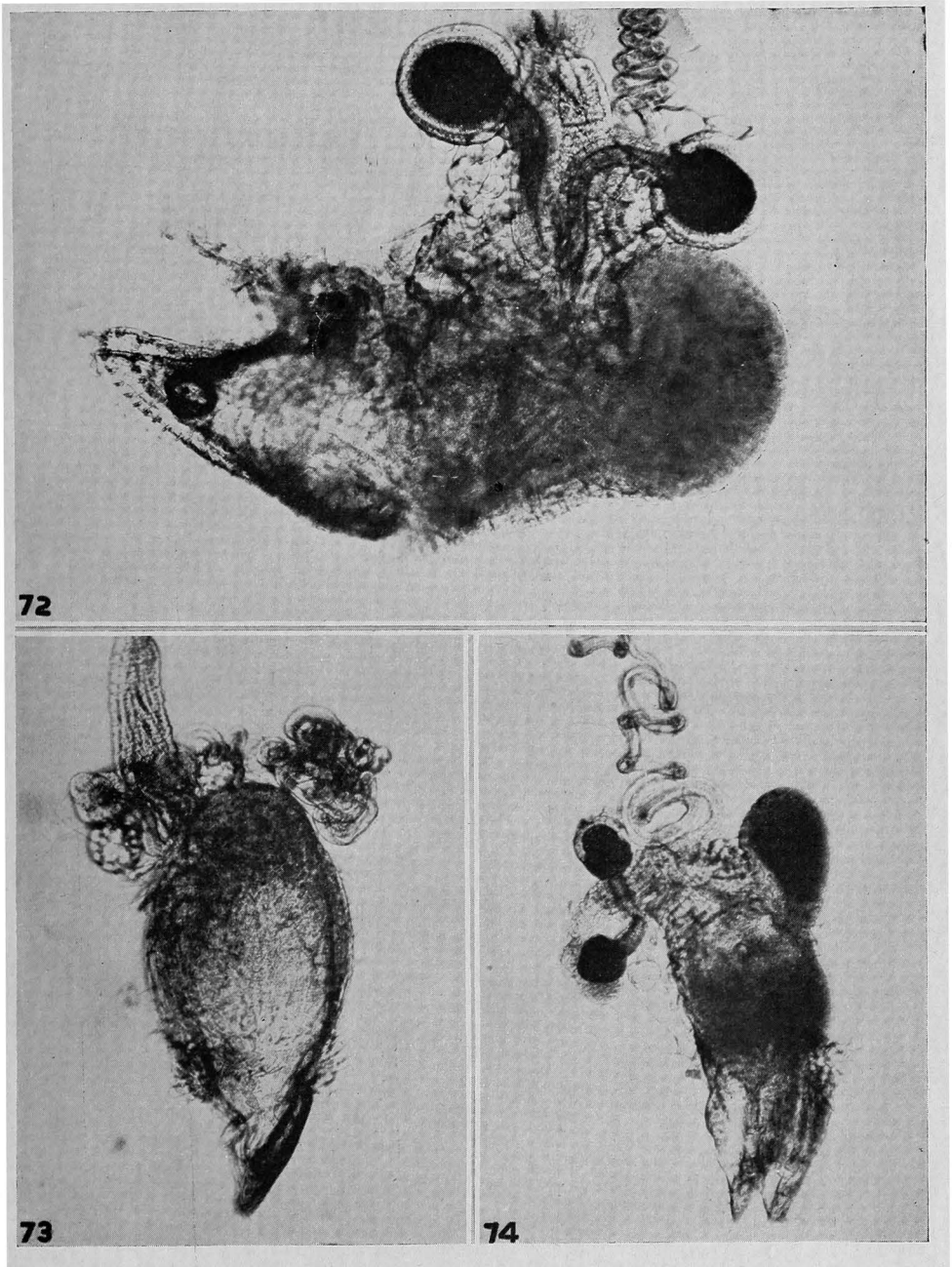

PLATE XIX. Vaginae from homogamic matings of three species of Drosophila. Fig. 72, *D. gibberosa* at two hours. Fig. 73, *D. anceps* at thirty minutes. Fig. 74, *D. carbonaria* at thirty minutes.

10. *melanica group*

Drosophila melanica Sturtevant.

One mating was secured with flies 16 days old. Copulation lasted 3 minutes and 45 seconds. This specimen, dissected at 30 minutes, is shown in Fig. 75 (Pl. XX). The main body of the vagina was only slightly enlarged but a pronounced pouch was present. Before the photograph could be taken most of the mass in the pouch escaped through the oviduct and can be seen in the illustration around the broken end of this organ. The reaction mass was relatively clear except in the pouch where many sperm were visible. Both the ventral receptacle and spermathecae contained many sperm. Additional dissections of stock females chosen at random revealed various stages of clearing. Apparently the mass in the main cavity of the vagina clears first; this is followed by a decrease in density of the mass within the pouch, and eventually the loss of this mass.

Drosophila melanica paramelanica Patterson.

Dissections were made of females selected at random from the stock culture bottle. One specimen was observed with a reaction of about the same severity as that observed in *D. melanica* described above. This individual is illustrated in Fig. 76 (Pl. XX). The vagina exhibited a pronounced pouch filled with a granular reaction mass which was not particularly dense in appearance. There were many motile sperm around its edges. The mass extended into the lumen of the vagina where more motile sperm were observed. A small narrow band of this material was seen leading toward the ovipositor as if expulsion of the mass was in progress. Both the ventral receptacle and spermathecae contained many sperm.

Drosophila nigromelanica Patterson and Wheeler.

One mating was observed among previously unmated flies of various ages. Dissection was performed at 30 minutes. The vagina was moderately enlarged and rounded with a slight pouch at the base of the ventral receptacle. The entire vaginal cavity was filled with a rather clear granular mass, motile sperm being visible only in a pocket in the pouch area. Many sperm were visible in the ventral receptacle and in the stalks of the spermathecae.

Drosophila micromelanica Patterson.

A single copulation was observed in a vial containing two-day-old unmated flies. The female was isolated and dissected at 30 minutes. The vagina was enlarged in the form of a pouch which was filled with a granular, clear reaction mass in which many sperm were observed. Although the ventral receptacle was teeming with sperm, none were visible in the spermathecae. This specimen is shown in Fig. 77 (Pl. XX). Dissections of two- and three-day-old flies which had not been separated since emergence, revealed that insemination occurs very early in the life of this species although the ovaries are still quite immature and these

females do not begin laying fertile eggs for some time. This condition, which has also been observed in several other species, has been described by Stalker (1942) for certain members of the virilis species complex as follows: ". . . Females of some *Drosophila* species pass through two

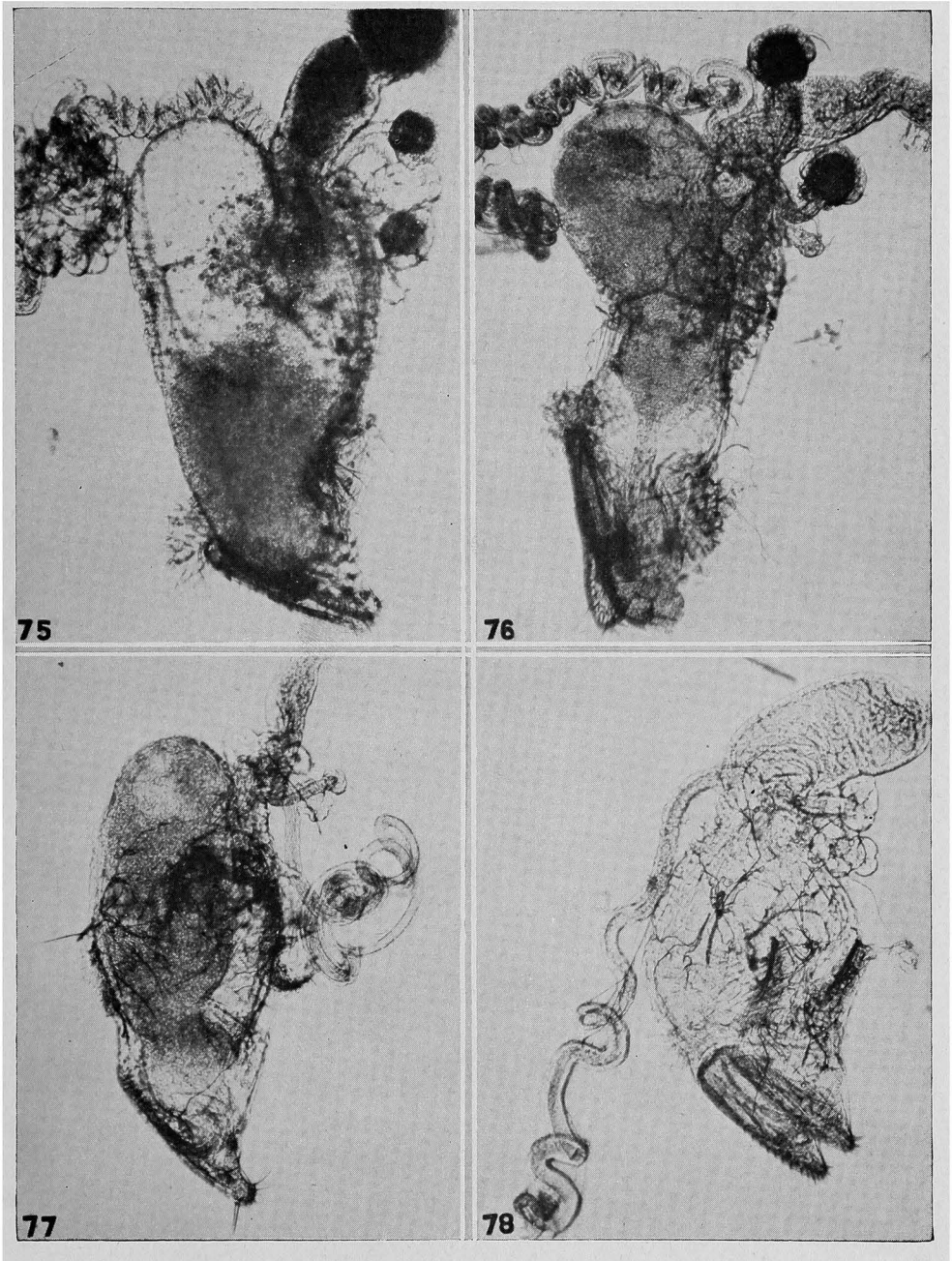

PLATE XX. Vaginae from homogamic matings of four species of Drosophila. Fig. 75, *D. melanica* at thirty minutes. Fig. 76, *D. melanica paramelanica* from stock. Fig. 77, *D. micromelanica* at thirty minutes. Fig. 78, *D. polychaeta* at thirty minutes.

distinct stages in their adult development. In the first stage they will mate, and can be inseminated, but are unable to lay any eggs due to the fact that their ovaries are undeveloped. In the second stage they can both mate and oviposit."

11. *polychaeta group*

Drosophila polychaeta Patterson and Wheeler.

The peculiar mating behavior of this species made it very difficult to know when copulation had occurred. The males seldom courted the females before mounting but usually walked up from behind and forcibly jabbed at the female with the tip of the abdomen. Many times they managed to secure a grip on the wings of the female and remain in position for several seconds without actually accomplishing penetration. Six- and 7-day-old flies, for example, were set up in matings and watched intently for two hours, during which time numerous jabs at the females were made. When the females were later dissected none had been inseminated. A copulation lasting 35 seconds was observed in a vial of 13-day-old flies and the female was dissected 30 minutes later. Insemination had been successful but all the sperm were in the ventral receptacle, the vagina being free of sperm or semen. This individual is shown in Fig. 78 (Pl. XX). In another instance, a 7-day-old female was placed with several 6-day-old males. There were numerous jabs by the males and then an apparent copulation which lasted 20 seconds. This was followed by more jabs and then another copulation lasting about 15 seconds. The female was isolated and dissected one hour after the first copulation. Insemination had been accomplished but no sperm were visible in the vagina or spermathecae while many were observed in the ventral receptacle.

12. *carbonaria group*

Drosophila carbonaria Patterson and Wheeler.

Matings were secured with flies 3 and 4 days old. The average length of copulation was about 26 minutes. Several pairs observed mating in the wild around exuding sap of the mesquite (*Prosopis* sp.) were captured and the females dissected. A specimen dissected at 30 minutes after mating is illustrated in Fig. 74 (Pl. XIX). The reaction mass was confined almost entirely to the elongated pouch, the remainder of the vaginal cavity appearing free of sperm and semen. Both the ventral receptacle and spermathecae contained many sperm. Other dissections at 30 minutes resulted in expulsion of most of the mass. The pouch of a specimen dissected at one and one-half hours was considerably smaller but still contained a fair sized reaction mass.

13. *cardini group*

Drosophila cardini Sturtevant.

Matings were secured with flies 4 days old. The average length of copulation was about 23 minutes and 45 seconds. A specimen dissected at

30 minutes is shown in Fig. 80 (Pl. XXI). The vagina appeared to be a little larger than that of a virgin but contained no observable semen. A very few non-motile sperm were visible along the ventral edge of the cavity. Sperm were present in both the ventral receptacle and spermathecae. Another specimen dissected at 30 minutes and one dissected at one hour appeared to have had some granular material in the vagina which escaped to the outside during dissection. In both cases a few motile sperm were observed in the saline solution. Dissections at one and one-half and at two hours showed no further evidence of an obvious reaction.

14. *immigrans group*

Drosophila immigrans Sturtevant.

Matings were secured with flies 3, 4, 5, and 9 days old. Considerable variation was found in the duration of copulation, the shortest recorded being 14 minutes, the longest, 64 minutes, with an average of about 30 minutes, although Sturtevant (1942) gives the average as 53 minutes. A dissection 4 minutes from the beginning of mating revealed that a small amount of granular material had been deposited in the vagina but no sperm were visible as yet. A dissection 10 minutes from the beginning of mating revealed many motile sperm in the vaginal cavity although none were seen in the receptacles. A specimen dissected immediately following a copulation of 30 minutes had an enlarged vagina filled with an emulsion-like material in which large numbers of motile sperm were visible. The vagina appeared rounded and somewhat elongated but showed no observable tendency for pouch formation. A specimen dissected one hour after a copulation of 36 minutes had a nearly normal vagina, this organ being approximately the size of that of a virgin and containing only a minute amount of granular material. All of the receptacles were filled with sperm. The vagina of a specimen dissected two hours after a 37-minute copulation was very large, however, and was filled with the reaction mass, motile sperm being observed only along the edges of the mass. A dissection 4 hours after mating once again revealed a vagina of normal size, free of all semen and sperm. Additional dissections were performed at varying intervals after mating in an effort to determine if there was any relation between the length of copulation and the duration of the reaction. Only conflicting results have been secured to date so that a discussion of this question must await further study.

15. *macroptera group*

16. *alagitans group*

17. *rubrifrons group*

No members of the above groups were available for study.

18. *guarani group*

Drosophila guarani Dobzhansky and Pavan.

A pair of flies was observed mating in the stock bottle and the female isolated. The dissection was made at 30 minutes with the ovaries attached.

The vagina was slightly enlarged and was filled with a rather clear granular material in which a few motile sperm were visible. There was a large mass of sperm in the median oviduct and some of these sperm were observed to have migrated into the lateral oviduct. The ventral receptacle contained a moderate number of sperm and the spermathecae were filled with these cells. Twelve females, 7 days old, were exposed for 16 hours

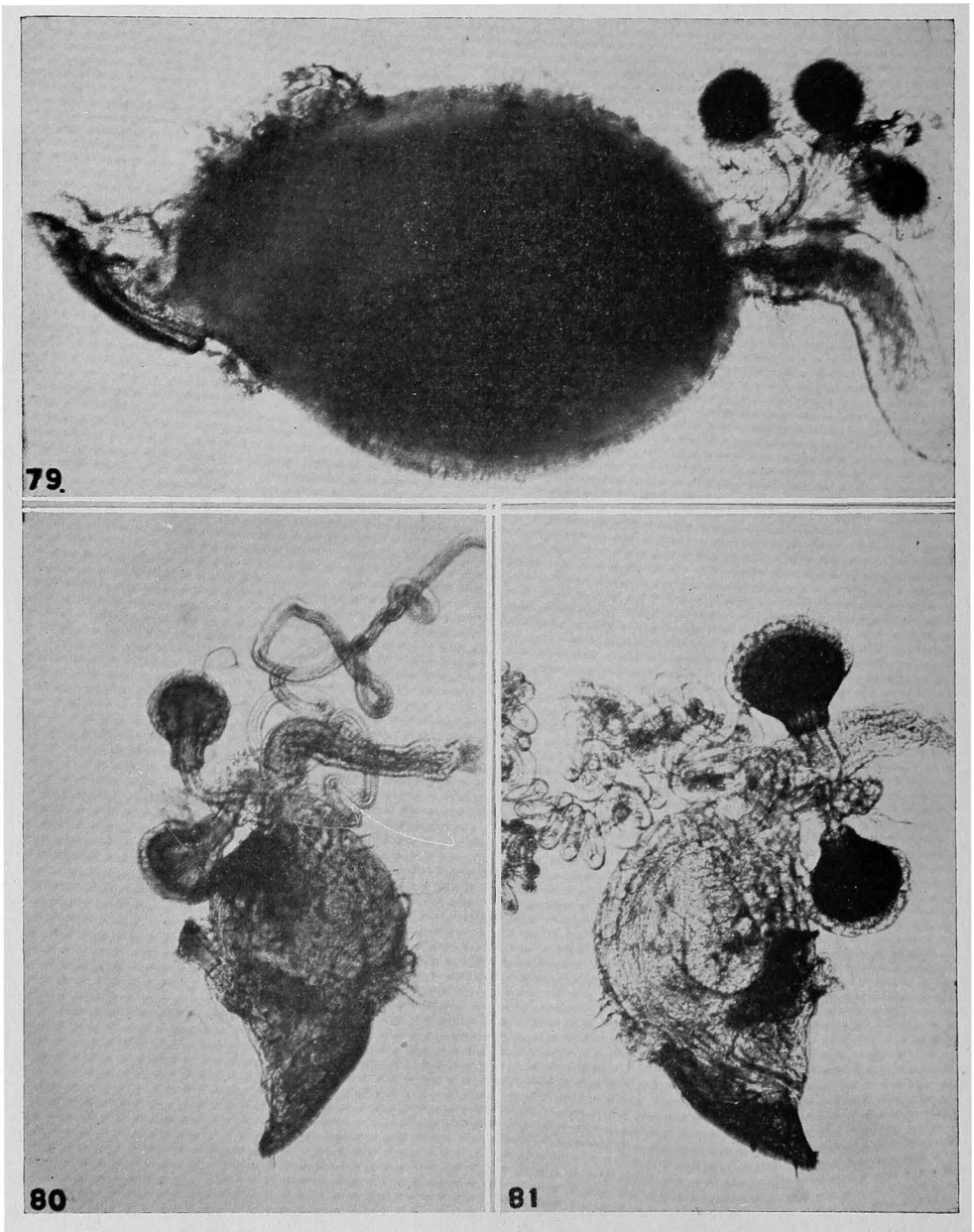

PLATE XXI. Vaginae from homogamic matings of three species of Drosophila. Fig. 79, *D. pallidipennis* at one hour. Fig. 80, *D. cardini* at thirty minutes. Fig. 81, *D. subbadia* at thirty minutes.

to males of the same age and then dissected. Only two were found to have been inseminated and no evidence of a reaction was found.

Drosophila subbadia Patterson and Mainland.

One mating was observed in a vial of previously unmated, 10-day-old flies. The female was isolated and dissected 30 minutes later. The vagina was not enlarged and contained no semen or sperm. Both the ventral receptacle and spermathecae were teeming with sperm. This specimen is illustrated in Fig. 81 (Pl. XXI).

Species unclassified as to grouping

Drosophila parachrogaster Patterson and Mainland.

One mating was secured with flies 22 days old. The length of copulation was 30 minutes and 25 seconds but it is likely that this is longer than the normal since the first part of the mating seemed to be faulty. The female was dissected 30 minutes after the completion of copulation. The vagina was nearly three times its normal size and appeared to be elongate and rounded with no tendency for pouch formation. Both the ventral receptacle and spermathecae were teeming with sperm. Before this specimen could be photographed at least half of the vaginal contents were expelled through the oviduct. Many motile sperm were then observed in the saline mounting fluid.

Drosophila pallidipennis Dobzhansky and Pavan.

Matings were secured with flies 4 to 6 days old. The average length of copulation was about 30 minutes. Dissections made at 30 minutes and at one, one and one-half, and two hours after mating were alike in nearly all respects. The preparation shown in Fig. 79 (Pl. XXI), dissected at one hour, is typical of this species. The vagina was exceedingly large and showed no tendency for pouch formation. The entire cavity was filled with a very dense, opaque reaction mass with a few motile sperm visible along the edges. Some of this mass and the contained sperm had moved into the oviduct. Both the ventral receptacle and spermathecae contained many sperm. In the specimen illustrated one spermathecal stalk was doubled at the tip and possessed two bodies.

Drosophila pallidipennis centralis Patterson and Mainland.

Matings were secured with flies 2 and 3 days old. The average length of copulation was about 32 minutes. Dissections at 30 minutes, and at one, and one and one-half hours after mating showed that the development of the reaction in this form follows very closely the pattern of the typical species discussed above. The dissection at one hour was of particular interest since the male did not appear to have delivered any spermatozoa in the ejaculate. The reaction developed, however, in the usual fashion, indicating that the presence of sperm is not a requisite for the formation of the insemination reaction.

DISCUSSION

In the present section it is proposed to discuss briefly the sequence of events following insemination in view of the varied expression of the insemination reaction and to present a classification of the species and species groups based on the type of reaction they show. Finally, an attempt will be made to show some possible functions of the reaction in homogamic matings.

An attempt to interpret the mechanisms involved in a reaction between the semen of the male and the reproductive tract of the female should logically begin with an inspection of the origin and development of the semen and contained sperm since it is obvious that there can be no insemination reaction without one or both of these products. The only other alternative would be the possibility that the physical act of copulation, *per se,* might inaugurate the reaction. The fact that many copulations, particularly those with old males, fail to result in ejaculation and, similarly, fail to result in the development of the typical reaction would tend to show that strictly mechanical processes are incapable of provoking this phenomenon.

Dr. Patterson has shown in the preceding article that when known sterile males are mated to females which would normally develop the reaction following copulation the typical form of the insemination reaction follows even though no mature sperm are delivered. Further evidence that sperm are not a requisite for the development of this reaction is presented by the dissection of a *D. pallidipennis centralis* female, described in the previous section of this paper, in which it was observed that the male had apparently failed to deliver any sperm in the ejaculate, but, nevertheless, the typical reaction developed.

According to Nonidez (1920), who studied the passage of the spermatozoa from the time of their formation to ejaculation, the liquid part of the ejaculate is composed of the secretions of the paragonia. He describes this material as a dense, sticky fluid in which float abundant refractive granules of varied size. The similarity of this description to that of the material observed in the vaginal cavity of many species immediately following copulation is strikingly obvious. There can be but little doubt that the material seen in these vaginae at this time is composed largely of the paragonial secretions and the spermatozoa. The emulsion-like material observed in certain species, e.g., *D. mercatorum,* merely represents a specific modification of the type of secretion.

It seems likely, then, that any observed reaction is largely a reaction between these secretions and the female reproductive tract and, furthermore, that the severity of the visible reaction is determined by those changes in the paragonial secretions which are observable. In some species, e.g., *D. polychaeta,* one is unable to detect any change, the ejaculate disappearing from the vaginal cavity with remarkable rapidity. In other forms, members of the obscura group, for example, this material disappears much less rapidly, while in species like *D. ananassae* it seems

to harden into a gel-like mass. In the most extreme cases, e.g., in members of the mulleri subgroup, the vagina becomes tremendously swollen and the contents become consolidated into a dense, formed mass which may remain in the vagina for many hours. It is difficult to explain the relatively sudden swelling of the vagina in these latter forms. Patterson (1946) concluded that the introduction of the semen into the vagina was followed almost immediately by a reaction of the mucous membrane which secreted a relatively large amount of fluid into the cavity, thus bringing about the characteristic swelling of this organ. This explanation adequately explains the increase in size but should not be interpreted as resulting in a dilution of the ejaculate but rather as resulting in a further reaction which is evidenced by the consolidation of the material into a formed, persistent mass.

In view of the many observed species differences with respect to the age at mating, the duration of copulation, the speed with which the sperm migrate into the receptacles, etc., it is not surprising that the observed severity of the insemination reaction should also vary among the different forms. Such variation is apparent among the species described in the preceding section of this paper. Table 1 represents an attempt to classify the species under consideration into three groups based on the type of insemination reaction they exhibit. Generally speaking, class 1 is composed of those forms in which no reaction is apparent; class 2 contains those in which a slight or moderate reaction develops; and class 3 contains those in which the severe enlargement of the vagina and the

TABLE 1

Classification of seventy-eight species with respect to the type of insemination reaction present

Class 1	Class 2	Class 3	
C. procnemis	C. amoena	D. duncani	D. mojavensis
S. graminum	D. busckii	D. victoria	D. buzzatii
D. sturtevanti	D. prosaltans	D. transversa	D. hamatofila
D. rectangularis	D. cordata	D. munda	D. ritae
D. nebulosa	D. elliptica	D. quinaria	D. peninsularis
D. melanogaster	D. emarginata	D. subquinaria	D. mercatorum
D. simulans	D. willistoni	D. suboccidentalis	D. m. pararepleta
D. tripunctata	D. equinoxialis	D. innubila	D. meridiana
D. crocina	D. sucinea	D. subpalustris	D. anceps
D. repleta	D. fumipennis	D. guttifera	D. hexastigma
D. melanopalpa	D. ananassae	D. virilis	D. gibberosa
D. hydei	D. pseudoobscura	D. americana	D. melanica
D. robusta	D. persimilis	D. a. texana	D. m. paramelanica
D. polychaeta	D. affinis	D. montana	D. nigromelanica
D. cardini	D. azteca	D. lacicola	D. micromelanica
D. guarani	D. tolteca	D. putrida	D. carbonaria
D. subbadia	D. neorepleta	D. funebris	D. immigrans
	D. "limensis"	D. subfunebris	D. parachrogaster
	D. canapalpa	D. macrospina	D. pallidipennis
		D. mulleri	D. p. centralis
		D. aldrichi	
		D. arizonensis	

dense, opaque nature of the mass are easily observed. Obviously, attempts to classify the borderline cases can only reflect personal opinion since there are no definite rules that may be relied upon in such cases. Where considerable doubt existed as to the proper category to which certain forms belonged they have been placed in the lower one of the two classes concerned. In addition to the seventy species considered in this paper, the eight members of the mulleri subgroup have been added in order to include all species for which information was available.

The major distinction between members of class 1, in which no reaction of any sort can be observed, and members of class 2, in which only a slight reaction can be observed, is largely the speed with which the ejaculate disappears from the vaginal cavity. Certainly, if a female of class 1 is dissected at the appropriate time during copulation the vagina will be seen to contain a certain amount of ejaculate. Frequently, however, this material is so slight in amount and disappears so rapidly that by the end of copulation no signs of it are left. On the other hand, a like amount of material present in the vagina of a female of class 2 persists for a longer time and is visible after copulation. Furthermore, in none of the members of class 1 does there appear to be a consolidation of the ejaculate to form an observable persisting mass. One cannot say, however, that the presence of the ejaculate in the tract of the female does not stimulate secretions by the female organs nor exert some influence on the vagina that may affect fertilization or oviposition.

The members of class 1 form a rather heterogeneous group. The tripunctata, robusta, polychaeta, cardini, guarani, and part of the repleta species group of the subgenus *Drosophila* are included here as well as parts of the saltans and melanogaster groups of *Sophophora.* Sturtevant (1942, p. 29) separates the saltans group into subgroups on the basis of their mesonotal markings. With the exception of *D. prosaltans,* which has doubtfully been placed in class 2, the members of this group which are here considered as belonging to class 1 occur in the same subgroup, while the members of the second subgroup have been placed in class 2, thus adding evidence that the division is a natural one.

Class 2 consists of those forms which are intermediate between species showing no observable reaction and those in which a violent and persistent reaction develops. Most of the species included in class 2 exhibit a certain amount of ejaculate in the vaginal cavity at least thirty minutes after mating but do not show any obvious enlargement of this organ. Frequently the ejaculate seems to gel, as in *D. ananassae,* and this formed mass may be retained for several hours before expulsion.

With the exception of *C. amoena, D. (Dorsilopha) busckii,* and certain members of the repleta section of the repleta group of the subgenus *Drosophila,* class 2 contains those members of the subgenus *Sophophora* not included in the first class, i.e., the willistoni (except for *D. nebulosa*) and obscura groups, and the remainder of the saltans group.

Class 3 contains those forms in which enlargement of the vagina is usually accompanied by the development of a dense reaction mass. Quite frequently the posterior portion of the vagina enlarges to form a pouch. This may be accompanied by enlargement of the entire vagina, as in *D. mercatorum,* or the enlargement may be confined to the pouch, as in *D. carbonaria.* A few forms included in this class, e.g., *D. anceps,* fail to develop the dense, opaque mass, this material appearing clear and crystalline when viewed under the microscope.

Inspection of the members included in class 3 reveals that all but two belong to the subgenus *Drosophila,* the exceptions being *D. (Hirtodrosophila) duncani* and *D. (Pholadoris) victoria.*

It is of interest to compare the hypothetical relationships of the members of the genus as suggested by Sturtevant (1942) in the light of the insemination reaction. Two possible evolutionary interpretations may be considered: (a) independent origin of the character at various times in different phylogenetic lines, and (b) a single appearance of the character early in evolution followed by modification along the various lines. It is important to remember here that, as was pointed out by Patterson (1946), if the insemination reaction arose as a mutation, irrespective of any selective value it might have to the species as a whole, it would spread throughout the population. As a consequence, once such a mutation arose in an evolutionary line it would tend to remain. This does not imply that such a mutation could not occur several times, and, quite possibly, in different ways, but it does imply that once the reaction character developed in a form it would remain in the lines of descendance even though modified by further changes.

With respect to Drosophila phylogeny, then, certain lines of descent, as interpreted by Sturtevant, seem to fit this pattern very well, as, for example, the virilis-quinaria-testacea-guttifera groups. It seems probable, however, that the origin of the subgenus *Dorsilopha (D. busckii)* from this stem is doubtful. Similarly, a study of the supposed lines of descent through the virilis-tripunctata-funebris-repleta groups suggests that the tripunctata group is out of place in this series and that what is generally termed the repleta group is probably composed of several independent species groups, the hydei-repleta portion having evolved along one line, the mulleri-mercatorum portion along another.

The arrangement of the groups of *Sophophora* fits the pattern quite well while the origin of the *Hirtodrosophila* from this stem requires the assumption of an independent mutation for this character.

Several possible functions of the insemination reaction in homogamic matings may be mentioned. It has been pointed out that the lack of an observable reaction in members of class 1 does not necessarily mean that the only function of insemination is sperm delivery. The comments of Patterson (1946, p. 207) are especially pertinent: "It may have the effect here of preparing the reproductive tract for the fertilization mechanism

which is to follow. It should be pointed out, in this connection, that even in forms which show no visible reaction there still may be a change in the mucous membrane which has the same effect."

The retention of the ejaculate in members of class 2 appears to be correlated in some instances with the slower movement of the spermatozoa into the receptacles. It seems logical that in these forms the carrier fluid is retained until most of the sperm have had time to reach the receptacles. Evidence is presented that after a certain length of time this material is expelled by the female along with the excess sperm. This expulsion (in *D. melanogaster,* for example) may account in part for the discrepancy in the number of sperm deposited by the male of this species in relation to the number utilized by the female as determined by Kaufmann and Demerec (1942). They found that each fertile egg layed accounted for twenty to thirty sperm cells whereas numbers greater than seven or eight per egg were rarely seen, the conclusion being that large numbers of sperm were squandered during the laying of the first eggs. It now appears likely that expulsion by the female of the excess sperm may account, at least in part, for this apparent squandering.

In *D. ananassae* the reaction material seems to form a plug at the posterior orifice, effectively preventing expulsion for some time. It is suggested that secretions of the female tract may eventually soften this plug and allow its expulsion or it may be forced out due to muscle contractions.

Among members of class 3 it has been frequently observed that motile sperm were visible around the edges of the mass while only non-motile sperm could be seen within the mass. When the mass was forced into the saline solution, however, many of the sperm within the mass became motile. This suggests two possibilities. It may be that the sperm trapped within the mass are immobilized, due, probably, to the greatly increased viscosity of the mass, and are eventually expelled along with the mass. It seems more likely, however, that dissolution of the mass takes place rather slowly, thus gradually freeing more and more sperm cells into the cavity. This interpretation is in agreement with the observation in many forms that expulsion is gradual and that the mass seems to drain out a little at a time.

One further possible function remains. The reaction process may in some way prepare the vagina for fertilization or oviposition. The laying of large numbers of eggs by virgin females of certain species would tend to show that such a reaction is not a prerequisite to egg-laying but further study is needed to determine what correlation exists, if any, between virgin egg-laying and the development of an obvious reaction. Superficial examination indicates that those females which lay large numbers of eggs as virgins do not show an obvious reaction while most members of class 3 lay only small numbers of virgin eggs or none at all. Similarly, the question of an effect on fertilization must await further study, since no experimental procedures for testing this problem are available as yet.

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V. TWO STRAINS OF DROSOPHILA PENINSULARIS WITH INCIPIENT REPRODUCTIVE ISOLATION

J. T. PATTERSON AND MARSHALL R. WHEELER

INTRODUCTION

To anyone interested in the problem of speciation the most important material to be found is among forms which are morphologically similar or identical, but which show some degree of reproductive isolation. In the genus Drosophila there are a number of pairs, or even groups, of species which fulfill these conditions. These forms constitute borderline cases and furnish critical material for the study of evolutionary divergence. All such cases should be fully investigated. From among several cases of similar pairs in the genus Drosophila, we have selected for comment three which have recently been reported in the literature.

The first case is one of the most extreme examples of two very similar forms which have developed complete reproductive isolation. The case was recently reported by Dobzhansky (1946), and concerns two members of the willistoni group, *D. willistoni* Sturtevant, which has a wide distribution extending from southern United States to Brazil, and *D. equinoxialis* Dobzhansky from Teffe in the state Amazonas, Brazil. These two species are extremely similar morphologically; the only established difference between the two is that, on the average, *equinoxialis* is smaller than *willistoni*. A strong sexual isolation exists between the two species. In one series of tests, in which the flies were exposed from 6 to 47 days without choice of mate, *willistoni* males inseminated only 8 out of 545 *equinoxialis* females, and in the reciprocal cross *equinoxialis* males inseminated 28 out of 729 *willistoni* females. No viable offspring resulted from these interspecific inseminations. Reproductive isolation between the two forms is, therefore, complete, and Dobzhansky correctly ranks them as full species.

The second case, which is represented by a pair of similar subspecies, was recently reported by Patterson and Dobzhansky (1945). The two forms are *D. pallidipennis pallidipennis* Dobzhansky and Pavan from the state of São Paulo, Brazil, and *D. pallidipennis centralis* Patterson and Mainland from the state of Vera Cruz, Mexico. The two forms are very similar morphologically, but careful measurements show that *pallidipennis* has a larger body size and a more rounded wing shape than *centralis*, and they are thus distinguishable in living material. The metaphase chromosome configurations are practically identical in the two subspecies; each has one pair of very large V-shaped elements (the sex chromosomes), four pairs of rods, and a pair of very fine dots. The salivary gland chromosomes of the hybrid larvae show very complete synapsis, with a single inverted section in one of the autosomes. The subspecies cross and produce viable hybrids, with little evidence of sexual isolation. However,

the F_1 males are completely sterile, but the females are fertile in backcrosses. A few of the males obtained in the progeny of the first backcross are fertile, and the proportion of fertile males increases in the offspring of the succeeding backcrosses. If the distribution ranges of these subspecies were to overlap, hybrids between them could be produced, and there would be an opportunity for gene exchange through the female hybrids.

The third case was studied by Wharton (1944) and includes the subspecies *D. mercatorum mercatorum* Patterson and Wheeler from North America, and *D. mercatorum pararepleta* Dobzhansky and Pavan from Brazil. These two forms are much alike morphologically, but they can be separated in living material. Moreover, the metaphase chromosome configurations are different in the two forms. The females of *mercatorum* have a pair of large V's, two pairs of short rods, a pair of long rods (X chromosomes), and a pair of small V's. The males have the same pattern, except that there are but nine chromosome elements, the X having no synaptic mate. In contrast to this condition, females of *pararepleta* have a pair of large V's, two pairs of short rods, a pair of large rods (X chromosomes), and a pair of large dots. In the males the Y is much shorter than the X. Wharton demonstrated that while both series of the *mercatorum-pararepleta* crosses were fertile and gave F_1 flies which showed hybrid vigor, yet there was great reduction in fertility, fecundity, and viability among the F_2 flies. She further demonstrated that the apparently XO condition in the males of *mercatorum* had been brought about by a fusion or translocation of the Y chromosome to a dot-like element, forming a small, V-shaped chromosome. Hence, the formulae for the sexes of this subspecies are, females 2A:XXYY and males 2A:XYY. Through hybridization crosses, Wharton was able to obtain true, although sterile, XO males, which lacked the small, Y-bearing V of *mercatorum* and the unattached Y of *pararepleta*. Finally, she synthesized a pure-breeding strain, differing from either parent and having females with the formula 2A:XXYY and males with the formula 2A:XYYY.

THE TWO STRAINS OF DROSOPHILA PENINSULARIS

In 1942 we described a new species from Florida under the name *Drosophila peninsularis* (Patterson and Wheeler 1942). Last May, 1946, Dr. Harrison D. Stalker of Washington University, St. Louis, sent us a culture of a new geographic strain of this species from Tarpon Springs, Florida, where he had collected twenty-nine specimens. We are grateful to Dr. Stalker for his generosity in sending this stock with his permission to use it in any way we might desire.

A preliminary examination of these flies confirmed Stalker's identification that they belonged to the species *D. peninsularis*. A more detailed study of this stock revealed that the strain from Tarpon Springs showed certain differences when compared with the type strain from Lake McKethan, a stock of which was still available in the laboratory. Specimens

of the new strain have a lighter body and eye color than those of the Lake McKethan strain. Another difference observed concerns the nature of the spermathecae stalks. In the strain from Tarpon Springs these stalks are separated, as in most species of Drosophila, with their points of origin from the vagina usually some little distance apart. In contrast to this condition, these stalks of the Lake McKethan strain are *fused,* and arise from a common point on the wall of the vagina. The phenotypic expression of this character is variable and shows different degrees of fusion of the two stalks. On the basis of these observations, it was decided to carry out a series of cross tests with the two strains to see if there was any genetic difference between them.

Five different types of crosses were carried out with the two strains, and the results obtained are recorded in Table 1. The five types of crosses were, controls, P_1's, F_1 inbred, first backcrosses, and the inbred progeny of these backcrosses. The letters M and T are used to designate the Lake McKethan and Tarpon Springs strains, respectively. In the table the pedigree of each type of hybrid is indicated as follows: The original symbols show the female first. Thus, MT is the hybrid from the cross Lake McKethan ♀ × Tarpon Springs ♂, and TM indicates the reciprocal cross. For the hybrid progeny of the backcrosses, the symbol (MT)M is the offspring of an F_1 hybrid female crossed to a Lake McKethan male, while the symbol M(MT) represents the offspring of a Lake McKethan female crossed to an F_1 hybrid male.

TABLE 1

Crosses between Lake McKethan (M) and Tarpon Springs (T) geographic strains of *Drosophila peninsularis*

Crosses	Matings ♀ ♂	Percentage fertile	Sex ratios ♀ ♂	Number per culture
Controls	M × M	89	47.8%:52.2%	26.1
	T × T	35	48.7%:51.3%	25.6
P_1 crosses	M × T	76	47.8%:52.2%	24.2
	T × M	52	47.1%:52.9%	21.4
F_1 inbred	MT × MT	70	44.5%:55.5%	69.8
	TM × TM	73	53.9%:46.1%	53.8
First backcrosses	MT × M	51	49.7%:50.3%	30.6
	MT × T	68	44.6%:55.4%	33.6
	TM × T	76	44.2%:55.8%	21.2
	TM × M	56	49.2%:50.8%	29.5
	M × MT	67	46.7%:53.3%	37.2
	M × TM	45	50.4%:49.6%	35.2
	T × MT	16	50.2%:49.8%	26.3
	T × TM	32	50.7%:49.3%	29.2
Inbred progeny of first backcrosses	(MT)M × (MT)M	47	50.0%:50.0%	43.3
	(MT)T × (MT)T	35	50.7%:49.3%	34.5
	(TM)M × (TM)M	60	51.5%:48.5%	61.0
	(TM)T × (TM)T	40	49.7%:50.3%	33.1
	M(MT) × M(MT)	44	50.3%:49.7%	38.0
	M(TM) × M(TM)	65	52.4%:47.6%	47.6
	T(MT) × T(MT)	40	50.9%:49.1%	32.8
	T(TM) × T(TM)	50	51.0%:49.0%	39.0

In addition to the types of crosses and matings, the table also shows the fertility percentages, the sex-ratios, and the number of offspring per culture for each type of mating. The fertility percentage is based on a check of one-hundred tested pairs, and the number per culture is based on the average number of offspring from a sample of twenty-five fertile pairs.

The control crosses gave fertility percentages of 89 and 35 for the M × M and T × T matings, respectively. The fertility percentage of 35 for Tarpon Springs seems low for homogamic matings, but this result is not unique. In the *pallidipennis-centralis* tests the homogamic matings of *centralis* also gave a fertility percentage of only 35. It is a rather common experience to find strains of Drosophila which breed well in mass cultures, but fail to do so in pair mating tests. This was found to be true for several strains of members of the virilis group (Patterson and Griffen 1944). The sex-ratios for the two control crosses are similar, each with slightly more males than females, and the average number of offspring is also similar for the two crosses. The P_1 crosses gave percentages of fertility of 76 for the M × T mating, and 52 for the T × M mating. The sex-ratios and the number of offspring per culture are similar to those for the control crosses.

The two F_1 inbred crosses gave very similar fertility percentages, and both showed a much higher average per culture than either the control or P_1 matings, indicating hybrid vigor. The fertility percentages of the eight backcrosses varied from 16 to 76, with a general average of 51.3. The general average per culture was 30.4. Both of these averages are higher than the corresponding ones for the control or P_1 matings. The fertility percentages of the eight inbred tests varied from 35 to 65, with an average of 47.6. The general average of the number per culture was 41.1. The difference in the fertility percentages of these two sets of matings is not significant (51.3 vs. 47.6), but the difference in the average per culture is (30.4 vs. 41.1). While the sex-ratios for the different matings vary, yet none of them deviate significantly from a 1:1 ratio.

TABLE 2

Dissection records of males and females from sterile matings

Crosses ♀ ♂	Males		Females	
	motile sperm	non-motile or immature sperm	inseminated	offspring
MT × MT	9	1	5	none
TM × TM	2	8	0	none
TM × T	2	8	4	none
TM × M	7	3	7	none
M × TM	10	0	0	none
T × TM	5	5	0	none
(MT)M × (MT)M	0	10	0	none
(TM)M × (TM)M	10	0	4	none
(TM)T × (TM)T	10	0	5	none
M(TM) × M(TM)	9	1	4	none
T(TM) × T(TM)	10	0	2	none

In order to determine why so many of the hybrid crosses resulted in sterile matings, samples of ten pairs of such matings from each of eleven different crosses were dissected. The results obtained from these dissections are recorded in Table 2. Of the two F_1 inbred crosses, the MT$\times$ MT matings had nine males with motile and one with immature sperm. Five of the females had been inseminated, but, like all of the inseminated females found in this series of dissections, none produced offspring. The TM $\times$ TM matings had two males with motile and eight with immature sperm. None of the ten females had been inseminated.

Four samples of the eight different backcrosses were dissected. Two of these represent backcrosses of TM females to the parent types of males, and two represent TM males backcrossed to the parent types of females. In the TM $\times$ T matings two of the males had motile and eight had non-motile sperm. Since the ventral receptacles of four of the females contained non-motile sperm, and only two of the males had motile sperm at the time the dissections were made, two of these females must have mated before the sperm had become inactive in the testes. In the TM $\times$ M matings seven of the males had motile and three had non-motile sperm. Seven of the females had been inseminated, the receptacles of four containing motile sperm. In the M $\times$ TM matings all of the males had motile sperm, but none of the females had been inseminated. In the T $\times$ TM matings five of the males had motile and five had non-motile sperm. None of the females had been inseminated. Five different types of matings from the inbred tests of the progeny of the backcrosses were examined. In the first of these, the (MT)M $\times$ (MT)M matings, all ten males had immature sperm, and naturally the females contained no sperm in their ventral receptacles. In the four remaining types of matings, all of the males had motile sperm except one. In the M(TM) $\times$ M(TM) matings a single male had immature sperm in its testes.

The results obtained in the dissections of the flies from fertile matings show that the sterility was due to two or more causes. In some of the crosses it was the result of sexual isolation. This is evidently true for matings in which none of the females had been inseminated, although most of the males contained many motile sperm in their testes. The matings M $\times$ TM and T $\times$ TM belong in this category. A second cause of sterility is seen in crosses in which the process of sperm formation had not been completed. In such males spermiogenesis proceeds to the point at which the strands of immature spermatozoa are formed, but stops just short of motile-sperm formation. Good examples of this type of sterility are found in the TM $\times$ TM matings, in which eight of the ten males had immature sperm, and the (MT)M $\times$ (MT)M matings in which all ten males had immature sperm. In two matings only one male out of ten had such sperm. These were MT $\times$ MT and M(TM) $\times$ M(TM). Finally, a few cases were observed in which a single cyst had succeeded in producing motile sperm, while the rest of the testis contained only immature stages. A third possible cause of sterility is the inactivation

of the sperm in the ventral receptacle of the female. Several cases of this type were found, two of which were seen in the TM × T and TM × M matings.

The two geographic strains were tested for sexual isolation by the multiple choice method. Five females of each strain were mated to a single male of one of the strains. The results from these tests are presented in Table 3. The Lake McKethan males showed a distinct preference for their own kind of female. The χ^2 value and the isolation index both indicate a significant difference between the homogamic and heterogamic matings. In the reciprocal cross, Tarpon Springs males showed a slight preference for Lake McKethan females, giving a negative isolation index, but the χ^2 value of 4.0 and the isolation index of — 0.19 do not indicate a significant difference. We have found two crosses in which males of one species preferred the alien females to their own. Thus, *texana* males prefer *americana* females, and *buzzatii* males prefer *arizonensis* females. The same was found to be true for several different strains among members of the virilis group (see Article I). Negative isolation indices have been reported for geographic strains of *D. willistoni* (Dobzhansky and Mayr 1944), and for those of *D. prosaltans* (Dobzhansky and Streisinger 1944).

TABLE 3

Number of female dissected (*n*) and per cent inseminated (%) in crosses between the two strains of *D. peninsularis*

Females	Males	Homogamic		Heterogamic		χ^2	Isolation Index
		n	%	*n*	%		
McKethan, Tarpon	McKethan	100	50.0	102	26.4	11.6	0.31
Tarpon, McKethan	Tarpon	101	28.7	102	42.1	4.0	—0.19

THE FUSED MUTATION

As stated above, the strain from Lake McKethan contains a mutant factor which causes a fusion of the spermathecae stalks. This character is variable in expression. In some specimens the proximal ends of the stalks are united, forming a V-shaped figure; in others the union involves varying lengths of the proximal ends, giving Y-shaped figures; in still others the two stalks are fused throughout their entire lengths, with the spermathecae occupying a terminal position. In contrast to this condition, the stalks of the Tarpon Springs strain arise at separate points in the wall of the vagina, as is the usual condition in most species of the genus Drosophila.

In Table 4 are listed the conditions of fused in seven different female genotypes. An analysis of these data shows that non-fused is dominant, or nearly so, over fused. The lack of complete dominance may be due to the presence of heterozygotes in the Tarpon Springs strain. This is indicated by the fact that three of the one hundred dissected females of this

strain showed the character fused. Since the original flies of the two strains were collected at points only about thirty-six miles apart, crossing between the two strains might well have taken place. The data also reveal that fused is not sex-linked, and that only one or a few factors are involved.

TABLE 4

Determination of the character *fused* in different female genotypes

Type of female	non-fused	fused		
		V-shaped	Y-shaped	Terminal
M	0	6	90	4
T	97	2	1	0
MT	68	21	11	0
TM	83	13	4	0
(MT) M	50	22	26	2
(MT) (MT)	67	12	19	2
(TM) (TM)	48	34	17	1

DISCUSSION AND CONCLUSION

The two geographic strains of *D. peninsularis* add another member to the growing list of pairs of similar Drosophila forms which have attained some degree of reproductive isolation without at the same time undergoing marked morphological differences. This pair of strains may be compared with the three other cases cited in the introductory section of this article. Of the four cases, the *willistoni-equinoxialis* pair has reached the highest degree of divergence, and, having developed complete reproductive isolation, the two forms represent full species. Next in line would be the *pallidipennis-centralis* pair, in which the F_1 males are completely sterile, and most of the males of the succeeding backcross generations are also sterile. The members of this pair have been ranked as subspecies. Close to this case would be the *mercatorum-pararepleta* pair. The reciprocal crosses of the two forms produce viable hybrids which show hybrid vigor, but the F_2 crosses show a great reduction in fertility, and the F_2 flies are less fecund and viable than the F_1 individuals. These forms have also been ranked as subspecies. The two geographic strains of *peninsularis* show at least three different types of isolating mechanisms operating in the direction of divergence, but they have not reached the taxonomic level of subspecies.

The two members of each of the four pairs may be compared with reference to their metaphase and salivary gland chromosomes. According to Dobzhansky (1946), the metaphase chromosome patterns of *willistoni* and *equinoxialis* are alike, but the latter species was not favorable for the study of salivary gland chromosomes. Patterson and Dobzhansky (1945) found that the metaphase patterns of *pallidipennis* and *centralis* were similar. A study of the salivary gland preparations of the F_1 larvae showed that synapsis was complete. The only difference in gene arrangement of the two forms is a long inversion in one of the

autosomes. Wharton (1944) has shown that the metaphase patterns of *mercatorum* and *pararepleta* differ. The females of both forms have in common two large V's, a pair of long rods, and two pairs of short rods, but *pararepleta* has a pair of dots, whereas *mercatorum* has a pair of small V's instead. The male patterns are similar, except that the short Y chromosome is absent in *mercatorum*. She suggests that the condition in *mercatorum* has been brought about by a fusion or translocation of the short Y to a dot-like chromosome of a pararepleta-like form, thus forming a small V-shaped element. Both forms show five long euchromatic arms and the dot-like element in salivary gland preparations. Dr. W. S. Stone kindly made preparations of brain cells of the Tarpon Springs strain, and of the salivary glands of the F_1 hybrid larvae of the two strains of *peninsularis*. He reports that the metaphase patterns of the two forms are alike, and that the pairing of the salivary chromosomes is complete, with no apparent differences in gene arrangement.

The degree of divergence exhibited between the members of each of these four pairs of forms must have been brought about by genetic factors, rather than by chromosomal differentiations. This seems evident from the fact that very little difference in metaphase patterns and gene arrangement could be detected. Even in the *mercatorum-pararepleta* forms, in which the short Y and dot-like chromosomes had fused to form a small, V-shaped element, the change does not represent a gene rearrangement of any great magnitude.

Some of the more important components of speciation in Drosophila are found among the four cases under consideration. We have shown, however, that two of these components, morphological differentiation and divergence of gene arrangement, are not involved in these forms, at least to any great extent. Sexual isolation, which is one of the most common isolating mechanisms in Drosophila, is almost complete between *willistoni* and *equinoxialis*, and although interspecific inseminations sometimes occur, hybrids are never produced. Sexual isolation between *pallidipennis* and *centralis* is very weak and is restricted to only one of the two possible reciprocal crosses. It has developed between the two strains of *peninsularis*, as is shown by the results from the dissections of the flies from sterile matings, and from the sexual preference tests. Thus far, no critical experiment has been carried out to determine whether sexual isolation has developed between the members of the *mercatorum-pararepleta* pair of subspecies.

The development of hybrid sterility is also of common occurrence in Drosophila. It is not involved in the *willistoni-equinoxialis* crosses, for the reason that hybrids are never produced. The F_1 hybrid males from the reciprocal crosses of *pallidipennis* and *centralis* are completely sterile, and a large majority of those from succeeding backcrosses are also sterile. There is no conclusive evidence that the F_1 flies from the reciprocal crosses between *mercatorum* and *pararepleta* are sterile, but Wharton observed a striking reduction of fertility in nearly all F_2 crosses. There is evidence

that some of the hybrids from crosses of the two strains of *peninsularis* are sterile. In the case of male hybrids the sterility is due to a failure of gamete formation.

The failure of spermatogenesis to produce functional gametes has been observed in the hybrids of several different forms. It occurs in the F_1 sterile hybrids of *pallidipennis* and *centralis*. In these males the first and second meiotic divisions are abortive, and this results in the formation of groups of abnormal spermatids which finally degenerate to form granular masses (Patterson and Dobzhansky 1945). This case was worked out by Dobzhansky, who previously had reported (1934) a similar degenerative process in the hybrids between weak strains of *D. pseudoobscura* and *D. persimilis*. A somewhat similar degenerative process has been observed in the sterile male hybrids of the two strains of *peninsularis*. In this case the degeneration does not begin until near the end of spermiogenesis, and results in the formation of bundles (strands) of nonmotile sperm. A very similar phenomenon has been found by Stone in sterile F_2 males between members of the virilis group (see article IX in this publication).

Another mechanism which adversely affects reproduction in Drosophila is the inactivation of the spermatozoa after they have been introduced into the receptacles of alien females. This was first established in crosses between members of the virilis group (Patterson 1942; Patterson, Stone, and Griffen 1942). It has frequently been observed in crosses between the two strains of *peninsularis*. A similar phenomenon has been reported by Oliver and his students in *D. melanogaster*. In this case, however, the loss of sperm motility in the female is associated with one of the multiple effects of the mutant lozenge gene (Oliver and Green 1943; Oliver and Anderson 1945; Anderson 1945).

The geographic relationships of the two members of each of the four pairs of similar forms are not altogether clear. Dobzhansky is uncertain as to whether *willistoni* and *equinoxialis* are wholly allopatric or sympatric in parts of their distribution ranges. The question is not too important, since they represent full-fledged species. In the case of *pallidipennis* and *centralis*, not too much is known about their distribution ranges. The former was taken at two places in southern Brazil, and the latter at one place in the state of Vera Cruz, Mexico. From the results of cross tests it seemed best to regard them as subspecies. Of the third pair, *mercatorum* is widely distributed over southwestern United States, and its range extends down through Mexico and Central America to as far south as Lima, Peru, while *pararepleta* has been reported from southern Brazil. These two forms are also regarded as subspecies. The pair of geographic strains of *peninsularis* came from Florida, where the species has been collected at eight different places. The experimental tests show that these two forms have not diverged to the subspecific level.

Various degrees of evolutionary divergence are found among pairs of similar, closely related forms in the genus Drosophila. At the upper level

are forms like the *melanogaster-simulans* and *willistoni-equinoxialis* pairs which represent full-fledged species, no longer capable of gene exchange. At the middle, or subspecific, level are several pairs in which gene exchange between the two members would be possible wherever their distribution ranges might overlap. Among these are the *pallidipennis-centralis* and *mercatorum-pararepleta* pairs. Finally, at the lower level are geographic strains in which gene exchanges can take place rather readily, but which show the very beginning of evolutionary divergence. Such a case is represented by the two strains of *peninsularis* from Florida.

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VI. A STUDY OF THE ISOLATING MECHANISMS FOUND IN DROSOPHILA ARIZONENSIS AND DROSOPHILA MOJAVENSIS

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INTRODUCTION

From a complete study of only one stage of any dynamic process, for example, evolution, it is impossible to determine the past interactions of events leading to this particular stage; neither is it possible to predict accurately the characteristics of a future stage in the process, nor the future interactions of mechanisms observed to be operative during a particular stage. Obviously, a thorough knowledge of a single process can only be obtained by a study of all stages in the course of events. Because of the time limitations placed upon experimental investigations of evolution, it is impossible to study more than "one" stage of this process in a particular form. In order to partially circumvent this difficulty, studies are made of different stages in the evolutionary process in forms where the pattern of evolution is thought to be comparable, i.e., closely related species. The present investigation is an attempt to determine some of the inherent factors existing at the present time in different populations of *Drosophila arizonensis* which might serve to isolate these strains, as well as an attempt to discover the nature of isolating mechanisms which are effective between this species and its very close relative, *Drosophila mojavensis.*

MATERIALS AND METHODS

The collection records of this laboratory (Patterson and Wagner 1943) indicate that the bulk of the *arizonensis* population is concentrated in the region of the Sonoran Desert which covers southern Arizona and most of the state of Sonora, Mexico. Two other apparently isolated populations of this species exist in the state of Tamaulipas near the east coast of Mexico and in the state of Guerrero on the southern coast. Further collections along the Pacific Coast of Mexico will be necessary in order to determine whether there is a connecting link between the latter population and the large population located in the Sonoran Desert. The distribution of *mojavensis* is not as well known. Spencer (1941) indicates that it is common in the Mojave Desert and Death Valley regions of southern California, and Sturtevant (see Patterson and Wagner 1943) reports that this species has been found in the part of the Sonoran Desert comprising Baja California. Collections in southwestern Arizona have failed to reveal a zone of overlap in this region between these two species.

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In the experiments to be described, the stocks listed below were utilized. Along with the geographical origin, the symbol for each stock, as used in the tables, is given.

D. arizonensis Patterson and Wheeler, 1942.

A—Tucson, Arizona

As—San Bernardino, Arizona

Am—Magdalena, Sonora, Mexico

Ag—Guaymas, Sonora, Mexico

At—Rio Purificacion, Tamaulipas, Mexico

D. mojavensis Patterson and Crow, 1940 (redescribed by Patterson and Wheeler, 1942).

Md—Mesquite Springs, Death Valley, California

Mc—Chocolate Mountains, just east of Salton Sea, California.

In order to determine qualitatively the type of isolating mechanisms, if such should exist, which would be effective in curtailing gene exchange between the different strains of *arizonensis*, the following procedure was used in the experiments to be discussed. Pair matings of virgin flies from three to six days old were made in shell vials containing a banana-Karo-yeast-agar medium which was inoculated with a heavy suspension of yeast. Fourteen days after the matings were made any vials in which both parents were not living or vials which were contaminated with mold were discarded. At this time the vials were examined under the stereoscopic microscope, if necessary, to determine the presence or absence of larvae. Within forty-eight hours from the time of this examination, the females of the pairs which did not produce any progeny were dissected and their reproductive tracts examined under the compound microscope for the presence of spermatozoa. If less than thirty pairs of a particular cross did not produce any progeny, all of the females were dissected; however, if more than thirty pairs produced no offspring, dissections were made on a sample of at least twenty-five females. Twenty days after matings were made, ten vials were selected at random from the pairs which had produced offspring and the number of imagines per vial was recorded.

The same general procedure was used in obtaining the data on the hybrid *arizonensis-mojavensis* crosses, as presented in Table 4, with these exceptions: virgins from six to eight days old were used and dissections were made of all females which did not produce progeny; these dissections were made on the fourteenth day after making the matings. In these crosses the reproductive tracts of the females were examined to determine the presence of the "insemination reaction" (Patterson 1946) as well as the presence of motile or immotile sperm.

From the preceding paragraphs it is evident that these experiments were designed to measure the following three quantities: (1) the number of females fertilized—a measure of sexual isolation, (2) the rela-

tion between the number of females fertilized and the number producing progeny—a measure of the effect of isolating mechanisms acting between the time of insemination and the hatching of larvae from the eggs, (3) the number of imagines produced in twenty days—a partial measure of the fecundity of the cross.

MATING RESULTS

In Table 1 are recorded the results of intraspecific matings of the five strains of *D. arizonensis*. The first column of this table indicates the cross being tested. In the second column is recorded the number of vials in which both parents were living fourteen days after the particular mating was made. The percentage of these pairs which produced offspring in the fourteen-day period is given in the third column. The fourth column is a tabulation of the percentage of the females tested which were inseminated. This includes the females producing offspring as well as the number of females, as estimated by the sample of non-fertile females dissected, which were inseminated but did not produce progeny. Recorded in the last column is the average number of adult progeny produced within the twenty-day period after the matings were made. In none of the crosses being reported were any significant deviations in the sex ratio of the offspring apparent; therefore, in the fifth column the number of males and females produced are lumped together. Tables 2 and 3 are arranged in the same manner as Table 1.

TABLE 1

Fertility Relationships in P_1 Crosses of *D. arizonensis*

Nature of Cross	No. Pairs Tested	% ♀♀ Producing Progeny	Estimated % ♀♀ Inseminated	No. Progeny per Vial
A ♀ × A ♂	152	78	84	42
As ♀ × As ♂	87	26	37	39
Am ♀ × Am ♂	175	75	88	23
Ag ♀ × Ag ♂	141	55	62	38
As ♀ × A ♂	152	68	74	39
A ♀ × As ♂	128	54	70	36
Am ♀ × A ♂	165	60	69	66
A ♀ × Am ♂	134	63	71	62
Ag ♀ × A ♂	72	32	43	----
A ♀ × Ag ♂	112	70	75	60
At ♀ × A ♂	56	70	82	30
A ♀ × At ♂	59	86	90	22
As ♀ × Am ♂	148	34	48	27
Am ♀ × As ♂	164	66	77	13
As ♀ × Ag ♂	124	33	35	31
Ag ♀ × As ♂	95	32	47	29
As ♀ × At ♂	50	56	74	12
At ♀ × As ♂	54	72	88	16
Am ♀ × Ag ♂	123	71	76	39
Ag ♀ × Am ♂	117	37	50	6
Am ♀ × At ♂	69	72	77	26
At ♀ × Am ♂ *	----	----	----	15
Ag ♀ × At ♂	59	86	90	32
At ♀ × Ag ♂	41	59	88	24

*This cross was partially contaminated with mold. Progeny data were taken from mold-free vials.

An examination of Table 1 discloses the fact that in many of these intraspecific crosses, namely, As × At, As × A, A × At, Ag × At, Ag × A, Ag × Am, and As × Am, the percentage of females producing progeny differs in the reciprocal crosses. The differences in the first three of the crosses mentioned may not be real; however, in the last four cases the differences are certainly significant. The percentage differences in the reciprocal crosses of Ag × A, Ag × Am, and As × Am are evidently caused by sexual isolating mechanisms since the variations in the percentage of females fertilized are in the same direction and of the same general magnitude as the differences in the percentage of females producing offspring. However, in the crosses As × A, A × At, and Ag × At, if the differences are real, they must be attributed mainly to isolating mechanisms which are effective after insemination has taken place. This is also the case in the crosses between the As and Ag strains since, in the reciprocal crosses, practically the same percentage of females produce progeny although more of the females are inseminated if the As strain is used as the male parent. The cross As × At produces a condition intermediate between the two mentioned cases; i.e., both types of isolating mechanisms are effective to about an equal degree. It is worthy of note that there is no apparent correlation between the strain used as the female parent and the fertility of the cross. For example, when the Ag strain is used as the female parent in crosses to A or Am, the number of progeny produced is reduced in comparison with crosses using this strain as the male parent; however, the cross Ag ♀ × At ♂ produces more offspring than the reciprocal cross. Similar situations exist with the As, Ag, and A stocks.

Sexual isolation, as denoted by a reduction in the number of heterogamic inseminations as compared with the number of homogamic inseminations, is especially evident in the Am and A stocks. The Am females are inseminated by males from the same strain more frequently than by males of the other four strains. Likewise, Am males show sexual isolation with all the other stocks. The A strain is sexually isolated to all strains with the exception of At. Males and females of the At strain mate extensively with each of the other four strains, in fact, more frequently than any other stock. This probably indicates that the Tamaulipas strain, which is geographically widely separated from the other strains, is the least sexually isolated; however, control values of this strain are not available.

The intraspecific hybrids produced in these crosses between the various *arizonensis* strains were quite vigorous. In order to determine whether a breakdown in the isolating mechanisms acting between strains accompanied this heterosis, pair matings were made between the F_1 sibs of the various crosses involving the Sonoran Desert strains. It is obvious from the results of these experiments, as presented in Table 2, that a radical reduction in the effectiveness of sexual isolating factors had taken place. The fact that practically all of the females which were inseminated

were fertile also indicates a breakdown of the isolating mechanisms acting after insemination. The differences in the percentage of fertile females in the reciprocal P_1 crosses have disappeared with the possible exception of crosses involving the As strain with the A and Am strains. In these cases the differences, which may not be significant, were accompanied by differences in the same direction of the percentage of females inseminated. In addition, the increase in the fecundity of the crosses is quite striking. For example, upon inbreeding the F_1 sibs of the cross A ♀ × Ag ♂, one female produced over 230 adult offspring in the twenty-day period.

TABLE 2

Fertility Relationships in F_1 Crosses of *D. arizonensis*

Nature of Cross	No. Pairs Tested	% ♀♀ Producing Progeny	Estimated % ♀♀ Inseminated	No. Progeny per Vial
AAs ♀ × AAs ♂	109	90	94	75
AsA ♀ × AsA ♂	89	78	83	94
AAm ♀ × AAm ♂	124	91	95	80
AmA ♀ × AmA ♂	120	97	99	81
AAg ♀ × AAg ♂	71	94	97	131
AgA ♀ × AgA ♂	122	87	93	61
AsAm ♀ × AsAm ♂	121	100	100	63
AmAs ♀ × AmAs ♂	171	89	94	64
AsAg ♀ × AsAg ♂	40	95	97.5	68
AgAs ♀ × AgAs ♂	70	100	100	63
AmAg ♀ × AmAg ♂	106	88	93	58
AgAm ♀ × AgAm ♂	123	93	93	93

Since *arizonensis* and *mojavensis* are apparently very closely related, crosses were made between each of the Sonoran Desert strains of *arizonensis* and the strain of *mojavensis* from Death Valley in order to determine if the intraspecific variation in the effectiveness of isolating factors in *arizonensis* would be more pronounced in crosses to *mojavensis*. The fertility relations in these crosses as well as the control values of Md strain of *mojavensis* are given in Table 3. Control values of the *arizonensis* stocks are given in Table 1. Although the Ag females when

TABLE 3

Fertility Relationships in *arizonensis-mojavensis* Crosses

Nature of Cross	No. Pairs Tested	% ♀♀ Producing Progeny	Estimated % ♀♀ Inseminated	No. Progeny per Vial
Md ♀ × Md ♂	123	72	98	11
A ♀ × Md ♂	139	20	44	----
As ♀ × Md ♂	139	20	36	12.5
Am ♀ × Md ♂	134	27	41	14
Ag ♀ × Md ♂	133	38	44	16
Md ♀ × A ♂	105	9	67	----
Md ♀ × As ♂	118	8.5	41	6
Md ♀ × Am ♂	90	10	52	5
Md ♀ × Ag ♂	83	12	53	4.5

inseminated by Md males are somewhat more fertile than the other *arizonensis* strains, the data indicate that each of the *arizonensis* strains behaves in the same general manner as was previously reported for the A strain by Crow (1942); i.e., in the cross *arizonensis* females with *mojavensis* males a majority of the inseminated females produced progeny, while in the reciprocal cross more females are inseminated but a much lower percentage of the females produced offspring.

With the discovery by Patterson (1946) of the insemination reaction and its significance as an isolating mechanism in the mulleri subgroup (Patterson 1947), it was deemed worth while to augment the data given by Crow (1942) on crosses involving the hybrids between *arizonensis* and *mojavensis*. The strain of *mojavensis* from Chocolate Mountains, California, was used in these tests. Table 4, which is a presentation of the data found in various crosses of these hybrids, is arranged in a manner similar to the previous tables with the exception of the fourth and fifth columns. In the fourth column is given the actual percentage of females inseminated. These data were made possible by dissection of all the females which failed to produce progeny. In this table the term "inseminated" is used to include those females whose genital tracts showed either presence of sperm or the insemination reaction. In the last column is tabulated the percentage of the females which were inseminated but produced no progeny in the fourteen-day period but which showed the reaction mass. The figures given in parentheses are the total number

TABLE 4

Fertility Relationships in Hybrid *arizonensis-mojavensis* Crosses

Nature of Cross	No. Pairs Tested	% ♀♀ Producing Progeny	% ♀♀ Inseminated	% ♀♀ Inseminated Showing Reaction Mass	
A ♀ × A ♂	118	76	79	67	(3)
Mc ♀ × Mc ♂	119	4	100	32	(112)
A ♀ × Mc ♀	115	49	66	25	(20)
Mc ♀ × A ♂	124	2	73	93	(74)
AMc ♀ × A ♂	113	33	86	29	(55)
A ♀ × AMc ♂	Males Sterile				
AMc ♀ × Mc ♂	153	75	97	28	(32)
Mc ♀ × AMc ♂	Males Sterile				
McA ♀ × A ♂	108	37	75	22.5	(40)
A ♀ × McA ♂	108	0	51	40	(45)
McA ♀ × Mc ♂	135	77	97	19	(26)
Mc ♀ × McA ♂	110	0	86	89	(84)
AMc ♀ × AMc ♂	Males Sterile				
McA ♀ × McA ♂	59	0	59	85	(33)
AMc ♀ × Mc ♂	89	0	79	62	(66)
McA ♀ × AMc ♂	Males Sterile				

of non-fertile, inseminated females on which the percentage figures are based.

The male hybrids produced from the cross *arizonensis* female to *mojavensis* male are sterile (Crow 1941). Smears of the testes of these males reveal that mature and motile spermatozoa are never formed. However, Patterson (1946) has demonstrated that these males copulate with females of either parent species and that the presence of the sperm-free semen in the vagina causes a typical reaction mass. Another graphic demonstration of this same behavior of sterile hybrid males was uncovered when males from the reciprocal of the above-mentioned cross were mated to *mojavensis* females, i.e., the cross Mc ♀ × McA ♂. Among the ninety-seven suitable dissections of the Mc females, it was found that forty-nine of them contained sperm somewhere in their reproductive tract, usually in the ventral receptacle. However, thirty-five cases were found in which the vagina of the female was swollen and granular (typical of the reaction mass of the insemination reaction), but a careful inspection of all parts of the reproductive tract of each female failed to reveal the presence of a single sperm. In order to determine if the McA males which were mated to these particular females were producing motile sperm, testes from fifteen of them were smeared and examined. It was found that thirteen of the males contained no motile sperm in their testes. Of the other two males, only a single small group of motile sperm was found in the one, while in the other there was an abundance of motile sperm. In this last case transfer of sperm during copulation had evidently not been successful. In this connection, it was noted, during dissection of the females of this particular cross, that in most of the cases where females contain sperm, only a relatively small number were present.

Therefore, in studies of sexual isolation where sterile males may be involved, the presence or absence of sperm in the female is not a reliable criterion of whether copulation has taken place. For example, in the cross being discussed, Crow (1942), prior to the discovery of the insemination reaction, reports that 56% of Md females were fertilized; while these tests, using the Mc strain, indicate that 86% were inseminated. If, in the present experiments, the insemination reaction is disregarded as evidence of previous copulation, a figure of 51% which is comparable with Crow's data would be obtained. Actually the sexual isolation is probably even less than reported because the reaction mass caused by these sterile males had probably cleared in some cases and these females were recorded as not inseminated. Although none of the pair matings involving McA males produced progeny in the fourteen-day period, all of these crosses will produce some offspring if mass matings are used. The fact that the majority of these hybrid males are fertile indicates the presence of still other mechanisms operating to reduce the production of progeny.

An examination of the results tabulated in Table 4 indicates that there is but a weak correlation between the variations in the percentage of

females inseminated and the variations in the percentage of females producing progeny. Superficially this would apparently provide evidence that sexual isolating mechanisms play a relatively minor role in limiting the fertility of these crosses. However, the possibility that sexual isolating factors may be involved is evident from the results of crosses in which a large percentage of the females which were inseminated contained a reaction mass (see the last column of Table 4). Patterson (unpublished) has found that in the cross Mc ♀ × A ♂ and in the reciprocal cross the insemination reaction lasts between forty-eight and seventy-two hours. In crosses of the McA males to either A, Mc, or McA females the reaction lasts less than twenty-four hours (Wheeler, unpublished). Thus in the crosses Mc ♀ × A ♂, Mc ♀ × McA ♂, McA ♀ × McA ♂, and AMc ♀ × McA ♂ this would indicate that either most of the pairs did not mate for the first twelve days they were together or that most of the pairs had remated shortly before the dissections were made. It is impossible to state which of these two events took place since copulations were not observed; but the possibility exists that sexual isolating mechanisms are effective for a long period of time. A few additional days must be added to this possible period of sterility since Patterson (1946) has shown that the presence of the reaction mass in the vagina of all female Drosophila where this phenomenon has been studied prevents the laying of normal viable eggs.

After a consideration of sexual isolating factors, male sterility and the insemination reaction, there are still crosses, such as McA ♀ × A ♂, AMc ♀ × A ♂, Mc ♀ × Mc ♂, and A ♀ × McA ♂ where the reaction mass in about two-thirds of the females inseminated has already cleared but still the production of progeny is curtailed. The action of this isolating factor, which is effective after insemination has taken place, is not known although it might be related to unknown after-effects of the insemination reaction. Associated with this sterility might be the fact that *mojavensis* females when mated in pairs are reluctant to lay eggs on the laboratory food. For example, experiments designed to determine if the presence of foreign sperm in *mojavensis* females caused a reduction in the number of eggs layed were discontinued after three attempts because adequate egg counts could not be obtained from the *mojavensis* controls. It is doubtful if immotile sperm caused by a reaction between the sperm and the vagina, as reported in crosses within the virilis group (Patterson, Stone, and Griffen 1942), are causing the isolation in these cases. Over 530 dissected females contained sperm but only in ten scattered cases were all the sperm found to be immotile.

DISCUSSION

The results reported above provide evidence that there exists in *D. arizonensis* factors which are potentially able to effect a certain degree of isolation between different populations of this species. The presence of intraspecific isolating factors has been widely demonstrated in the genus *Drosophila*. The existence of these mechanisms has been clearly shown

in *D. miranda* (Dobzhansky and Koller 1938) ; *D. americana* (Stalker 1942, and Patterson, Stone, and Griffen 1942) ; *D. macrospina* (Mainland 1942) ; *D. melanica, D. paramelanica, D. nigromelanica* (Griffen 1942) ; *D. repleta* (Wharton 1942) ; *D. sturtevanti* (Dobzhansky 1944) ; *D. pallidipennis* (Patterson and Dobzhansky 1945) ; and *D. peninsularis* (Patterson and Wheeler 1947). A more complex condition persists in *D. prosaltans* (Dobzhansky and Streisinger 1944), *D. willistoni* (Dobzhansky and Mayr 1944) and *D. nebulosa* (Dobzhansky 1944) since some strains show a preference for homogamic while other strains prefer heterogamic mating. It is evident from the data presented that the situation in *arizonensis* is similar to these latter cases. However, a strict comparison cannot be made since Dobzhansky *et al.* measured sexual isolation by allowing the males in a given culture a choice of conspecific or alien females with which to mate. This is undoubtedly a more sensitive method of discovering sexual isolation than the method employed in the present investigation. For example, Stalker (1942) was unable to demonstrate any preferential mating among various strains of *D. americana* unless a choice of females was offered the males. Thus it can be concluded that, although all cases of sexual isolation among the five strains of *arizonensis* may not have been disclosed, the ones demonstrated might be very effective in limiting gene exchange between populations which are not ecologically or geographically isolated.

Isolating mechanisms have been crudely divided, for the purpose of this paper, into those acting before insemination (sexual isolation—there is no evidence of mechanical isolation in any of these crosses) and those effective at a latter stage of the reproductive or ontogenetic process (reproductive isolation). The almost complete absence of any sexual isolation in the intraspecific F_1 sibling crosses of *arizonensis* indicates that the factors responsible for this behavior are for the most part autosomal and recessive although the differences in the percentage of females inseminated between certain reciprocal P_1 crosses signifies that some of these isolating factors are associated with sex. The parallel breakdown in the F_1 hybrids of the reproductive isolating mechanisms implies that these factors are similarly controlled. In view of the fact that these hybrids produce more progeny than either of their parents, it must be assumed that each strain also contains a distinct set of autosomal recessive factors which reduces the production of progeny within a strain. The question then arises as to what role such a system could play in intraspecific evolution. The net effect of this system would be to permit some independent divergence of the strains at the expense of maximum reproductive capacity and at the same time provide a means, if an intraspecific cross occurs, whereby the recombinations of factors between populations could be "tested" for adaptive value by building up a large number of F_2 hybrids. It is not apparent, unless the size of each population is rather restricted, how such a system is retained in a given population since those factors which reduce the reproductive capacity of

a strain are *per se* selected against. Wright (1932) has postulated that if a widely distributed population is composed of more or less isolated subgroups, the rate of evolution is relatively rapid. Such a population structure permits both adaptive and nonadaptive explorations among the subgroups making possible the attainment of particularly suited genotypes without the loss of plasticity within the species. The Sonoran Desert population of *arizonensis* appears to have such a structure. In this connection, it is interesting to note that the mulleri subgroup is composed of a collection of perhaps the most closely related species in the genus *Drosophila*.

In the interspecific crosses there is not only a difference in degree of effectiveness of the isolating mechanisms found to be operative within *arizonensis* but also a difference in quality. Within the limitations of the experimental technique employed, sexual isolating factors are relatively ineffective in curtailing gene exchange as compared to reproductive isolating mechanisms and hybrid sterility. In the cross *mojavensis* female to *arizonensis* male only three per cent of the inseminated females produce offspring although seventy-three per cent of the females are inseminated (see Table 4). In the reciprocal cross the reproductive isolating factors are relatively ineffective since seventy-four per cent of the inseminated females produce progeny; however, in this case the gene exchange is limited by the sterility of the hybrid male offspring. Therefore, it seems safe to assume that, even if the geographical distributions of *arizonensis* and *mojavensis* overlap, no pronounced gene exchange takes place.

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VII. RELATIONSHIPS WITHIN THE QUINARIA SPECIES GROUP OF DROSOPHILA

JACK W. SEARS

INTRODUCTION

The genus Drosophila presents to investigators in the field of speciation many advantages not found in any other genus which is known at the present time. These special advantages have been outlined by Patterson (1942a) and are as follows: first, in the genus there are many species living under a variety of environmental conditions in nature and capable of being cultured without great difficulty in the laboratory; second, the members of this genus have short life cycles and high fecundity and are adapted to exacting genetic analyses so that their genetic differences may be observed and quantitative data obtained; third, the chromosome number of all the species which have been studied cytologically is low and, in addition, the salivary gland chromosomes make more exact cytological analysis possible; fourth, the species of the genus have populations of different sizes and densities and occupy different distributional ranges; fifth, interspecific hybrids can be obtained, some of which are fertile or partially so, making it possible to study the genetic mechanisms by which species are isolated and differentiated.

Sturtevant (1942) has divided the genus Drosophila into six subgenera and several "species groups." Other workers in the field of speciation have emphasized the importance of these natural groups (Sturtevant and Dobzhansky 1936; Stalker and Spencer 1939; Sturtevant 1940; Patterson, Stone, and Griffen 1940, 1942; Patterson 1942a and b). As Patterson (1942a) has pointed out, it is within such species groups that interspecific hybrids can be obtained and the mechanism of species formation analyzed.

MATERIALS

As a part of the speciation studies being conducted at The University of Texas, the quinaria group was chosen for analysis, and the results of the investigation comprise the subject matter of this paper. This group includes *Drosophila quinaria* Loew, *D. subquinaria* Spencer, *D. occidentalis* Spencer, *D. suboccidentalis* Spencer, *D. palustris* Spencer, *D. subpalustris* Spencer, *D. innubila* Spencer, *D. munda* Spencer, *D. transversa* Fallén, *D. deflecta* Malloch, *D. suffusca* Spencer, and *D. tenebrosa* Spencer.

The first nine of these species are used in the present investigation. The remaining three species were not used since *deflecta* has never been cultured successfully in the laboratory and the other two were not available.

Sturtevant (1921) and Spencer (1942) classify this group ecologically as "fungus feeders." The species *transversa, suboccidentalis, subquinaria, munda, innubila, tenebrosa,* and *suffusca* have all been taken from fleshy

fungi (Spencer 1942). Sturtevant (1921) indicates that *quinaria* is a "fruit feeder," being found around tomato plants and in windfall apples. Spencer (1942) states that *palustris* and *subpalustris* larvae have been found in the decaying leaf stalks of the water plant *Sagittaria latifolia*. *Drosophila deflecta* also lives on decaying water plants.

The group has not been used extensively for genetic studies. Spencer (1942) reports that hybrids can be obtained between *occidentalis* and *suboccidentalis* and between *palustris* and *subpalustris*. In the latter case the hybrids were partially fertile (Spencer 1940). The ease with which a given combination will produce hybrids varies with the different strains originating from different pairs of wild flies.

Wharton (1943) worked out the metaphase chromosome configurations of the species used in this investigation. In the present study the cytological data were used in confirmative checks on all cases of hybridization. All of the species except four can be distinguished readily through an examination of the smears of larval brain tissue. In the cases of hybridization between any of the four species with similar metaphase plates the salivary gland chromosomes of the hybrid larvae were examined. Morphological studies of the hybrids were used in addition to the cytological and genetic tests in all portions of the work.

DISTRIBUTION OF STOCKS USED

The distribution of the species belonging to the quinaria species group was discussed by Spencer (1942) and more fully by Patterson and Wagner (1943). It should be noted that the group is divided at about the 98th meridian into an eastern and a western division.

The species found in the eastern portion of the United States include *quinaria, palustris, subpalustris, deflecta,* and *transversa*. *Drosophila quinaria* has been taken from Tennessee, Indiana, Ohio, Virginia, Pennsylvania, New Jersey, Connecticut, Massachusetts, Rhode Island, New Hampshire, New York (and Quebec). *Drosophila palustris* has been found in Ohio, New Jersey, and New York. *Drosophila subpalustris* has been taken only from the swamp land of Ohio, and *deflecta* has been recorded for the District of Columbia. The range occupied by *transversa* is more extensive than that of any other eastern species and includes all of the deciduous forest area of the eastern United States. The fact that it is found in Central Texas can be explained by the presence of the Edwards Plateau and its vegetation which forms a western extension of eastern plant associations. *Drosophila transversa* has been taken from the woodland of the Edwards Plateau but not west or south of it.

The species known for western United States and northern Mexico include *occidentalis, suboccidentalis, subquinaria, innubila, munda, suffusca,* and *tenebrosa*. *Drosophila occidentalis* has been taken from two localities in California and from one in Lower California. *Drosophila suboccidentalis* and *subquinaria* are Rocky Mountain species occupying the Transition and Canadian Zones of those mountains. *Drosophila subocci-*

dentalis has been collected from the Santa Clara Mountains of Chihuahua and the Federal District in Mexico, from the Rocky Mountain ranges of New Mexico, Colorado, Utah, Idaho, and Wyoming, and from the Black Hills of South Dakota. *Drosophila subquinaria* is somewhat less widely distributed, recorded only for the mountain ranges of Wyoming, Utah, Colorado, and New Mexico. *Drosophila munda, innubila, tenebrosa,* and *suffusca* occupy the highland of the Southwest. In Mexico *munda* was taken from fungus growing on willows along desert streams; it was found also in the highlands of New Mexico and Arizona. *Drosophila innubila* was collected from the mountains of Chihuahua and Michoacán, Mexico, and from the highlands of New Mexico and Arizona. *Drosophila suffusca* has been taken from Arizona and *tenebrosa* from the Sacramento Mountains in New Mexico and the Santa Clara Mountains of Chihuahua, Mexico.

An analysis of the data given by Patterson and Wagner (1943) dealing with the percentages of the various species in the Drosophila populations of different areas shows that none of the species of the quinaria group is very numerous in the population. Since their records usually include only one collection for each area, it is possible that a complete picture is not given. Patterson (1943) has shown that there are seasonal fluctuations in Drosophila population and that, unless numerous collections are made throughout the year, an accurate picture of the importance of any species in any given population may not be obtained. Further collection may increase the known ranges and the importance of the species of the quinaria group.

The writer is deeply grateful to Professor W. P. Spencer of the College of Wooster, Ohio, for the stocks of *palustris, quinaria,* and *subpalustris* used in this investigation, and to Professor Th. Dobzhansky for the stock of *occidentalis,* collected at Monterey, California. The others, collected by members of the Texas laboratory, are as follows: *subquinaria,* 1202.3 (Manitou Springs, Colorado); *subquinaria,* 1210.5 (Jackson's Hole, Wyoming); *suboccidentalis,* 1206.4 (Black Hills, South Dakota); *transversa,* 1062.6 (Great Smoky Mountains National Park, Tennessee); *innubila,* 1324.6 (Bonita Canyon, New Mexico); and *munda,* 929.8 (Cave Creek, Arizona).

METHODS

The methods used in this investigation were, with slight modifications, those outlined by Patterson (1942b). Adult flies that had been aged for ten or twelve days were mated in reciprocal crosses in small mass matings, using ten pairs per vial and ten vials per cross. It was necessary to age the flies since none of these species mate until the individuals are several days old. The flies were changed to new cultures at five-day intervals. At the end of thirty days, if no larvae appeared in the cultures, all flies from the ten vials were put together into a half-pint milk bottle, and then transferred to new food in a new bottle at five-day intervals. All

crosses were held for a minimum of thirty days or, in many cases, until the flies died. Some tests lasted as long as three months. At the end of the test period all crosses that did not produce larvae were recorded as sterile or incompatible.

In the event that offspring were produced in these primary crosses, pair matings were made for quantitative data. One hundred and fifty-two pairs were used for each of these crosses. These matings were allowed to stand for twelve days, then checked for living flies and for fertility. All vials in which one or both flies were dead were discarded. At the end of sixteen days the remaining vials were checked again; from the first one hundred vials which were examined, those showing larvae or pupae were counted and the percentage of fertility was recorded. In the cases in which the number of living pairs was less than one hundred at the end of the twelve-day period the per cent of fertility was calculated from the number remaining.

On the sixteenth day also ten of the fertile vials were taken at random and the pupae were counted to estimate the average number of progeny per vial (see Table 1). In some cases ten vials could not be obtained since the cross was less than ten per cent fertile, and in such cases the average number of pupae was obtained from the available fertile vials.

TABLE 1

P_1 ♀ \ P_1 ♂	q	sq1	sq2	p	sp	o	so	t	i	m
quinaria (q)	45% 56.1	<1%	st	st	st	st	st	st	st	st
subquinaria 1210.5 (sq1)	st	4%	2%	st	st	st	st	st	st	st
subquinaria 1202.3 (sq2)	st	3%	46% 55.6	st	st	st	st	st	st	6% 8.4
palustris (p)	st	st	st	27% 36.5	19% 35.1	st	st	st	<1%	st
subpalustris (sp)	st	st	st	16% 6.3	38% 43.7	st	st	st	st	<1%
occidentalis (o)	st	st	st	<1%	<1%	83% 66.6	35% 65.8	st	st	st
suboccidentalis (so)	st	st	st	<1%	st	79% 85.0	80% 53.2	st	st	st
transversa (t)	st	st	st	st	st	st	st	63% 55.9	<1%	st
innubila (i)	st	st	st	st	st	<1%	st	st	9% 59	st
munda (m)	st	st	10% 42.6	st	st	<1%	<1%	st	st	75% 56.3

After repeated experiments it was found that crosses which had produced only one fertile vial in mass matings, and which had produced very few hybrids from that single vial, would not show fertility in pair matings. All of these cases were then considered to be indicative of a fertility of less than one per cent.

All F_1 hybrids obtained were examined morphologically and then inbred in small mass matings in vials. Backcrosses were also made to both parent stocks. In the event that the original P_1 did not yield enough hybrids to make both tests, the $F_1 \times F_1$ cross was made first, and if after twenty days no larvae were produced, this cross was considered sterile. At the end of this time the male and female F_1 hybrids were separated, held for five days, and then backcrossed to the parent strains. These back crosses were held until the flies died. In the event that the $F_1 \times F_1$ cross was fertile in small mass matings, pair matings of this cross were made in order to determine the percentage of fertility. From the fertile $F_1 \times F_1$ crosses the F_2 hybrids were obtained and were inbred in small mass matings.

In order to guard against non-virginity, care was taken from the beginning to determine the period of time between the emergence of the adult from the pupae and the beginning of mating. It was found that if the flies were taken every twenty-four hours the females were virgins. In all cases of cross fertility, smears of the salivary gland and brains of the F_1 hybrid larvae were made and examined as final corroboration of hybridization.

An experiment was made to determine the amount of sexual isolation existing between species. All possible crosses were mated in vials, using one female and five males per vial, and made in duplicate. The cultures were inspected constantly in order to determine the extent of the attempts at copulation. Records were made showing frequency of these attempts and indicating, where possible, whether sexual isolation was unilateral or bilateral. After these matings had been allowed to remain undisturbed for three days all the females were dissected and examined for sperm. The results are shown in Table 4.

All experiments were carried out in a room kept at twenty-two degrees centigrade, using the standard agar-chondrus-karo-yeast-banana food employed in the Texas laboratory.

RESULTS OF FERTILITY TESTS

The results of the quantitative tests are given in Table 1. From this table it can be seen that all members of the group are linked by cross fertility to one or more of the others. *Drosophila occidentalis* shows the highest degree of interspecific fertility. With the exception of three cases, *subquinaria* × *munda*, *occidentalis* × *suboccidentalis*, and *palustris* × *subpalustris*, the interspecific crosses were fertile in one direction only.

TABLE 2

Cross ♀	♂	Number of Hybrids ♀	♂	Total	$F_1 \times F_1$
quinaria	× subquinaria 1210.5	25	25	50	Sterile
subquinaria 1210.5	× subquinaria 1202.3	13	12	25	Sterile
subquinaria 1202.3	× subquinaria 1210.5	27	32	59	Sterile
subquinaria 1202.3	× munda	29	13	42	Sterile
munda	× subquinaria 1202.3	172	169	341	Sterile
munda	× occidentalis	88	110	198	Fertile
munda	× suboccidentalis	8	6	14	Fertile
occidentalis	× palustris	24	23	47	Sterile
occidentalis	× subpalustris	45	39	84	Sterile
suboccidentalis	× palustris	18	20	38	Sterile
subpalustris	× munda	0	1	1	----------
suboccidentalis	× occidentalis	635	540	1175	Fertile
occidentalis	× suboccidentalis	256	178	434	Fertile
subpalustris	× palustris	55	40	95	Fertile
palustris	× subpalustris	325	342	667	Fertile
innubila	× occidentalis	1	2	3	Sterile
transversa	× innubila	272	256	528	Sterile
palustris	× innubila	24	25	49	Fertile

The determination of cross fertility percentages through the standard pair-mating procedure proved to be a very difficult task because of the reluctance of the flies to breed as isolated pairs. This reluctance is markedly shown in the controls and therefore is not confined to the attempts at hybridization. It is assumed that a laboratory food and other conditions more nearly approaching the natural environment of the species would obviate the difficulty. Mass cultures of the control stocks are highly fertile in both bottle and vial cultures. The following crosses showed some degree of fertility:

quinaria ♀ × *subquinaria* (1210.5) ♂ showed a small degree of fertility in mass matings. In pair matings the cross did not produce hybrids. All hybrids were sterile (see Tables 2 and 3).

subquinaria (1202.3) ♀ × *munda* ♂ was six per cent fertile in pair matings and gave an average of 8.4 flies per vial. Forty-two completely sterile hybrids were obtained.

munda ♀ × *subquinaria* ♂ was ten per cent fertile, yielding an average of 42.6 flies per vial. The hybrids were sterile.

munda ♀ × *occidentalis* ♂ in mass matings produced one hundred and ninety-eight hybrids which were fertile when inbred, but in backcrosses the males were incompatible to each parent type of female.

munda ♀ × *suboccidentalis* ♂ produced only fourteen hybrids, all from mass matings. They were fertile on inbreeding and on backcrossing, except when crossed to *suboccidentalis* females. The fertility was very low in each case.

subpalustris ♀ × *munda* ♂. From twenty mass matings of this cross only two pupae were obtained. From one of these a very weak male emerged; it showed some of the characters of both parents but died in two days, leaving no offspring. No other hybrids have been obtained from this cross and the question of hybrid fertility is not settled.

occidentalis ♀ × *suboccidentalis* ♂ in pair matings showed thirty-five per cent fertility and an average of 65.8 flies per vial. The hybrids were one hundred per cent fertile when inbred and crossed readily to either parent stock. The F_2 hybrids were quite fertile on inbreeding.

TABLE 3

Cross ♀	♂	Backcrosses: Hybrid Females		Backcrosses: Hybrid Males	
quinaria × sq 1210.5		F_1 ♀ × q	♂ = st	q ♀ × F_1	♂ = st
		F_1 ♀ × sq 1210.5	♂ = st	sq 1210.5 ♀ × F_1	♂ = st
sq 1210.5 × sq 1202.3		F_1 ♀ × sq 1210.5	♂ = st	sq 1210.5 ♀ × F_1	♂ = st
		F_1 ♀ × sq 1202.3	♂ = st	sq 1202.3 ♀ × F_1	♂ = st
sq 1202.3 × sq 1210.5		F_1 ♀ × sq 1210.5	♂ = f	sq 1210.5 ♀ × F_1	♂ = st
		F_1 ♀ × sq 1202.3	♂ = f	sq 1202.3 ♀ × F_1	♂ = st
sq 1202.3 × munda		F_1 ♀ × sq 1202.3	♂ = st	sq 1202.3 ♀ × F_1	♂ = st
		F_1 ♀ × munda	♂ = st	munda ♀ × F_1	♂ = st
munda × sq 1202.3		F_1 ♀ × munda	♂ = st	munda ♀ × F_1	♂ = st
		F_1 ♀ × sq 1202.3	♂ = st	sq 1202.3 ♀ × F_1	♂ = st
munda × occidentalis		F_1 ♀ × munda	♂ = f	munda ♀ × F_1	♂ = st
		F_1 ♀ × occidentalis	♂ = f	occidentalis ♀ × F_1	♂ = st
munda × suboccidentalis		F_1 ♀ × munda	♂ = f	munda ♀ × F_1	♂ = f
		F_1 ♀ × suboccidentalis	♂ = f	suboccidentalis ♀ × F_1	♂ = st
occidentalis × palustris		F_1 ♀ × occidentalis	♂ = f	occidentalis ♀ × F_1	♂ = st
		F_1 ♀ × palustris	♂ = f	palustris ♀ × F_1	♂ = st
occidentalis × subpalustris		F_1 ♀ × occidentalis	♂ = f	occidentalis ♀ × F_1	♂ = st
		F_1 ♀ × subpalustris	♂ = f	subpalustris ♀ × F_1	♂ = st
suboccidentalis × palustris		F_1 ♀ × suboccidentalis	♂ = st	suboccidentalis ♀ × F_1	♂ = st
		F_1 ♀ × palustris	♂ = f	palustris ♀ × F_1	♂ = st
subpalustris × munda		F_1 ♀ × subpalustris	♂ = *	subpalustris ♀ × F_1	♂ = †
		F_1 ♀ × munda	♂ = *	munda ♀ × F_1	♂ = †
suboccidentalis × occidentalis		F_1 ♀ × suboccidentalis	♂ = f	suboccidentalis ♀ × F_1	♂ = f
		F_1 ♀ × occidentalis	♂ = f	occidentalis ♀ × F_1	♂ = f
occidentalis × suboccidentalis		F_1 ♀ × occidentalis	♂ = f	occidentalis ♀ × F_1	♂ = f
		F_1 ♀ × suboccidentalis	♂ = f	suboccidentalis ♀ × F_1	♂ = f
subpalustris × palustris		F_1 ♀ × subpalustris	♂ = f	subpalustris ♀ × F_1	♂ = f
		F_1 ♀ × palustris	♂ = f	palustris ♀ × F_1	♂ = f
palustris × subpalustris		F_1 ♀ × palustris	♂ = f	palustris ♀ × F_1	♂ = f
		F_1 ♀ × subpalustris	♂ = f	subpalustris ♀ × F_1	♂ = f
innubila × occidentalis		F_1 ♀ × innubila	♂ = st	innubila ♀ × F_1	♂ = st
		F_1 ♀ × occidentalis	♂ = st	occidentalis ♀ × F_1	♂ = st
transvera × innubila		F_1 ♀ × transversa	♂ = st	transversa ♀ × F_1	♂ = st
		F_1 ♀ × innubila	♂ = st	innubila ♀ × F_1	♂ = st
palustris × innubila		F_1 ♀ × palustris	♂ = st	palustris ♀ × F_1	♂ = st
		F_1 ♀ × innubila	♂ = f	innubila ♀ × F_1	♂ = f

*No F_1 ♀ were obtained to make this cross.
†The F_1 ♂ died before this cross could be thoroughly tested.

TABLE 4

♀ ♀ \ ♂ ♂	q.	sq. 1210.5	sq. 1202.3	p.	sp.	o.	so.	i.	t.	m.
quinaria	Cop. sperm +	0 sperm —	♂ + + ; ♀ — sperm —	♂ + ; ♀ — sperm —	0 sperm —	0 sperm —	♂ + * ; ♀ —* sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + ; ♀ — sperm —
subquinaria 1210.5	♂ + ; ♀ — sperm —	Cop. sperm +	♂ + ; ♀ — sperm —	0 sperm —	♂ + * ; ♀ — sperm —	0 sperm —	♂ + ; ♀ — sperm —	0 sperm —	0 sperm —	0 sperm —
subquinaria 1202.3	0 sperm —	♂ + ; ♀ — sperm —	Cop. sperm +	0 sperm —	0 sperm —	0 sperm —	0 sperm —	0 sperm —	0 sperm —	♂ + ; ♀ — sperm —
palustris	♂ + ; ♀ — sperm —	♂ + ; ♀ — sperm —	♂ + ; ♀ — sperm —	Cop. sperm +	0 sperm —	Cop. sperm*	♂ + ; ♀ — sperm —	0 sperm —	0 sperm —	♂ + * ; ♀ —* sperm —
subpalustris	♂ + * ; ♀ —* sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + + ; ♀ — sperm —	Cop. sperm +	Cop. sperm*	♂ + ; ♀ — sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + — ; ♀ — sperm —
occidentalis	0 sperm —	♂ + ; ♀ — sperm —	0 sperm —	♂ + ; ♀ — sperm —	Cop. sperm +	Cop. sperm +	Cop. sperm*	0 sperm —	0 sperm —	♂ + ; ♀ — sperm —
suboccidentalis	0 sperm —	0 sperm —	0 sperm —	0 sperm —	0 sperm —	Cop. sperm +	Cop. sperm +	0 sperm —	♂ + * ; ♀ —* sperm —	0 sperm —
innubila	0 sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + ; ♀ — sperm —	0 sperm —	0 sperm —	♂ + ; ♀ — sperm —	Cop. sperm +	0 sperm —	♂ + ; ♀ — sperm —
transversa	♂ + ; ♀ — sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + — ; ♀ — sperm —	0 sperm —	0 sperm —	0 sperm —	0 sperm —	Cop. sperm +	♂ + ; ♀ — sperm —
munda	0 sperm —	0 sperm —	♂ + ; ♀ — sperm —	♂ + — ; ♀ — sperm —	♂ + — ; ♀ — sperm —	Cop. sperm +	♂ + ; ♀ —* sperm —	0 sperm —	♂ + + ; ♀ —* sperm —	Cop. sperm +

0 = No interest on the part of either sex.
♂ + = Males showed interest, began courting.
♂ + + = Males very interested, persistent courting.
♂ + * = Males attempted to copulate without success.
♂ + — = Males showed doubtful interest.
♀ — = Females indifferent to advance of males.
♀ — * = Females antagonistic to advance of males.
sperm — = No sperm in female reproductive tract.
sperm + — = Sperm present and active in female tract.
sperm * = Sperm present but inactive in female tract.

suboccidentalis ♀ × *occidentalis* ♂ gave the highest percentage of fertility encountered in any of the interspecific crosses which were made. Seventy-nine out of a possible one hundred vials were fertile and produced an average of 85 flies per vial. These hybrids also are one hundred per cent fertile, and the F_2 hybrids show fertility.

occidentalis ♀ × *palustris* ♂ yielded six female and four male hybrids from mass matings. The male hybrids were completely sterile but the female hybrids were fertile to both parental types of males.

occidentalis ♀ × *subpalustris* ♂ produced eighty-four hybrids from mass matings; only the female hybrids showed fertility.

innubila ♀ × *occidentalis* ♂ hybridized in pair matings only after many repetitions of the standard pair-mating test. One vial eventually yielded three offspring, one female and two males. These hybrids were tested by inbreeding and backcrossing and were completely sterile. However, this test is insufficient.

suboccidentalis ♀ × *palustris* ♂ produced fertile hybrid females and sterile hybrid males. The female hybrids showed fertility only when crossed to *palustris* males and were sterile when crossed to *suboccidentalis* males.

palustris ♀ × *subpalustris* ♂. Nineteen out of the first one hundred counted vials produced offspring for an average of 35.1 flies per fertile culture. These hybrids were fertile in all crosses made. The F_2 hybrids also showed some fertility.

subpalustris ♀ × *palustris* ♂. This cross was sixteen per cent fertile and yielded 6.3 flies per fertile vial. The hybrids were fertile on inbreeding and on backcrossing to all parent types. The F_2 hybrids also were fertile.

palustris ♀ × *innubila* ♂ yielded forty-nine hybrids from mass matings. This cross did not go in pair matings. The hybrids were fertile when inbred or when crossed to *innubila* males or females, but the fertility was very slight in each case. Within the limits of this experiment the hybrids were incompatible with *palustris*.

transversa ♀ × *innubila* ♂ produced hybrids which were completely sterile in all tests.

The intraspecific crosses *subquinaria* (1210.5) ♀ × *subquinaria* (1202.3) ♂ and *subquinaria* (1202.3) ♀ × *subquinaria* (1210.5) ♂ showed two and three per cent fertility respectively. Only female hybrids from the last cross showed fertility in backcrosses, and all hybrids were sterile when inbred.

Out of the ninety possible cross-matings eighteen showed fertility and seventy-two produced no hybrids.

CYTOLOGICAL RESULTS

Larval ganglionic tissue from both the parent stocks and the F_1 hybrids were examined with the use of the acetocarmine smear technique. The results of the study are in complete agreement with those of Wharton (1943). The descriptions given below summarize the metaphase configurations which were observed in the several stocks and hybrid classes.

Drosophila quinaria has two V-shaped autosomes, two J-autosomes, two dots, and long rod-shaped X-chromosomes with proximal constrictions. The Y-chromosome is a J-shaped element with a constriction approximately at the center of the long arm.

Drosophila subquinaria has eight short rods, long rod-shaped X-chromosomes, and two dots. The Y-chromosome is a small V.

Four species, *occidentalis, transversa, palustris,* and *subpalustris,* have identical metaphase configurations, showing eight medium-length rods, two long rod-shaped sex-chromosomes, and two dots. The male and female brain smears showed the same configuration.

Drosophila innubila males and females exhibit eight medium-length rods, long sex chromosomes with proximal constrictions, and two dots.

Drosophila suboccidentalis has eight medium-length rods, long rod-shaped X-chromosomes with proximal constrictions, and two dots. The Y-chromosome of the male is a long simple rod without the proximal constriction which is seen in the X-chromosomes.

Drosophila munda males and females have four large V-shaped autosomes, two rod-like sex chromosomes, and two dots.

Figure 1 shows the metaphase configurations of nine female hybrids and of the corresponding male and female parents; the hybrid chromosome configurations are as follows:

quinaria-subquinaria hybrids. The female hybrids had one large V-shaped autosome, a J-shaped autosome, four short rods, two dots and two long rods, one of which had a proximal constriction. The V, J, one dot, and the long rod with the constriction were contributed by the *quinaria* parent; the remaining elements were from *subquinaria.*

subquinaria-munda hybrids. The configuration of the female hybrid showed two large V's, four medium-length rods, two long rods, and two dots. The *munda* parent had contributed the two V's, one long rod, and one dot. The four medium-length rods, one long rod, and one dot were from the *subquinaria* parent.

munda-occidentalis hybrids. The metaphase configuration of these hybrids showed the two large V-shaped autosomes from *munda,* four medium-length rod-shaped chromosomes from *occidentalis,* and two long rods and two dots contributed by both parents.

munda-suboccidentalis hybrids. These hybrids had metaphase plates like those described immediately above except for a constriction at the

proximal end of one of the long rods. This represents the X-chromosome from *suboccidentalis.*

suboccidentalis-occidentalis hybrids. The larval brain smears of female hybrids showed four pair of medium-length rod-like autosomes, two long rods (one with a proximal constriction), and two dots. The larvae were selected with particular care since only female larvae would be diagnostic. The hybrid male larvae would be indistinguishable from male larvae of *suboccidentalis* since they both would have the same metaphase configuration if *suboccidentalis* were used as the female parent. If *occidentalis* were used as the female parent the male hybrid larvae would have a metaphase configuration like that of *occidentalis.* In the reciprocal crosses involving *occidentalis* and *suboccidentalis* the female hybrid larvae, however, would have an X-chromosome from each parent. Since the X-chromosomes of the two species can be distinguished from each other, that of *suboccidentalis* having a proximal constriction, it is possible to distinguish hybrid female larvae from female larvae of either parent stock by examining the brain smears (see Figure 1).

transversa-innubila hybrids. Male and female larvae showed in the metaphase plates four pairs of medium-length rods, two long rod-like X-chromosomes (one with a proximal constriction), and two dots. The long rod with the proximal constriction was from the male parent, *innubila.*

palustris-innubila hybrids. Male and female hybrid larvae showed the same configuration as that described above for the *transversa-innubila* hybrids. The long rod with the constriction again came from *innubila.*

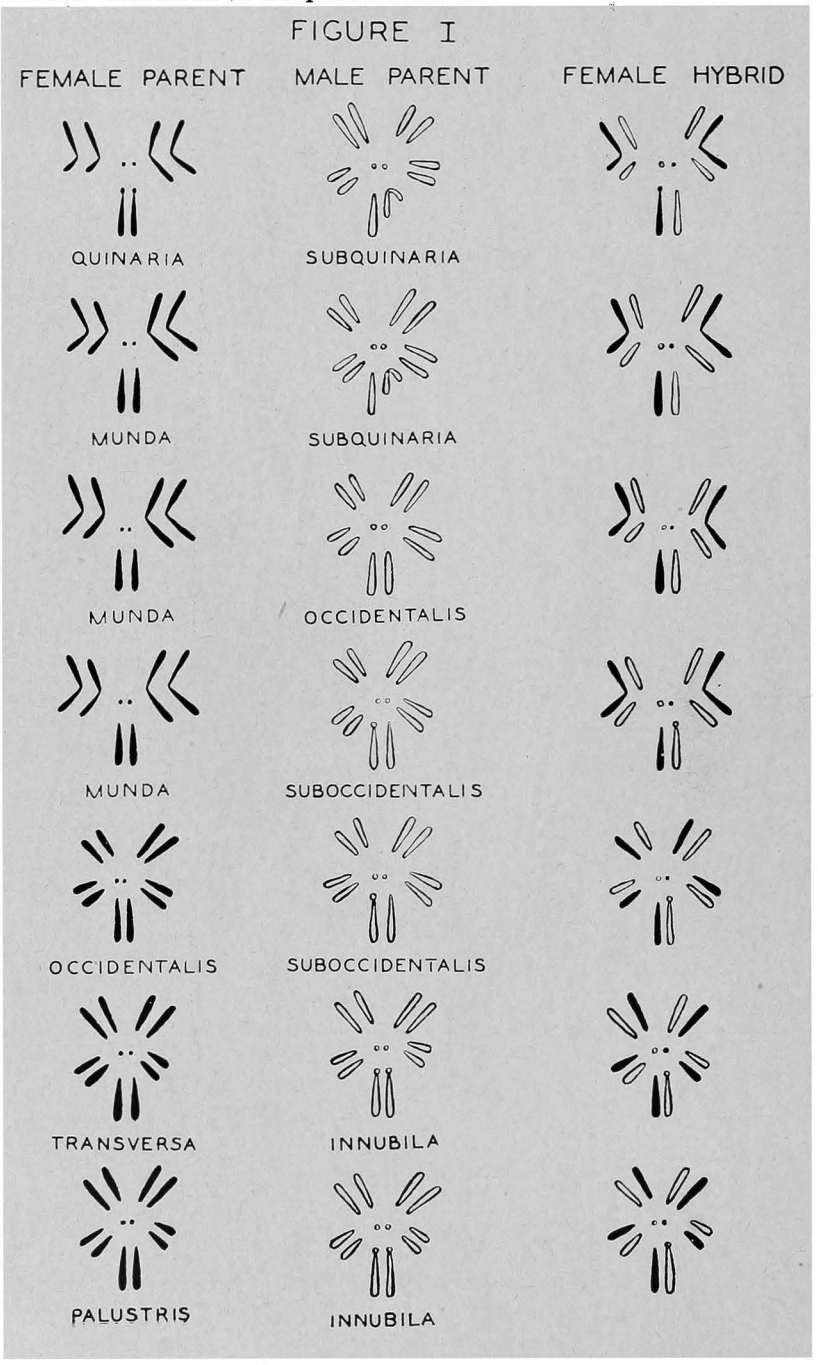
The metaphase configuration of hybrid larvae from crosses involving *occidentalis, palustris* and *subpalustris* are not useful in checking hybridization since these three species have identical metaphase plates. Hybrid larval salivary gland chromosomes were used instead. These showed in each case numerous inversions not found in the salivary chromosomes of the parent species.

The metaphase configurations of hybrid larvae from the crosses *suboccidentalis* × *palustris, subpalustris* × *munda,* and *innubila* × *occidentalis* are not given since too few larvae were produced to obtain both brain smears and adult flies. The adult flies proved by their appearance to be hybrid.

The salivary gland chromosomes of the species studied in this group are not well adapted to exacting analysis since they tend to remain knotted into small masses when the smears are made. Inversions were found within all the stocks used in this investigation. This made the analysis of the inversions found in the hybrids so difficult that it will be left for later work. It should be noted, however, that many more complicated inversions were found in the hybrids than in the parent stocks. The salivary gland chromosomes of F_1 larvae from the crosses between *palustris* and *subpalustris* are a *tangled* mass of poorly synapsed strands with many complex inversions in each chromosome. This is in agreement with

the observations reported by Spencer (1940). In all crosses the F_1 hybrid salivary chromosomes were similar to those described above in that they revealed numerous inversions and very poor synapsis. It is not possible at this time to state whether these complicated configurations are the result of overlapping, tandem, or included inversions; on the other hand, it is obvious that none is simple in nature.

FIGURE I



DESCRIPTION OF HYBRIDS

Some hybrids obtained in this investigation were intermediate in appearance between the parent forms, others were mixtures. Only those that were most striking in appearance will be dealt with here.

transversa-innubila hybrids. The abdomen of *transversa* is yellow with dark spots on each tergite. The eye color is an orange-red and the wings have clouded cross veins. *Drosophila innubila* has a yellowish-grey abdomen with tergites four, five, and six a dark brown. Its eye color is a dull red and the wings are not clouded. The hybrids are larger than either of the parent species but have a body shape more like that of *transversa* with yellowish-grey abdomens and with dark spots on each tergite. The eye color is intermediate between an orange-red and a dull red. The wings have very slight clouds on the cross veins, lighter than those of *transversa* but darker than those of *innubila*.

palustris-innubila hybrids. *Drosophila palustris* has dark clouds on the cross veins and at the apex of the second, third, and fourth longitudinal veins. In *innubila* the wings are clear. The abdomen of *palustris* is a dark brownish-grey with three broad yellow stripes running lengthwise. The abdomen of *innubila* is a yellowish-grey with narrow bands interrupted in the mid-dorsal line on tergites two and three; tergites four, five, and six are dark brown. The eye color of *palustris* is red, and that of *innubila* is a dull red. The eye color of the hybrid is intermediate, the wings are clear, and the abdomen is more like that of *innubila* although not quite the same color. The hybrids were more like *innubila* than *palustris* but could be distinguished from either parent.

suboccidentalis-palustris hybrids. The abdomen of *palustris*, as was stated above, is a dark brownish-grey with three yellow bands running lengthwise. The lateral edge of each tergite is tipped with black. The wings of *palustris* have dark clouds on the cross veins and at the apex of the second, third, and fourth longitudinal veins. The abdomen of *suboccidentalis* is a shiny yellow with dark brown apical bands, interrupted in the mid-dorsal line, on tergites two to five. The wings are clouded on the cross veins only. The lateral margins of the tergites are not tipped with black. The hybrid body shape and wing clouds were of the *palustris* type. The abdominal banding and color was intermediate, being nearly like *suboccidentalis* except for the presence of dark spots on the lateral tips of the tergites.

occidentalis-palustris hybrids. *Drosophila palustris* has already been described. *Drosophila occidentalis* has a shiny yellow abdomen with dark brown apical bands on tergites two, three, and four, slightly interrupted in the mid-dorsal line. The lateral edges of the tergites are not tipped in black. The wings have clouds on the cross veins only. The hybrids have a body shape like that of *palustris*, but wings with clouds only on the cross veins. At the lateral margin of each tergite there is a black edge like that found in *palustris*. The abdominal color, abdominal banding, and the eye color are intermediate.

occidentalis-subpalustris hybrids. *Drosophila subpalustris* is very similar to *palustris* in abdominal color and banding, and in the wings clouds on the cross veins and at the apex of the second, third, and fourth longitudinal veins. At the lateral tip of each tergite in *subpalustris* there is a black spot. The hybrids have the *subpalustris* body shape and an intermediate abdominal color and banding. The three yellow bands running the length of the tergites of *subpalustris* are present in the hybrids. The tips of the tergites are edged in black as in *subpalustris,* but the wings have clouds only on the cross veins, as in *occidentalis.*

quinaria-subquinaria hybrids. *Drosophila quinaria* has clouds on the cross veins and at the tip of the longitudinal veins. The hairs on the median side of the male fore-tarsus are longer than twice the width of the tarsus. In *subquinaria* the clouds on the wings are restricted to the cross veins and the hairs on the fore-tarsus of the male are shorter than twice the width of the tarsus. The hybrid has clouds on the cross veins and at the apex of the longitudinal veins, but the hairs on the median side of the fore-tarsus of the male are short as in *subquinaria.*

DISCUSSION

In order for organic diversity to lead to the formation of new species, it is necessary for some type of isolating mechanism to be established between populations. Numerous types of isolating mechanisms have been found to function in the genus *Drosophila* (Patterson 1942c). In the quinaria group several of these mechanisms can be seen at work: geographic isolation, ecological isolation, sexual isolation, gametic inviability, and hybrid sterility.

GEOGRAPHIC AND ECOLOGICAL ISOLATION

Geographic and ecological isolation has been demonstrated in several species groups of the genus *Drosophila.* In the *melanica* group Griffen (1942) found these types of isolation to be more or less effective. *Drosophila melanica paramelanica* occupies the northeastern part of the United States, while *melanica* is found in the south and southwest. *Drosophila nigromelanica* has a distributional range overlapping both of the ranges occupied by the other species, but seems to be ecologically isolated from them by a preference for fungi as food. It is also more restricted to the wooded areas than either of the other species.

In the macrospina group (Mainland 1942) geographic isolation is effective to some extent since the populations are divided into a western, a southwestern, and an eastern group. These groups, however, are connected by an intergrading series of geographical populations. It was found that cross fertility between strains decreased as their distance apart increased.

In the mulleri group (Patterson and Crow 1940) geographical and ecological isolation is quite effective. *Drosophila buzzatii* is found in

Sicily and Argentine, *mojavensis* in the deserts of California, and *arizonensis* in Arizona. Only *aldrichi* and *mulleri* occupy a common distributional range, an area extending throughout Texas and into Mexico. These are somewhat ecologically isolated since *aldrichi* feeds mostly on the prickly pear, *Opuntia lindheimeri,* while *mulleri* is more cosmopolitan in its food habits and feeds on various other kinds of fruit.

In the virilis group (Patterson, Stone, and Griffen 1940, 1942) geographical and ecological isolation has been demonstrated. *Drosophila virilis* is a cosmopolitan species. *Drosophila montana* and *novamexicana* are found in the Rocky Mountains. *Drosophila montana,* which occurs at high altitudes, ranges from central New Mexico north to Montana, while *novamexicana* has been taken near Silver City, New Mexico. *Drosophila americana* and *texana* occupy the eastern part of the United States, with *americana* in the north and *texana* in the south around the Gulf of Mexico.

In the quinaria group geographical and ecological isolation has undoubtedly played a part in evolution. *Drosophila suboccidentalis* and *subquinaria* are completely isolated from all other species since they occur in the Transition and Canadian zones of the Rocky Mountains. *Drosophila occidentalis,* as far as collection records show, is restricted to California and Lower California. *Drosophila munda* and *innubila* are found in the Highlands of the southwest and Mexico. *Drosophila quinaria, palustris,* and *subpalustris* occupy the northeastern United States, and *transversa* is found throughout the entire eastern part of the country. As was pointed out in the section on materials these four species are more or less separated ecologically. *Drosophila quinaria* is a "fruit-feeder" and lives in the moist forest areas. *Drosophila palustris* and *subpalustris* inhabit swampy areas, feeding on decaying water plants. *Drosophila transversa* is a "fungus-feeder," living in the dryer parts of the deciduous forest of the East.

It has been pointed out that geographic isolation in itself is insufficient to bring about diversification of a population into two or more species. There must, therefore, be some actual genetic mechanism present which will prevent or reduce appreciably the transfer of genes before any new species can be said to be established. Several of the possible genetic isolating mechanisms are operative between members of the quinaria group.

SEXUAL ISOLATION

One type of genetic isolation that has been demonstrated in the genus *Drosophila* is sexual or psychological isolation. Species that are sexually isolated show no desire to mate. Perhaps the elaborate behavior pattern before mating is different in each species, and if this pattern is interrupted or is incorrect the desire to mate is not aroused. There may be other factors involved. Whatever the cause, this type of isolation has been shown to be extremely effective since it prevents the transfer of

genes and since there is no waste of reproductive efficiency through the production of possible sterile offspring.

Wharton (1943) has shown that sexual isolation is effective both between species and between strains within a species of the repleta group. The complex cross-fertility relationship within this group indicates that there must be several different factors within the various species and strains that cause this isolation.

In the melanica group Griffen (1942) has shown that this is the principal type of isolation present. If the initial isolation of the P_1 crosses can be broken down, the hybrids produced are fertile. Sex isolation seems to be the major genetic mechanism separating the species in this group.

Sturtevant (1921) showed that when males and females of two species of the melanogaster group, *melanogaster* and *simulans,* were placed together in one vial they invariably mated with their own species and not with individuals of the other species. Only when individuals of their own species were removed did crossing occur. Sexual isolation has been demonstrated in the pseudoobscura group (Dobzhansky and Koller 1938), in the macrospina group (Mainland 1942), in the virilis group (Patterson, Stone, and Griffen 1940, Stalker 1942, Spencer 1940), and in the mulleri group (Patterson and Crow 1940, Crow 1942).

In the quinaria group this type of isolation is very effective. From an examination of Tables 1 and 4 it can be seen that in most cases the interspecific crosses are fertile in one direction only and that sexual isolation is largely responsible for the failure of the other crosses to go. In the interspecific crosses the females showed little or no interest in the males; in fact, in several instances they showed a decided antagonism. The males, on the other hand, often showed a great deal of interest in the females and began their courting reactions, in some cases attempting to copulate in spite of the hostility of the females. In certain cases the sexual isolation existing between species can be broken down if the males and females of different species are forced to remain together in small food vials with no other mates for a sufficient period of time.

Drosophila quinaria is normally isolated from all other species, although occasionally the cross *quinaria* ♀ × *subquinaria* 1210.5 ♂ will produce some sterile hybrids. *Drosophila subquinaria* 1210.5 is sexually isolated from all other species and even shows isolation from *subquinaria* 1202.3. *Drosophila subquinaria* 1202.3 will cross to *munda* in reciprocal matings but will not cross to *quinaria.* Without choice of mate *palustris* will mate with *subpalustris* in reciprocal crosses and fertile hybrids are obtained. Apparently the only isolation present between these species is sexual isolation, since the F_1 and F_2 hybrids are fertile.

Sexual isolation is indicated again in the cross *palustris* females to *innubila* males. This cross is slightly fertile, determined as less than 1%. In this cross hybrid larvae can be detected at the end of a period which

is approximately twice as long as that for either control stock. The reciprocal cross is incompatible.

The crosses between *occidentalis* females and *palustris* or *subpalustris* males showed a high degree of sexual isolation. After repeated experiments the fertility was determined as less than 1% in each case. The hybrid females from both crosses were fertile but the males were completely sterile. The reciprocal crosses were sterile.

Drosophila transversa females cross only with *innubila* males, but here again the cross is difficult to obtain because of the presence of sexual isolation. The reciprocal cross is sterile, as are all other crosses to *transversa*. Table 4 shows this incompatibility to be due to sexual isolation.

Sexual isolation is indicated in the crosses between *munda* and *subquinaria* 1202.3. These crosses went reciprocally, showing 6% fertility when *subquinaria* was used as the female parent and 10% when *munda* was the female parent. The experiment on sexual isolation indicates that this low fertility is due to the lack of desire of the female to mate.

Drosophila munda females cross occasionally to both *occidentalis* and *suboccidentalis* males, but here again the fertility is low because of the reluctance of the females to mate. In fact, the *munda* females were antagonistic to the advances of the *suboccidentalis* males. The results of the cross-fertility tests and of the experiment dealing with sexual isolation indicate that there are different factors causing the isolation between the various species.

HYBRID STERILITY

It will be seen from an examination of Tables 2 and 3 that hybrid sterility, shown particularly in the males, is of importance in the isolation of the various species of this group. The cross *quinaria* ♀ × *subquinaria* 1210.5 ♂ produced completely sterile hybrids. The cross *subquinaria* 1210.5 ♀ × *subquinaria* 1202.3 ♂ likewise gave sterile hybrids, but from the reciprocal cross hybrid females were obtained which showed a slight degree of fertility when backcrossed to both parent types. Perhaps if more hybrid females from the former cross had been available for testing, they also would have shown a slight fertility, since genetically the hybrid females from the two crosses are the same.

The cross *subquinaria* 1202.3 ♀ × *munda* ♂ and the reciprocal cross *munda* ♀ × *subquinaria* 1202.3 ♂ produced completely sterile hybrids. An examination of the cross fertility of the two strains of *subquinaria* reveals that they are different genetically. *D. subquinaria* 1210.5 crosses to *quinaria*, if the latter is used as the female parent, but *subquinaria* 1202.3 does not; *subquinaria* 1202.3 crosses reciprocally to *munda* and produces sterile hybrids, but *subquinaria* 1210.5 does not. When *subquinaria* 1210.5 and *subquinaria* 1202.3 are crossed reciprocally the fertility is only 2% to 3%. The female hybrids from the cross *subquinaria* 1202.3 ♀ × *subquinaria* 1210.5 ♂ are slightly fertile, but the other hybrids are sterile. Morphologically, the two strains of *subquinaria* can be separated with

ease. These facts make it probable that *subquinaria* 1210.5 should be made a new species.

The cross *munda* ♀ × *occidentalis* ♂ produced hybrids that were fertile when inbred. The male hybrids, however, were sterile when backcrossed to either parent type. The cross *munda* ♀ × *suboccidentalis* ♂ seems to be another case of unbalance similar to the preceding one, except that the hybrid males were fertile when backcrossed to *munda* females.

The cross of *occidentalis* females to *palustris* or *subpalustris* males produced fertile female and sterile male hybrids. In the cross *suboccidentalis* ♀ × *palustris* ♂ the female hybrids were slightly fertile if backcrossed to the *palustris* parent type. The male hybrids were completely sterile. The cross *transversa* ♀ × *innubila* ♂ gave sterile hybrids, as shown by the tests of five hundred twenty-eight hybrids.

The cross *palustris* ♀ × *innubila* ♂ produced hybrids that were slightly fertile when inbred and when backcrossed to *innubila* in both directions, but completely sterile when backcrossed to *palustris* in both directions. It is possible that, since only forty-nine hybrids were obtained, the crosses were not sufficiently large to show any slight fertility that might exist.

The crosses *occidentalis* ♀ × *suboccidentalis* ♂ and *suboccidentalis* ♀ × *occidentalis* ♂ were 35% and 79% fertile, respectively. The hybrids from both crosses showed heterosis and were 100% fertile when inbred. The F_2 hybrids were also fertile. Table 4 indicates that there is little if any sexual isolation present between these species. In the first cross there is some gametic mortality which reduces the fertility of that cross but not sufficiently to make the cross sterile. Morphologically these species are very similar, but cross fertility tests show that they are slightly different. The difference is illustrated by the fact that *occidentalis* crosses with *suboccidentalis, palustris, subpalustris, munda,* and *innubila,* while *suboccidentalis* crosses with *occidentalis, palustris,* and *munda* only.

The crosses *palustris* ♀ × *subpalustris* ♂ and *subpalustris* ♀ × *palustris* ♂ showed 19% and 16% fertility, respectively. This low fertility is not surprising when it is noticed that the controls are quite low, that for *palustris* being 27% and for *subpalustris* 38%. The hybrids from the crosses were 19% and 82% fertile, respectively, when inbred; this fertility percentage was obtained by testing only twelve pairs of flies, since no others were available. The F_2 hybrids in both cases were fertile. From an examination of Table 4 it can be seen that there is some sexual isolation present but it is not sufficient to separate these forms completely.

IV. GAMETIC MORTALITY

Gametic mortality was found to be effective in reducing the fertility in crosses between *D. virilis* ♀ and *D. americana* ♂ (Patterson, Stone, and Griffen 1940). The *americana* sperm were inactivated and perhaps killed in the reproductive tracts of the virilis females so that only the eggs laid during the first day after copulation were fertilized. In a

comparable study in the quinaria group no hybrids were produced from the crosses *subpalustris* ♀ × *occidentalis* ♂ and *palustris* ♀ × *occidentalis* ♂, although copulation was observed. Upon the dissection of the females it was found that the sperm in each case were inactivated in the reproductive tracts of the females. In the cross *occidentalis* ♀ × *suboccidentalis* ♂ the first eggs which were laid after copulation produced larvae, but when the females were dissected at the end of three days the unused sperm in the genital tracts were immotile. In the reciprocal cross the sperm were still motile at the end of the three-day period.

SUMMARY

1. Only fifteen of a possible eighty-eight interspecific crosses tested have proven fertile. The most important cause of this lack of cross-fertility is sexual isolation.

2. Seven out of the fifteen interspecific hybrid combinations were sterile on inbreeding, and only twenty-seven of the sixty possible back-crosses were fertile. Therefore, the interspecific hybrids usually had a much reduced reproductive efficiency.

3. Two cases are noteworthy if verified by further investigation. These are *munda* ♀ × *occidentalis* ♂, and perhaps *munda* ♀ × *suboccidentalis* ♂. In these two cases the hybrid males did not fertilize the parent type females, although they were fertile with their hybrid sibs. These cases represent the origin of a cross sterility due to a new combination of genes present in these hybrid males.

4. The metaphase chromosome configurations of these species vary quite widely and fusion must have been an important factor in the change in chromosome number (e.g., *occidentalis* and *munda*). The chromosome patterns, including constrictions, are the properties of the chromosomes themselves for they persist in the hybrids. In addition to fusions, there are many inversions present in this species group.

5. Numerous phenotypic differences exist between these species. The hybrids show that both blending and dominant relations exist between the several gene systems which determine these phenotypes.

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VIII. THE SPECIES RELATIONSHIPS IN THE VIRILIS GROUP

WILSON S. STONE AND J. T. PATTERSON

Sturtevant (1942) raised a question as to the species status of the five members of the virilis species group known at that time. He classifies *Drosophila virilis* as the type species with *americana, texana, novamexicana,* and *montana* as additional subspecies. Patterson, Stone, and Griffen (1942) disagreed with Sturtevant on this classification and regarded all five members of the group as separate species. Additional evidence leads us to propose certain revisions of this group.

There is an overlap zone where *americana* and *texana* occur, which we know extends through Tennessee, Arkansas, and into Texas (Patterson and Wagner 1943). In the fall of 1946, Mr. Marshall R. Wheeler and Mr. William K. Baker collected a number of females of the virilis group at Morrilton, Arkansas. These females were separated from the males as soon as they were trapped, and each female was placed in a separate container to establish a stock. In case the female was not fertile, she was mated to males of a *virilis* stock with the following markers in the major autosomes: Rounded (*R*, 2 chromosome), cinnebar (*cn*, 3), plexus (*px*, 4), peach (*pe*, 5). In case the captured female was fertile, the F_1 females were mated to the *R cn Px pe* males. The F_1 males from each cross to the marker *virilis* stock were testcrossed individually to *cn px pe* females. This allows us to determine in the next generation the segregation of the X chromosome and each of the major autosomes. The small sixth chromosome was not followed in these experiments. The effectiveness of this test depends on the chromosome architecture of these forms (see Wharton 1943, for illustrations of the metaphase chromosome configurations of the known species of this group).

Drosophila virilis has the primitive chromosome configuration for Drosophila, i.e., five pairs of rods and a pair of dots. In this species the X and Y and the four major autosome pairs are rod-shaped chromosomes, indistinguishable in the ordinary metaphase configuration. In *americana* Stalker (1940), and Patterson, Stone, and Griffen (1940) reported that females have two pairs of V-shaped chromosomes and a pair of rods, while the males have three V-chromosomes and four rods. The pair of V's common to both male and female are fused 2 and 3 chromosomes. The other pair of V's in the female are the fused X and 4 chromosomes, while the male has one V representing the fused X and 4 chromosomes and two free rods which are the unfused Y and the other 4 chromosome. Therefore, *americana* is a species with a multiple Y complex (the Y and free 4 chromosome).

Patterson, Stone, and Griffen (1940) described the ordinary metaphase chromosome pattern of *texana* as one pair of autosomal V's, three pairs of rods (one of these is the X–Y pair), and a pair of dots. In that publication the autosomal fusion was described as linking chromosomes

3 and 4 and was so referred to in later publications. Further investigation has revealed that this is not correct for a number of *texana* stocks, and that the autosomal fusion involves chromosomes 2 and 3 as in *americana*. Segregation tests with *texana* (stock number listed) were used to determine this association, as follows:

The *texana* stock used was from Georgetown, Texas (Stock 84.7).

P_1 84.7 ♀ × *R*(2) *cn*(3) *px*(4) *pe*(5) ♂.
F_1 84.7/*R cn px pe* ♂ × *cn px pe* ♀.
F_2 *R cn px pe* = 48; + = 54; *R cn px* = 40; *pe* = 51; *R cn* = 51; *px pe* = 46; *R cn pe* = 45; *px* = 41.

Similar results were obtained with strains of *texana* from New Orleans, Louisiana (1128.10), from Caddo Lake, Texas (1593 B.6a), and from Lake McKethan, Florida (1148.9). In these cases also *R* and *cn* segregated together, indicating that chromosomes 2 and 3 are fused.

We desired to determine whether *americana* and *texana* hybridized in nature. The segregation test outlined above allows us to check this possibility. Stocks of *americana*, where the X and 4 chromosomes are fused, will show this linkage: If *americana* females are crossed to *R*(2) *cn*(3) *px*(4) *pe*(5) males and the F_1 males testcrossed to *cn*(3) *px*(4) *pe*(5) females, all the F_2 females will be non-plexus(4), and the F_2 males will be plexus(4) for that reason. As the autosomes 2 and 3 are fused, *R*(2) and *cn*(3) will segregate together. On the other hand, as *texana* does not have this X–4 fusion, plexus(4) will occur at random in both sexes. In case a hybrid female between *texana* and *americana* is tested, one half the F_1 males will have the *texana* linkages and the other half the *americana* linkages. Such hybrids were present in the collection from Morrilton, Arkansas.

The stock 1586(8).3e from a single fertile female proved to be hybrid. The F_2 progeny from test of individual F_1 males were.

(1) males = 4 *R cn px pe*; 4 *R cn px*; 5 *px pe*; 6 *px*.
females = 5 *R cn pe*; 4 *R cn*; 6 *pe*; 6+ (*americana* linkage).
(2) males = 3 *R cn px pe*; 3 *R cn px*; 2 *px pe*; 6 *px*; 3 *pe*; 2+.
females = 1 *R cn px pe*; 5 *R cn px*; 4 *px pe*; 5 *px*; 1 *R cn pe*; 2 *R cn*; 5 *pe*; 5+ (*texana* linkage).
(3) males = 5 *R cn px pe*; 4 *R cn px*; 7 *px pe*; 5 *px*.
females = 5*R cn pe*; 4 *R cn*; 4 *pe*; 3+ (*americana* linkage).

One test was made with a female which was virgin when captured 1591(12)1a. She was mated directly to *R cn px pe* males. The F_2 progeny were:

(1) males = 7 *R cn px pe*; 6 *R cn px*; 9 *px pe*; 7 *px*.
females = 6 *R cn pe*; 7 *R cn*; 6 *pe*; 9+ (*americana* linkage).
(2) males = 2 *R cn px*; 1 *px pe*; 2 *px*; 2 *R cn pe*; 1 *pe*; 1+.
females = 1 *R cn px pe*; 2 *R cn px*; 1 *px pe*; 2 *px*; 1 *R cn pe*; 2 *R cn*; 3+ (*texana* linkage).

(3) males = 8 *R cn px pe;* 9 *R cn px;* 11 *px pe;* 11 *px;* 7 *R cn pe;* 9 *R cn;* 10 *pe;* 13+.
females = 8 *R cn px pe;* 7 *R cn px;* 10 *px pe;* 12 *px;* 8 *R cn pe;* 9 *R cn;* 9 *pe;* 11+ (*texana* linkage).

This female proved to be heterozygous for *texana* (free X and 4 chromosomes) and *americana* (fused X and 4 chromosomes). Therefore she was a hybrid captured in nature. The first stock tested may have been established either from a hybrid female, or from hybridization in nature. Several other stocks tested proved to be only *americana* or *texana* in so far as linkage tests could show. We could not distinguish several possible types of back cross hybrids by this method, so we cannot determine the history of these strains.

There is very little comment necessary on these results. All the *americana, texana,* and hybrid stocks tested showed linkage between chromosomes 2 and 3, so this probably is the autosomal fusion present in these two forms. Strains of *texana* and *americana* cross readily with each other in the laboratory. These tests show that they hybridize under natural conditions in the overlap zone of their respective distribution areas. We do not have quantitative data on the extent of such hybridization. There exists certain cytological information on the hybridization in the overlap zone between *texana* and *americana.* These two forms differ in their gene sequence, due to inversions in the X and 4 chromosomes. Warters (1944) demonstrated that certain stocks, which had only *texana* fusions, had the *americana* gene order in one or the other chromosome where their gene orders differ. These stocks were from the overlap zone of these two forms, and certain of them must have come from natural hybridization.

These two forms are, therefore, best classified as subspecies, which in the main occupy different areas of distribution and cross where these areas join or overlap. These forms are sufficiently alike morphologically that it is almost impossible to distinguish on that basis. We cannot determine the status of *D. novamexicana* until a group of representative stocks are available for testing. A discussion of the origin and relations of *novamexicana* must be deferred until these relationships are tested.

We therefore suggest that the known relationships and differences can justify only subspecific rank, and these two forms should be designated *D. americana americana* and *D. americana texana.* The revision of the virilis group would then be as follows:

I. *D. virilis* Sturtevant
II. *D. montana* Patterson and Wheeler
III. *D. lacicola* Patterson
IV. (a) *D. americana americana* Spencer
(b) *D. americana texana* Patterson, Stone, and Griffen
V. *D. novamexicana* Patterson—undetermined relationship to *texana* and *americana*

This group is composed of four distinct species which can be separated on the basis of their morphology, and which genetic tests show to be separate species. The status of the fifth member remains undetermined.

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IX. GENE REPLACEMENT IN THE VIRILIS GROUP

WILSON S. STONE

This paper reports preliminary investigations of the ability of a group of genes (one or more chromosomes) from one species to replace effectively the equivalent group of genes in a second species. This work also includes some measurements of the effect of the chromosomal differences that exist between these species on the fertility of their hybrids.

MATERIALS AND METHODS

These tests were made with three members of the virilis group, *Drosophila virilis* Sturtevant, *Drosophila americana americana* Spencer, and *Drosophila americana texana* Patterson, Stone, and Griffen. The species relationships have been discussed elsewhere in this volume.

The *virilis* stocks used were Mexico City (1341.13) and two marked stocks: (1) *Rounded* (*R* = 2 chromosome); *cardinal* (*cd* = 4 chromosome), and *peach* (*pe* = 5 chromosome); (2) a stock of *cd pe* for testcrossing heterozygotes. The Anderson, Indiana, stock of *americana* was used. Three strains of *texana* were tested, the one from Georgetown, Texas (84.7); a second from Newton, Texas (841.10); and a third from New Orleans, Louisiana (1128.10).

The experimental procedure consisted of crosses between *texana* (or *americana*) and *R cd pe*. The F_1 hybrids and F_2 segregants were crossed to *cd pe*. As an alternative, certain of the F_2 combinations were crossed to the unrelated Mexico City stock so that the effect of stock lethals could be minimized. Egg counts and hatch counts were made in certain crosses to determine the effect of the recombination on reproductive efficiency.

The differences in chromosome architecture that exist between these species must be considered in analyzing the results of these tests. *Drosophila virilis* has the primitive chromosome complex for *Drosophila* of five pairs of rods and a pair of dots. This species will be used as the base in describing differences that occur between these forms. In *texana* and *americana* chromosomes 2 and 3 are fused to form a large autosomal V in place of the two rods in *virilis*. In addition there is a medium size inversion in 2, involving about one-third of the chromosome. Chromosome 5 in *americana* (and usually in *texana*) differs from *virilis* by a small inversion near the free end of the chromosome and a large inversion nearer the centromere, involving about two-thirds of the chromosome. The X in *texana* has a double inversion in the proximal half and *americana* has an additional overlapping inversion extending further toward the free end of the chromosome. Finally, the X and 4 chromosomes are fused in *americana* although the Y and 4 are not, so that *americana* has a complex Y chromosome.

In making reciprocal crosses, the direction of the initial cross determines the X and Y chromosomes of the F_1 and F_2 hybrids; for example, the X — 4 fusion and lack of Y — 4 fusion leads to several differences in reciprocal crosses involving *americana*. (See Patterson, Stone, and Griffen (1940) for Griffen's chromosome maps and analysis of the inversions.)

RESULTS

Hybrids between *virilis* and *texana* or *americana* are obtainable if sufficient crosses are made even though the egg hatch is quite low and sexual isolation decreases the amount of cross fertilization. These experiments have not indicated a great differential in viability between combinations, for if the F_1 males from matings of *americana* or *texana* to *R cd pe* are testcrossed to *cd pe*, the different possible recombinations occur with nearly equal frequencies. Also, the fertile F_2 males of the different combinations produced the different possible F_3 classes in the expected ratios. Therefore, the viability of the different recombination classes does not seem to be markedly changed by the differences in association. It should be noted that all the tests in Tables 1 and 2 hold the foreign chromosome(s) in the heterozygous condition, and, therefore, at minimum possible selective disadvantage.

Table 1 shows that all female F_2 recombinations from all reciprocal crosses between *americana* or *texana* and *virilis* are fertile, i.e., produced offspring. Even though some of the tests involve small numbers, the number fertile (F) is always larger than the number sterile (S). In fact, in most cases the fertility of these F_2 recombinations seemed normal. In contrast to these results, the data for male recombinations were not uniform. In the crosses where *americana* and *texana* were used as the P_1 female all F_2 male combinations were fertile, with little, if any, reduction below normal. In reciprocal P_1 crosses, the F_2 males showed that the complementary Y-autosome combination previously reported was necessary for fertility (Patterson, Stone, and Griffen 1940, 1942). If the *texana* or *americana* Y-chromosome is present, the 2-3 chromosome fusion and the 5 chromosome from *texana* or *americana* must also be present for normal fertility. Occasionally combinations without all complementary chromosomes produce offspring. In Table 1, 15 out of 174 without 2-3 chromosome of *texana* and 1 out of 153 without either 2-3 or 5 chromosome were fertile. In Table 1, the semisterile males which produced offspring are starred. However, none of these males produced more than one or two progeny. The egg hatch which will be discussed later indicates that only a few sperm are functional.

Certain of these hybrid male combinations have been dissected and their descriptions follow:

In all cases, the sperm pump appeared normal but the paragonia appeared to be immature. In the classes without the 5 chromosome of *texana* or *americana* (the 2-3 fusion might or might not be present) the testes were small with many large abnormal cells and cell debris; usually

TABLE 1

Fertility Tests of F_2 Recombinations

	texana or *americana* autosomes; see cross for X chromosome	2–3, 4, 5		2–3, 4		4, 5		4		2–3, 5		2–3		5		0	
	Phenotype of F_2 =	+		*pe*		*R*		*R pe*		*cd*		*cd pe*		*R cd*		*R cd pe*	
		F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S
(1)	*R cd pe* ♀ × Anderson ♂; F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	15	2	12	2	23	0	17	7	20	1	17	0	25	1	28	1
(2)	Anderson ♀ × *R cd pe* ♂; F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	92	18	56	4	62	5	36	10								
(3)	Anderson ♀ × *R cd pe* ♂; F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀									41	10	61	10	35	10	27	4
(4)	*R cd pe* ♀ × New Orleans ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	16	0	10	2	23	1	18	5	16	2	15	1	26	6	27	0
(5)	*R cd pe* ♀ × Newton ♂; F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	32	3	23	1	36	0	60	2	26	0	53	6	58	6	66	3
(6)	*R cd pe* ♀ × Georgetown ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	22	4	17	6	22	1	26	7	13	9	10	4	32	0	37	3
(7)	Total F_2 ♀ testcrossed	70	7	50	9	81	2	104	14	55	11	78	11	116	12	130	6
(8)	*R cd pe* ♀ × New Orleans ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	26	4	0	23	3*	27	0	27	25	0	0	22	5*	33	1*	38
(9)	*R cd pe* ♀ × Newton ♂; F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	24	2	0	34	6*	27	0	54	36	6	0	65	1*	43	0	90
(10)	*R cd pe* ♀ × Georgetown ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	9	8	0	11	0	16	0	25	5	1	0	18	0	13	0	25
(11)	Total, ♂ = *texana* Y testcrossed	57	14	0	68	9*	70	0	106	66	7	0	105	6*	89	1*	153
(12)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	18	6	36	7	18	1	15	2	26	2	38	8	16	4	6	12
(13)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	27	4	20	5	13	5	12	3	31	3	39	4	17	1	23	8

the sperm bundles were abnormal, with a few long thick strands, but occasionally some strands approached the immature normal in appearance although no sperm or motility was observed. In the cases with the 2-3 fusion absent (but 5 present), the testes were more nearly normal in size and shape. The sperm bundles were more often abnormal with beaded sperm but a few were nearly normal and there was some motility. In no case did they appear normal, with the usual masses of motile sperm.

Obviously chromosome 5 has much more effect on the fertility of these males (the 1 male classed as *R cd pe* which produced an offspring may have been a mutation, a misclassified *R cd* male or have been mated to a non-virgin female). However, the 2-3 fusion is necessary for normal fertility for if it is missing, at best only a few sperm are functional.

The egg hatch counts deserve some comment. The eggs were collected from one to ten pairs of flies—usually over five pairs—to reduce the effect of extrinsic factors on fertility. Certain crosses have not been made and these are left blank. Others where no fertile crosses were obtained are indicated by 0. The egg counts for the semisterile males were usually made from a pair or pairs that had produced at least one larvae from an unknown number of eggs previously laid. In these cases, the number of such offspring (and tests also as only one offspring per pair was obtained) is indicated as (+ 2), (+ 4), and (+ 1) with the number of eggs subsequently counted without further offspring indicated. These males, therefore, produced a very few short-lived functional sperm.

There were certain complications due to genes in the related *virilis* marked stocks *R cd pe* and *cd pe*. Some few females were sterile because they produced abnormal eggs without filaments which failed to hatch. This made the hatch low in some crosses, especially affecting those where the number of eggs was low.

Despite these complications the effect of gene replacement and chromosomal differences on these hybrids can be determined. The 4 chromosome of *texana* (and 6, which may be present or absent in any one of these combinations) has no major detectable gene rearrangements as compared to *virilis*. It can replace the *virilis* 4 at least heterozygous without ill effects. For example, in an unlisted cross of *R cd pe* ♀ × Georgetown ♂; F_1 ♂ × *cd pe*; F_2 *R pe* ♀ × *Mexico* ♂, there were 1,359 hatched from 1,411 eggs, or 96.3%. This is a normal hatch, despite the fact that the *texana* 4 chromosome is heterozygous. Other similar crosses included in Table 2 numbers 6, 7, 8, (14) indicate that the 4 chromosome does not reduce the egg hatch below the normal if present in either male or female parent in the heterozygous condition. It should be noted that the *R cd pe* class is in effect a control even though the 6 chromosome may be heterozygous.

The 5 chromosome of *texana* or *americana* which has two inversions in comparison to the *virilis* 5, does not have much detrimental effect if heterozygous. The results with the semi-sterile males, of course are the exception, but this is the Y-autosome complementary action, not the effect

TABLE 2

Egg Counts with F_2 Recombinations

	texana or *americana* autosomes; see cross for X chromosome =	2–3, 4, 5	2–3, 4	4, 5	4	2–3, 5	2–3	5	0
	Phenotype of F_2 =	+	*pe*	*R*	*R pe*	*cd*	*cd pe*	*R cd*	*R cd pe*
(1)	*R cd pe* ♀ × Anderson ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	362/641 = 56.5%	135/348 = 38.8%	451/554 = 81.4%	626/793 = 78.9%	231/777 = 30.0%	117/413 = 28.3%	459/576 = 79.7%	475/730 = 65.1%
(2)	Anderson ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	597/1188 = 50.3%	394/611 = 64.5%	85/243 = 35.0%	177/228 = 77.6%				
(3)	Anderson ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × Mexico ♂	402/947 = 42.4%		577/953 = 59.9%					
(4)	Anderson ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀					428/713 = 60.0%	346/599 = 57.8%	319/504 = 63.3%	656/841 = 78.0%
(5)	Anderson ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × Mexico ♀					542/832 = 65.1%	571/875 = 65.3%	433/473 = 91.5%	613/639 = 95.9%
(6)	*R cd pe* ♀ × New Orleans ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	9/46 = 20.0%	233/540 = 43.1%	132/226 = 58.4%	570/676 = 84.3%	281/570 = 49.3%	19/101 = 19.0%	569/685 = 83.1%	430/468 = 91.9%
(7)	*R cd pe* ♀ × Newton ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	253/484 = 52.2%	56/106 = 52.8%	625/722 = 86.6%	529/607 = 87.1%	190/353 = 53.8%	125/295 = 42.4%	526/615 = 85.5%	386/449 = 86.0%
(8)	*R cd pe* ♀ × Newton ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × Mexico ♂	347/767 = 45.2%	366/869 = 42.1%	916/1346 = 68.1%	734/905 = 81.1%	355/649 = 54.7%	264/635 = 41.6%	407/446 = 91.3%	796/869 = 91.6%
(9)	*R cd pe* ♀ × New Orleans ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	125/317 = 39.4%	0	0(+2)/333(+) = 1.±%	0	70/172 = 40.7%	0	0(+4)/648(+) = 1.±%	0(+1)/165(+) = 1 ±%
(10)	*R cd pe* ♀ × Newton ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	165/388 = 50.3%	0	0	0		0	0	0
(11)	*R cd pe* ♀ × Newton ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × Mexico ♀	237/412 = 57.5%	0/806	3/437 = 2.1%	0/610	840/1301 = 64.6%	0/1152	0/984	0/1278
(12)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × *cd pe* ♂	235/466 = 50.4%	541/1097 = 49.3%	345/577 = 61.9%	470/594 = 79.1%	235/643 = 36.5%	260/564 = 46.1%	440/667 = 66.0%	247/317 = 77.9%
(13)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♀ × Mexico ♂	103/341 = 30.2%		198/511 = 38.7%	234/302 = 77.5%	826/1797 = 46.0%	385/667 = 57.7%	79/114 = 69.3%	163/265 = 61.5%
(14)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × *cd pe* ♀	61/178 = 34.3%	80/225 = 35.5%	179/234 = 76.4%	77/84 = 78.4%	120/268 = 44.8%	263/474 = 55.5%	14/33 = 42.4%	127/162 = 78.4%
(15)	New Orleans ♀ × *R cd pe* ♂ F_1 ♂ × *cd pe* ♀; F_2 ♂ × Mexico ♀	463/802 = 57.7%	792/1523 = 48.8%	365/592 = 61.7%					

of the 5 as such (see the data with the *virilis* Y-chromosome). If, however, we consider those combinations which have the 2-3 fusion of *americana* or *texana*, we find that the egg hatch in general is much below the normal or that of gene combinations without the fusion. There are two possible explanations. One is that the gene combination is less viable. The second and more probable explanation is that the 2 chromosome inversion and the 2-3 fusion cause abnormalities in meiosis. A similar situation has been investigated by Brown (1940) who demonstrated the general relation between crossing over and disjunction that exist in such cases. There is not enough data on the X-4 fusion of *americana* to determine its effect.

Only a few data are available on crosses back to the *americana* P_1 type. These suggest certain restrictions on the ability of certain chromosomes to replace others. The tests are as follows:

P_1 Anderson ♀ × *R cd pe* ♂
F_1 ♂ × Anderson ♀
(1) F_2 + ♂ × Mexico ♀ ;
2 fertile pairs laid 108 eggs (of which 34 hatched (31.5%)
(red and black pupae)
12 sterile pair laid over 1,700 eggs which did not hatch
(2) F_2 *R* ♂ × Mexico ♀
2 fertile pair laid 144 eggs of which 71 hatched (49.3%)
(red and black pupae)
25 sterile pairs produced over 1,300 eggs but none hatched

The initial cross was made reciprocally.

P_1 *R cd pe* ♀ × Anderson ♂
F_1 ♂ × Anderson ♀
(1) F_2 + ♂ × Mexico ♀
10 pairs produced no offspring from 904 eggs
(2) F_2 *R* ♂ × Mexico ♀
4 pairs went well and produced 234 progeny from 529 eggs (44.2%)
8 pairs went poorly and produced only 34 progeny from 802 eggs (4.2%)
14 other pairs were sterile, although we obtained over 1,000 eggs
(All fertile vials had red and black pupae)
(3) F_2 *R* ♀ × Mexico ♂
5 pairs produced 780 eggs, of which 332 hatched (42.6%)
(red and black pupae)
6 pairs were sterile although over 700 eggs were laid.

The implications of these data depend on the fact that the factor(s) for red versus black pupa color are in the 5 chromosome of *americana* with modifiers in the 2-3 chromosome (Stalker 1942). The production of black pupae by each of the fertile F_2 individuals indicates that all of them had the *virilis* 5 heterozygous. There are two possible explanations. The

first is that certain of these gene recombinations are sterile. The second is that certain combinations of genes—when chromosome 5 of *americana* is homozygous—are to a large degree sexually isolated from *virilis*. It should be pointed out that the 2-3 chromosome does not produce as much effect because both *R* and + combinations were fertile. It is not possible to decide which of these alternatives is correct without further evidence.

DISCUSSION

These data are of a preliminary nature so a general comparison with similar work will be deferred until more comprehensive data are available. Dobzhansky (1936) has investigated the effect of recombinations of genes on the fertility of *pseudoobscura* A and B *(pseudoobscura* and *persimilis)* hybrids. In that case he demonstrated that a number of genes in several autosomes were involved in the reduction of fertility of these hybrids. In this case the Y + 2-3 + 5 *texana* or *americana* relation involves genes in several chromosomes but the number of genes is unknown. No genes in the 4 chromosome affect this fertility relation, and those in the 2-3 have much less effect on normal development than those in 5. Only this Y-autosome complementary gene system, and the effect of the 2-3 fusion, probably due to difficulties in meiosis, produced a reduction in the reproductive efficiency of the F_2 combinations in *virilis* backcross hybrids. There is some evidence that *americana* backcross hybrids are less fertile, or that certain recombination genotypes are sexually isolated from *virilis*.

Patterson, Stone, and Griffen (1940, 1942) and Muller (1942) have discussed the role of gene transfer between nearly isolated species in evolution. This can be regarded as migration or mutation pressure as discussed by Wright (1940). It has one difference in effectiveness as it provides "selected" genes for testing in another genotype. The present work shows that such hybrid genotypes, once they occur, can be retained in the heterozygote for repeated testing of recombinations.

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X. INTERRELATIONSHIP BETWEEN EYE COLOR AND FACET ARRANGEMENT IN LOZENGE ALLELES OF DROSOPHILA MELANOGASTER

C. P. OLIVER

One of the methods available for the study of gene action is the analysis of the multiple effects of a gene. A mutant gene usually is first recognized and described as primarily controlling the development of some one characteristic. More exact examinations often reveal that the mutant gene produces several phenotypic effects. However, repeated mutations at that locus are detected and described by their effects on the characteristic first associated with the gene.

Increased information about the multiple effects of genes has raised the question of how the pleiotropic effects are related. Because the multiple effects sometimes occur in different parts of the body it is often difficult to see any association between the various results of the gene action. However it is conceivable that the gene controls a single developmental process which affects different parts of the body depending upon the place of action. In some instances, one affected characteristic may secondarily cause another one to develop. An alternative interpretation of gene action is that the gene produces more than one independent effect upon the organism. In his investigations of mutants in the rat and the mouse, Grüneberg (1938, 1943) in each case found that a single gene mutation caused several defects to develop and that the various anomalies were traceable to the control by the gene of a single effect. He concluded that there is always a unity of gene action and, therefore, that the pleiotropic effects of a gene are spurious rather than genuine.

The difficulty of ever proving that pleiotropism is genuine has been discussed by Dobzhansky and Holz (1943). Even though a study failed to show that one physiological process caused the pleiotropic effects of a gene, the proof that genuine pleiotropism was involved would still be lacking. Some evidence in support of genuine pleiotropism might be derived from the study of a series of multiple alleles. If some of the alleles affected several characters and others in the series only one or only certain ones of the traits, a multiplicity of primary gene products would be suggested. Alleles which show a gradation of effects on several characteristics offer an effective test for possible unity of action of the gene. If the alleles cause a single immediate product their effects on different characters should be parallel except for the threshold, and there should be no complementary effects in compounds of alleles. This is not necessarily true if there are different immediate products of the gene.

Pleiotropic effects have been observed in studies of the *R* alleles in corn (Stadler and Fogel—1945) and in several series of alleles in

Drosophila. Stern (1943) reported that *ci* has an effect on venation of the wings as well as on the legs and some of the bristles, and the effects were not always of the same grade in each case. Alleles of *vg* affect more than one trait (Mohr 1932) but the gradations for one allele are not always the same as those for another. Dobzhansky and Holz (1943) reported that four tested alleles of *w* and 9 of 10 alleles of *y* affect not only color but also shape of spermathecae in the female.

The recessive gene *lz* (lozenge) on the X-chromosome of *Drosophila melanogaster* and the known alleles of that gene affect many characters of the fly. The degree of expression is varied for the several alleles. Each described allele of *lz* has some effect upon the structure of the eyes. Gottschewski (1936) in a study of 14 alleles of *lz* referred to their varied effects on eye structure, eye pigmentation and fertility. In 10 lozenge alleles studied by Oliver and collaborators, those and several other characteristics are affected by the gene. All are recessive, under ordinary conditions, to the normal allele. Each allele affects the surface (facet structure) of the eyes and with rare exceptions the size and shape and the color of the eyes. Certain female reproductive structures, the spermathecae and parovaria, are completely absent or are abnormal (Anderson 1945). The claws and pulvilli on the tarsal segments of the legs are abnormal with some of the alleles, and in the four alleles tested by Cummings (1946), variations occurred among the legs on an individual. Decreased viability and lowered fertility also are effects of the gene.

Some relationship between certain effects of the gene have been suggested. Decreased fertility and fecundity of the females are related to the anomalies of the spermathecae. In one allele tested, females lacking spermathecae and parovaria had a constant daily egg production for a period of 9 days (Oliver and Green 1944), but only those eggs deposited during the first two days after copulation were fertilized. It was suggested that the low fertility was due to sperm inactivation. This was substantiated by a series of rematings of females which had passed through a short period of fertility and then became sterile (Oliver and Anderson 1945). In these tests females were first mated and then kept away from males for six days. During that time the females produced some progeny for approximately two days and became sterile although control females still continued to produce offspring. On the seventh day when new males were added, the females again had a short period of fertility before they became sterile. Anderson (1945) carried out a more extensive study of the relationship between genital anomalies and fecundity. In the females homozygous for either of 9 *lz* alleles, spermathecae were absent and the ventral receptacle was filled with sperm which gradually lost their motility. In lz^{37} the degree of anomaly of the spermatheca seemed to be correlated with the degree of fecundity. Those females lacking spermathecae had the same low fecundity found in the other alleles. Those which had spermathecae, although abnormal

in size and shape, retained motile sperm for several days and were fertile for the whole period of the test. The effect of the gene on fertility seems therefore to be secondary, depending upon whether the gene affects the spermatheca and to what degree the effect is expressed.

The lozenge series of alleles with their multiple effects are useful for the study of gene action. Gradation of the several effects varies with many of the alleles. Spurious pleiotropism is suggested in some effects and complementary effects in compounds of some of the alleles are known. The present paper reports a study of the interrelationship between the effects on the eye surface (facet arrangement) and eye color.

MATERIALS and METHODS

The ten mutant alleles at the lozenge locus used in the study are *lz*, the original mutant at the locus, lz^{g} (glossy), lz^{BS}, lz^{y4}, lz^{s}, lz^{34k}, lz^{37h} and the three spectacles (lz^{s}, lz^{sB}, and lz^{36c}). Seven of these are described in Bridges and Brehme (1944) but the origin of lz^{sB}, lz^{BS} and lz^{y4} is somewhat obscure. Spectacle-Bishop *(lz^{sB})* arose concurrently with an inversion as yet incompletely determined. Allele lz^{BS} occurred in a T(1:4) B^{S} (Bar-Stone) culture, and lz^{y4} in the y-4 inversion, but each has been crossed into a normal chromosome. Spectacle *(lz^{s})* and glossy *(lz^{g})* were induced by irradiation in an X-chromosome bearing the *dl-49* inversion. The other alleles are in chromosomes free of rearrangements. Because of reduced fertility and viability of the lozenge alleles, cultures are carried in balanced form by use of the *ClB* inversion. Isogenic cultures were prepared (by Kay Cummings Green) by first making *lz* isogenic for the sex, second and third chromosomes. This culture was then used to make the other *lz* alleles isogenic for chromosomes 2 and 3 from the *lz* culture. Because of inversions with three of the alleles it is not possible to have all alleles isogenic for the sex chromosomes. However each culture of an allele is isogenic for its own sex-chromosomes.

In test matings, 2 or 3 *ClB* females from a culture type were placed with 3 to 6 brothers in vials containing approximately 10 cc. of yeast food. Five or more matings of each culture were started as a precaution against the inviability of homozygous females which were used for the observations. At the end of 4 days the flies were transferred to new vials. All matings were kept in a constant temperature chamber at 23° ± 1C. During a period of years the same types of matings were repeated, using isogenic and non-isogenic cultures, and when homozygous females were observed in tests for other characteristics they were examined for eye surface and color. Because of the known effect of age on the concentrations of both the red and brown pigments (Ephrussi and Herold 1944) the females were examined within 48–72 hours after emergence except where mentioned in subsequent discussions. The descriptions therefore are a composite picture derived from repeated examinations. In general the females in isogenic strains did not appreciably

differ from non-isogenic strains in color of eyes and facet arrangement. Any noticeable differences are mentioned in the subsequent descriptions.

Examinations of the facets were made in two ways, by visual inspection of etherized flies under 20 and 40 magnifications and by the use of impressions of the eyes made in thin sheets of celluloid and plastic that had been dissolved in amyl-acetate. Examinations of pigmentation of the eyes as reported here were made by visual inspection although Green (1947) has made a spectral analysis of the red pigment.

As a further means of examining the amount and distribution of eye pigment, females of the *lz* allele series were made homozygous for either *v, st* or *bw*. According to Ephrussi and Herold (1944) *st* almost entirely suppresses the brown pigment without interfering with the red pigment; *bw* totally suppresses the formation of red and at the same time decreases the amount of brown pigment formed. The suppression effects of the three pigment genes on the quantity and distribution of the pigments have also been discussed by Johannsen (1924), Wright (1932) and Glass (1934). In order to counteract the effect of lack of isogeneity in these tests, repeated descriptions based upon several independent tests were made.

EXPERIMENTAL

The ten lozenge alleles vary in their effects upon the surface of the eyes. The effect is expressed in the size and shape of the compound eyes as well as in the facet arrangement. The gradations in size and shape range from normal to eyes which are narrow and pointed especially at the ventral portion. Gradations between these two extremes are difficult to separate because shape depends upon so many variables. In some it is the result of a normal anterior portion with a flattened middle posterior portion. In others, both the anterior and posterior margins do not extend the normal limits. Some of the alleles have a narrowed ventral portion in combination with either of the types mentioned, and others are narrowed throughout the length of the eye.

FACET ARRANGEMENT

The surface of the normal eye of *Drosophila melanogaster* is composed of hexagonally shaped facets which are the corneas of the ommatidia. Small hairs develop at the point where three facets meet (Johannsen). In the normal eye, the size and shape of the facets and the arrangement of the hairs give a smooth appearance to the surface of the eye. Each of the ten lozenge alleles has an effect upon the structure and arrangement of the facets, but the alleles differ in their degree of effect. In five alleles, no regular facets are formed. In the other five, the effect varies from the presence of a small roughened area to several large areas of fused facets, giving a distinguishable characteristic appearance to each of the alleles.

In the descriptions, the ten alleles are placed in seriation from the most normal to the least normal in appearance. Each description is based upon visual examinations of etherized flies and upon studies of the impressions of the eyes. The least effect on facets is observed in lz^{37}. Males of this genotype have a roughened area due to irregular facets in the posterior part of the eye and sometimes a wettish appearance in this area because of the fusion of a few facets. The eyes are normal in shape and size. Females lz^{37} are almost normal in appearance. In the isogenic strain the roughened area appears more regularly than in cultures which are not isogenic.

The arrangement of facets in *lz* and lz^{BS} is somewhat similar although lz^{BS} is the less normal in appearance and also the more variable. Both genotypes are rougher than normal. In general small areas in which the facets are irregular in shape and the hairs are unevenly placed are scattered over the eye. Other facets have a normal appearance. Some of the areas of irregular facets have a wettish, warty appearance caused by fused facets and occasionally these fused areas are pimply because of a few facets which are rounded instead of hexagonally shaped. The warty areas vary in size and appear in the impressions as relatively large spots which resemble molten glass. Especially in lz^{BS} these areas may on occasion approach the extremeness of lz^{9} and cover most of the eye, with only small islands of normal facets being present. If the fused area is large, the appearance is that of a blister on the eye. In size and shape, lz^{BS} eyes are smaller than normal but are rounded except for a flattened posterior margin. In *lz* the eyes vary from an ovoid to an almond shape even in isogenic cultures.

The rounded eye in lz^{9} is almost normal in size and has an overall glossy appearance. Areas of fused facets are surrounded by islands and streaks of normal facets. The hairs on the eyes are irregular in position. Some of the fused areas resemble blisters and may have a slight pimply appearance. The characteristic glossiness of the eye is caused by the several streaks and areas of fused facets which give the appearance of molten glass. This allele differs from *lz* and lz^{BS} in that it causes more blistery areas which tend to cover more of the surface of the eye.

A more extreme variation of the eyes occurs in lz^{34}. They are narrow and pointed at the dorsal and particularly at the ventral portion. Most of the facets are fused into irregularly shaped, glass-like blisters which vary in size. Some of these areas have the smoothness of glass but may have a few pimples scattered over the surface. Others, especially the large areas, have a creased central region which gives the appearance of erosion. Between the blisters are found one to three rows of normal facets with approximately five facets in a row. Although the number and size of the blisters vary, most of the eye is covered with the glass-like blisters which cause the eye to have a glazed appearance.

The other five alleles have eyes which are narrower than normal. In each of them true facets fail to form and the whole eye has a glass-like

surface. Two of these alleles, lz^{s} and lz^{y4} have a roughish, wettish appearance due to the presence of pimples on the eye and to a few scattered hairs. The pimples which seem to be single rounded facets are irregular in number and position, but they are never frequent enough to repress the general appearance of a glass-like surface. The pimples and hairs occur more frequently in lz^{s} than in lz^{y4} and the two usually can be distinguished phenotypically by the rougher surface of lz^{s} eyes.

In the three alleles, lz^{s}, lz^{sB} and lz^{36}, the surface of the eye is a smooth, hairless sheet. When light is reflected on the eyes, pimples are recognizable on some lz^{s} and lz^{sB} females, but in the impressions of the eyes no pimples or facets are observed. Of these three alleles, lz^{36} is the smoothest.

COLOR OF THE EYES

The characteristic red-brown color of the wild-type eye of Drosophila is due to the amount and distribution of two pigments, red and yellow, in the pigment cells of the ommatidia. Yellow pigment predominates in the outer cells of the ommatidia and red pigment in the lower cells.

Most of the ten lozenge alleles affect both pigments of the eye. It is not possible to seriate the alleles because of the variations in concentration of the pigment. One group of the alleles can be graded from normal to extreme dark, sepia-like color of the eye, but the others have a yellowish, straw-brown color associated with a deeper, web-like arrangement of red and a marginal rim of darker pigment. In each of the alleles gene *e* which causes the dark ebony body-color intensifies the red but does not suppress the brownish color of the eyes. All examinations were made on white paper.

Except for a wettish and sometimes shiny-red appearance in the small rough area, lz^{37} flies are uniformly normal in color. In *lz* flies the eyes are normal except for flecks of brown and sometimes of shiny red in the areas of fused facets. If these areas are numerous the whole eye has a dark color, or sometimes the color is a sharp red. The color in lz^{BS} varies according to the irregularity of the facets. In some flies the eyes have normal areas with spots of shiny red where the blisters of fused facets occur. In others the color in some areas and at times over the whole eye is an opaque, muddy-looking clay-brown, somewhat similar to lz^{g}. However, the eye seems to have more red than brown in it as though the red pigment was normal and the brown was decreased in spots. In isogenic cultures, lz^{g} ordinarily has no normal color in any part of the eye. The color is best described as a venous or dark blood-red with wettish, shiny spots occurring in some areas.

Two of the alleles are much darker than normal but the red pigment is concentrated in irregular fashion. A dark maroon-red color with occasional wettish spots characterizes lz^{34}. Variegation is observable in some eyes more than in others. In the heavier pigmented eyes, small, facet-size, yellowish spots are observed around which is a heavy aggregate

of red color, the whole appearance suggesting that the primary pigment cells surrounding the cone are almost pigmentless and the secondary pigment cells have a heavy concentration of granules. Other eyes have yellowish areas of varied sizes with streaks and spots of the darker red pigment throughout the lighter areas, somewhat resembling the web-like appearance in the facetless alleles except that lz^{34} has more red pigment. The margin of the eye in lz^{34} is always heavily pigmented but the streaks extending from it into the center of the eye prevent the appearance of a rim.

A dark, sepia-like color is found in the facetless eyes of aged lz^{44} flies. In contrast to lz^{9} and lz^{34}, the eyes are darker and seem more brownish in color although both red and brown pigments are observable in the eyes. In very young flies the color is not evenly distributed. A heavy and wide band of pigment is found at the margin of the eye and in the central portion, yellowish areas irregular in shape and size have streaks of dark color penetrating them. These streaks often are wide near the margin and come to a point nearer the center of the eye. As the flies increase in age, the yellowish areas become lost to view and the whole eye becomes darker. No true rim of pigment is present because of the heavy streaks of pigment extending from the marginal areas.

The other four facetless alleles have a greatly decreased amount of pigment in the eyes. The two spectacles, lz^{s} and lz^{sB}, are identical. Their color is dilute, resembling a very thin layer of dried blood. On close inspection the color is found to be an even yellowish, pale straw-brown color with a deeper web-like distribution of soft, red-brown which gives the appearance of a fine stipple. A narrow darker rim encircles the eye. Eyes darken with age due to darkening of the red-brown pigment but the pale yellow color is constant. Small variations in the depth of color are observed. In lz^{3} the color is heavier than in lz^{s} because of an increased amount of red distributed more evenly over the eye. The yellowish color is only a little darker than in lz^{s}. In older flies the color becomes a rusty brown. A rim is observed only in those flies which have a lighter center but ordinarily the color of the whole eye in lz^{3} is more nearly that of the rim of lz^{s}. The center of the eye in lz^{36} is tannish with the very fine web-like distribution of red pigment. This has the lightest eye of all the alleles and in older flies the color approaches lz^{s} but the rim persists.

EFFECT OF ALLELES ON BROWN PIGMENT

According to Ephrussi and Herold (1944) the recessive gene *bw* (brown) removes or suppresses all the red and also decreases the amount of brown pigment. Placing *bw* into the genotypes with the lozenge alleles, therefore, will show how these alleles affect the brown pigment which remains in the presence of *bw*. All except one of the lozenge alleles have an effect upon the brown pigment, and of these 9 all except one causes a definite decrease in the amount of pigment.

The allele with the most nearly normal facet arrangement, lz^{37}, has no observable effect upon the brown pigment. The brown color is uniformly distributed over the eye. In *lz*, however, there is an increased concentration of pigment but the opaque color appears more red than brown. In *lz* flies with the rougher eyes due to facet irregularities and in older flies, the color increases in redness. The distribution of the pigment over the eye is uniform.

Three of the alleles have a slightly reduced quantity of brown pigment. In lz^{g} the eyes have a foamy or frosted brown color and a glazed appearance. The rougher areas have a darker color, somewhat reddish in appearance. Age also darkens the color. The pigment is reduced over all the eye so that it is uniformly distributed. Although the facets are more regular than in lz^{g}, there is a greater reduction of brown pigment in lz^{BS}. The eyes of lz^{BS} are faded in color, with less brown pigment in central areas than in the marginal regions, although some color is present throughout the eye. In older flies, the depth of color increases especially in the marginal regions to a reddish tinge. Even less brown pigment is found in eyes of lz^{34}, the allele of these three which has the least regular facets. The eyes have a light, dirty, smeared brown appearance. This color is traceable to the irregular distribution of the pigment which ranges from very light areas to small reddish spots. Age increases the color of the eyes and causes the smeared appearance to disappear, but the increased color does not make the eye as dark as lz^{g} *bw* or lz^{BS} *bw*.

Five of the alleles have a greatly reduced amount of brown pigment in the eyes. In lz^{s} and lz^{v4} the distribution is more or less uniform over the whole eye. In color, lz^{s} *bw* is only slightly lighter than lz^{s} bw^{+}, and is uniformly pigmented with a heavy straw-brown color. A marginal rim is found only in the very young flies in which the central area has not become fully pigmented. The eye color in lz^{v4} *bw* flies is just a little lighter than that in lz^{s} *bw* flies. In general the pigment is uniformly distributed although occasionally a fly will have a small, yellowish area in the central portion of the eyes. This allele, lz^{v4}, which is sepia-like and therefore the darkest in color when both pigments are present, very closely resembles spectacle *(*lz^{s}*)*, one of the alleles least normal in color, when *bw* is added to the genotype to suppress the red pigment, the main difference being that lz^{v4} *bw* does not have the rim of heavier color characteristic of lz^{s}.

The least amount of brown pigment is found in the three alleles which have the smoothest, glass-like eyes. In lz^{s} *bw* and lz^{sB} *bw* flies, the eyes have a tannish-brown central area not much lighter than that of the lz^{s} bw^{+}. In the central area of the eye can be observed small spots of pigment more or less uniformly scattered over the eye. A narrow marginal rim of heavier color is present, this rim being narrower in the presence of *bw* than with bw^{+}, and in lz^{sB} *bw* the rim is narrower in the anterior margin than in the posterior margin. Color increases in in-

tensity with age, more so in lz^{sB} *bw* than in lz^{s} *bw*. Allele lz^{36} with *bw* differs from lz^{s} *bw* primarily by having smaller spots of pigment in the central area of the eye. There spots resemble fine stippling and are uniformly distributed over the eye. The resulting color is a light tan center with a thin rim of heavier color. Although lz^{36} *bw* is the lightest of all the alleles in the tests with *bw*, with age the color of the eyes is as dark as that in aged lz^{s} *bw*.

EFFECT OF ALLELES ON RED PIGMENT

The recessive gene *st*, scarlet, almost entirely suppresses the brown pigment and does not interfere with the red (Ephrussi and Herold). By adding *st* to the genotypes of the lozenge alleles, therefore, it is possible to determine the effects of the various alleles upon the red pigment of the eye. All except one of the ten alleles decrease the quantity of red pigment and some of them also cause very irregular distribution of the pigment. In many of the alleles black facet-size spots are found in the eyes.

Three of the alleles seem to have little effect upon the red pigment, as determined by visual inspection. A uniform scarlet color not differing from *st* flies is found in lz^{37} *st*. In lz^{9} and lz^{BS} the color is distinctly scarlet but the whole eye is lighter and more shiny than in lz^{37} *st* flies. The distribution of the pigment is not uniform although variegation is not very striking.

In combination with *st*, *lz* usually causes a light, scarlet color with wettish or shiny spots on the eye, but in some of the flies the whole eye has a uniform orangy appearance although definitely not an orange. This color seems to be due to a decreased quantity of pigment over the whole eye.

The red pigment is rarely concentrated enough in lz^{34} to cause a scarlet eye color. Ordinarily the eyes have an orangy tint, more orange than the lightest *lz st* but less so than lz^{v4} *st*. In the lz^{34} orangy eyes, the red pigment occurs in numerous spots and blotches which are irregular in shape and varied in size. These aggregates of pigment are scattered over the eye and sometimes seem to be attached by thin lines of red pigment. Whether the small spaces between the spots of pigment are colorless or are yellow can not be determined by inspection. The whole combination of the eye seems to be responsible for the color, with the greater concentration in a few eyes leading to a brilliant scarlet. A marginal rim rarely occurs, and when it does the inner margin is very irregular and the rim varies in width.

The eyes of lz^{v4} *st* flies are bright red-orange in color. The red pigment is distributed in irregular, anastomosing streaks which extend over the eye. Ordinarily the streaks are not wide although they may have points at which the aggregation of red pigment causes an irregular spot of some size. The colorless areas between the streaks are larger than those

in lz^{s4}. The pigment is always heavier along the margin of the eye causing the appearance of an orangy rim which however is very irregular along its inner margin.

The red pigment in lz^{s} *st* causes the eye to have a creamy-pink color. The small amount of red pigment that is present occurs in small spots which are distributed uniformly over the eye except in the marginal rim. The rim is very narrow especially in the dorsal margin of the eye.

The other three alleles, lz^{s}, lz^{sB} and lz^{s6}, have no observable red pigment in the presence of *st*. With each allele the color is whitish although it is not the same white as that produced by *w*. These alleles with *st* behave in the same manner as homozygous *st bw*. It would seem, therefore, that if *st* has no effect upon red pigment, either of these three alleles suppresses the development of all the red pigment which their pigment cells should have in the presence of *st*.

INTERACTION WITH VERMILION

The sex-linked recessive gene *v* also controls the development of brown pigment and leaves only red pigment. Although one of the lozenge alleles, lz^{sB}, cannot be tested with *v* because that gene cannot be crossed into the inversion associated with lz^{sB}, the other alleles have been tested. The main difference between the combinations of the lozenge alleles with *st* and with *v* are found in the more extreme alleles and the positions of aggregates of pigment with lz^{s4} and lz^{v4}. However, the *v* combinations have more pigment than do the *st* combinations.

In the lz^{s7} *v* flies, the color is a uniform vermilion. This color also occurs in lz^{g} *v* except that shiny spots occur on the eye. Ordinarily the red pigment is uniformly distributed but on occasions a small colorless spot occurs near the center of the eye, and the area around such a colorless spot is vermilion in color. The combination *lz v* has vermilion color with shiny (wettish) spots scattered over the eyes. Occasionally lighter vermilion areas occur in the center of the eye. Black facet-like spots are often observed in the eye, the number varying from one to as many as twenty.

Considerable variation is found in the lz^{BS} *v* combination. Many are vermilion in color although shiny spots and small areas of decreased pigment often occur. Sometimes several black facet-size spots are scattered over the eye. In some other flies a relatively large area and sometimes smaller twin areas occur in which pigment is entirely absent. Surrounding this region, the red pigment is aggregated into a thick, opaque wide marginal rim covering approximately one-third of the eye on the anterior and posterior margins and a lesser width on the dorsal and ventral portions. This rim, if it may so be called, is a dark, muddy-gumbo color.

In the three alleles lz^{s7}, *lz*, and lz^{g} with *v* and in those lz^{BS} *v* flies which are vermilion, the color as determined by visual inspection is the same.

However, Green (1947) has studied the absorption of the red pigment and determined that the quantity differs for the various alleles.

Both lz^{34} *v* and lz^{y4} *v* have colors similar to the *st* combinations. The color in lz^{34} *v* is orangy. Red pigment is always present in the marginal area but is distributed irregularly in the central portion. In many eyes small, facet-size clear but not colorless islands are surrounded by concentrations of red pigment. These give the impression of an absence of color in the primary pigment cells with as much as normal pigment in the other cells. Sometimes the clear yellowish areas are larger and streaks of red pigment of various widths, from fine lines to heavier streaks, extend over the eye in all directions, crossing and joining with other streaks. A rim as such is observable only if many thin streaks occupy the central portion of the eye. A heavy orange or tangerine color occurs in lz^{y4} *v*. Streaks of red pigment extend over the eye from a margin of concentrated pigment. The streaks vary in size and shape sometimes being wide near the margin and ending in a point nearer the center of the eye, although fine lines radiate out from these and cross or join with other lines. Where red pigment is not observable, the color is light yellow.

Only a small amount of red pigment is present in lz^{3} *v*, the color being a pinkish cream and having more red than that observed in lz^{3} *st*. The red pigment is distributed over the eye as a fine stipple but distribution is slightly irregular. A thin orange rim is present around the margin of the eye.

Only a very small amount of red pigment is present in lz^{s} *v* and lz^{36} *v*. The pigment is uniformly distributed over the eye as a very fine stipple and gives a creamy color to the eye. A thin rim is present around all the eye. In lz^{36} *v* the posterior ventral portion of the rim is wider than the other parts. The combination lz^{sB} *v* has not been observed.

DISCUSSION AND CONCLUSIONS

The ten lozenge alleles under study affect the structure of the eyes, as measured by facet irregularities and size and shape of the eyes. All except one of the alleles also cause an alteration in the quantity of both the red and brown pigments in the eyes. The distribution of the pigments varies markedly with the different alleles.

SERIATION OF INDIVIDUAL EFFECTS

Normal facet arrangement is not characteristic of any of the ten alleles. Their effects on facets can be seriated from almost normal arrangement to complete absence of facets (Table I), but some of the alleles duplicate each other. The only deviation from normal in lz^{37} is a small area of irregularly shaped facets in the posterior part of the eye. Small warty areas of rough and fused facets occur in *lz*, and in lz^{BS} the areas are larger and occur more frequently. An increased number of areas of

fused facets gives lz^{g} a glossy appearance. The fused areas cover more of the eye in lz^{34} and cause the eyes to have a glazed appearance. Two of the alleles, lz^{3} and lz^{94}, are similar in that their facetless eyes resemble rough, molten glass. The eyes have some pimples and occasionally have hairs which together make the eyes rough. The pimples seem to be rounded facets. Alleles lz^{s} and lz^{sB} are identical in appearance. In both the facets are fused into a glass-like surface without hairs, and although

TABLE 1

Relative Order of Effects of Lozenge Alleles on Surface and Pigment of the Eyes

Allele	Eye Surface	Brown Pigment Amt.	Brown Pigment Dist.	Red Pigment with *st* Amt.	Red Pigment with *st* Dist.	Red Pigment with *v* Amt.	Red Pigment with *v* Dist.	Color of eye
Wild-type	100	100	+	100	+	100	+	100
lz^{37}	95	100	+	100	+	100	+	100
lz	80	120	+	75	+	90	±	105
lz^{BS}	75	80	—	90	—	90	—	110
lz^{g}	50	90	+	90	—	95	+	120
lz^{34}	25	70	—	60	-----	70	-----	140
lz^{3}	10	40	+	10	+	15	+	60
lz^{y4}	9	35	+	40	-----	50	-----	170
lz^{s}	4	25	+	0.1	+	1	+	30
lz^{sB}	4	25	+	0.1	+	No test	No test	30
lz^{36}	3	20	+	0.1	+	1	+	25

LEGEND. The alleles are placed in order of severity of effect on facets from normal to least normal. Brown pigment was tested by using *bw*. The "Color of eye" is based upon amount of color. The numerical values are not percentages but merely show the relative degree of difference from normalcy for the specific category in the column. "Amt." refers to quantity of pigment; "Dist." to uniform distribution, if, + or irregular or variegated, if —.

small pimples seem to be present when light is reflected on the eye, pimples are not observable in impressions of the eyes. The smoothest glass-like eye is found in lz^{36} flies.

It is not possible to use the same seriation for eye color that is used for facet irregularity. One group of alleles can be graded from normal to increased dark color but in four of the alleles the color is considerably reduced (Table I). The color in lz^{37} is normal. Brown flecks in the eyes cause a slightly darkened color in *lz*. Eyes of lz^{BS} vary in color from that of *lz* to a clay-red and sometimes to a darker color. The color in lz^{g} is brownish and resembles venous blood. A heavy maroon-red is characteristic of lz^{34} flies. The darkest eyes are found in lz^{y4}, the color being sepia-like. In contrast to those alleles, color is reduced in lz^{s} to rusty, reddish brown. Both lz^{s} and lz^{sB} are yellowish straw-brown with an underneath, web-like scattering of red and a darker marginal rim; and lz^{36} is similar to them except that the center is a little lighter.

When the pigment components are followed separately, linear seriation of the effects upon the quantity of pigment can be made. The brown pigment is increased sufficiently to give a reddish color to *lz*. It is normal in quantity and distribution in lz^{37} and decreased in amount in lz^{g}, lz^{BS}, and lz^{34}, in that respective order. Distribution of the brown pigment is uniform over the eyes in lz^{g} but somewhat variegated in the other two. Both lz^{s} and lz^{y4} which are similar for facet arrangement are also similar for quantity and distribution of brown; both have a dilute color but lz^{y4} is slightly lighter. Allele lz^{y4}, which is the darkest of the series when both pigments are present, becomes similar to one of the spectacle series when only the brown pigment is present. The other three alleles have only a very small amount of brown pigment.

The alleles vary in the amount and distribution of red pigment which they produce and can be seriated according to their effects from practically normal to almost complete absence of the pigment. As is true with the brown pigment the seriation of the effects on red pigment does not follow exactly that of facet effects (Table I). In general the same degree of effect occurs whether *st* or *v* is used to remove the brown pigment, but slightly more pigment is present with *v* than with *st* in the genotype. No alteration of red pigment is observed in lz^{37}. The three alleles lz^{g}, lz^{BS}, and *lz* cause some reduction in the amount of red pigment. With *st*, the greatest reduction is likely to occur in *lz*, the reduced but evenly distributed pigment causing the eye to have an orangy appearance. The other two alleles have approximately the same degree of redness and do not differ greatly from *st*. With *v*, the three alleles are alike as determined by visual observation, but differ in the order lz^{g}, lz^{BS}, *lz* as shown by the absorption spectra of extracts of the eyes (Green 1947); this is just the reverse of the order expected if the seriation were the same as that for facet arrangement. With lz^{34} and lz^{y4} the red pigment is reduced and is irregularly distributed in spots, streaks and

blotches of heavy red concentrations surrounded by clear areas of varied sizes and shapes. Both the amount and distribution give an orangy tint to the eyes of lz^{34} and a definite orange color to lz^{v4}. Allele lz^{s} has a creamy color with *st* and pinkish center and orangy rim with *v*, due to the greatly reduced amount of pigment and its even distribution over the eye. The three alleles lz^{s}, lz^{sB}, and lz^{36} have very little red pigment. When combined with *st*, their color is whitish, although not the same white as that caused by *w*, and only a small amount of color (creamy) is observed when the alleles are combined with *v*. One, lz^{sB}, cannot be tested with *v*.

RELATIONSHIP OF SERIATIONS

Comparisons of the seriations indicate that the effects on facet disorder and on pigment production are interrelated to some extent. Differences in the behavior of duplicates and certain exceptions to linear seriation, however, suggest that some degree of independence between the two effects exists.

A relationship between the effects on the facets and pigmentation is suggested by the tendency for certain of the alleles to have the same severity of expression for each trait. In lz^{37}, only a small area of the eye is irregular and the red and brown pigments are normal in quantity and distribution. Normal facets occur infrequently in lz^{34}. Both pigments in flies having this allele are irregularly distributed and greatly reduced in amount. The three "spectacles" which have facetless, hairless, glass-like eyes also have very little brown pigment and practically no red pigment in the eyes.

Lack of complete relationship is suggested, however, when an attempt is made to fit the linear seriation of one effect to that of another effect. In a comparison of facet effects and color of the eyes, one group of alleles, lz^{37}, lz, lz^{BS}, lz^{9}, and lz^{34}, can be graded in that order from normal facets through increasing irregularity and in the same order from normal through increasing darkness of color. The two alleles lz^{s} and lz^{v4} which almost duplicate each other and are next after lz^{34} in severity of effect on facets break the relationship. Although lz^{v4} is even darker than lz^{34}, the opposite is true for lz^{s} in which the pigment is greatly reduced in amount. This difference in color between lz^{s} and lz^{v4} *is* primarily one of a difference in quantity and distribution of red pigment. The other three alleles (the spectacles) occur in a series with lz^{s}; that is, the more abnormal the facets, the greater the reduction in pigment.

If one follows the brown and red pigments separately, the alleles can be seriated in approximately the same order as they are for facet irregularity, although here too striking exceptions occur. Only lz, lz^{BS} and lz^{9} which follow that order for increasing facet irregularity fail to follow the same general gradation for brown pigment production. All three alleles are rougher than lz^{37} but lz has a heavier concentration of brown

pigment and both lz^{BS} and lz^{g} have less brown pigment than lz^{37}, with lz^{BS} having the least amount and the more irregular distribution. As determined by visual inspection lz^{g} and lz^{BS} have the same quantity of red pigment and *lz* with its more normal facets has a reduced amount of the pigment. Also lz^{3} and lz^{v4} which are similar for facet arrangement differ considerably in quantity and distribution of their red pigment. The other alleles have the same seriation for facet arrangement that they have for production of red pigment.

Some alleles fail to follow the same pattern of seriation for the production of the two pigments. In lz^{sB} and lz^{s}, lz^{36}, and lz^{3} the brown is not decreased as much as the red. The brown in *lz* is increased and the red decreased in quantity. The two alleles lz^{34} and lz^{v4} cause considerable variegation of the red pigment as well as a decrease in its quantity, but with the brown pigment variegation is observed only in lz^{34} and that is less so than with the red. Allele lz^{BS} has less effect on red pigment than it has on the brown.

In the lozenge alleles all of the effects reported here involve the eyes and therefore might be expected not to show very much independence. Nevertheless the lack of common seriation and also the behavior of some alleles which duplicate each other for certain effects indicate that there is some independence of the effects. Alleles lz^{3} and lz^{v4} are almost identical for eye surface and for their effect on brown pigment, but they differ greatly for effect on red pigment. In lz^{3} only a small amount of red is present and this is evenly distributed causing the eye to have a pinkish tint. On the other hand lz^{v4} has the red pigment aggregated in streaks scattered over the eye in such a manner that the color is an orange or tangerine. To a lesser extent *lz* and lz^{BS} are alike for facet irregularity although lz^{BS} is slightly the less normal. These two have greater differences in their production of brown and of red pigment when the latter is studied with *st*. It is also known that lz^{s} and lz^{sB} which are identical in their effect on facets, brown pigment and red pigment production do not behave alike in compounds with some of their alleles (Oliver 1945; Cummings 1946). One of them, lz^{s}, has a complementary effect with certain alleles which interact with lz^{sB} to produce a blending of the trait.

INTERPRETATION OF EYE COLOR

The gene may control the production of pigment by one of two methods or by a combination of the two. Decrease in pigment may be secondary to cellular disarrangement or it may be caused as a primary effect of the gene. Evidence that some ommatidia are pigmentless even though the cellular arrangement is normal, has been reported by Casteel (1929). Johannsen (1924) has also reported genic effects on pigment which are quite independent of structural arrangement of the ommatidia. Some of the ommatidia in the lozenge alleles may likewise have normal cellular

structure but lack pigment or have a decreased amount of pigment. Under those circumstances the effects on facet arrangement and color may be independent of each other, thus being similar to a condition reported in maize. The R allele in corn may have its effect on the aleurone, seedling or pericarp and the effect on one may be independent of the others (Stadler and Fogel 1945; Stadler 1946). Some of the alleles, however, affect more than one character although the degrees of expression may not be the same (Fogel 1946).

The irregularity of the facets and of pigment production may both be directly related to irregularities in the ommatidia. Waddington and Pilkington (1943) reported for lz^s, one of the alleles studied here, that the retinulae and cone cells are in disorder. Primary pigment cells are present and have produced pigment as well as a flat cornea. Basal pigment cells are present at the base of the ommatidia, but the secondary pigment cells are absent or disorderly arranged. Although it is unwise to assume that each allele of *lz* acts in a manner identical to that of lz^s, it is nevertheless conceivable and very probable that with the alleles facet irregularities are caused by cellular disarrangements and these disturbances may include the loss and irregular arrangement of some of the pigment cells. A gene control of cellular differentiation would therefore secondarily cause abnormal facet and pigment production.

The color of the eyes in the lozenge series of alleles is determined by the quantity and distribution of the two pigments, but another important factor is the contrast developed as a consequence of the distribution, in which one color intensifies an accompanying color. The small amount of red in lz^s, lz^{sB} and lz^{36} is not observable as such in the presence of *st* although the eye does not have the colorless appearance that is observed when gene *w* is present. When these alleles are present with bw^+ and st^+, they have a very light straw-brown color and the red pigment can be detected as a fine web-like stipple underneath. The orangy appearance of *lz st* is apparently the result of a dilution of the red pigment over all the eye, but in lz^{34} *st* it is due to the reflection of light through the clear areas around which the red pigment is aggregated in spots and fine streaks. When both pigments are present in lz^{34}, the contrast made between the smeared, somewhat irregularly distributed brown and the extremely variegated red pigment causes a dark maroon-red color. In lz^{v4} *st* the reflection of the light around the heavy streaks of red pigment causes an orange color. The brown pigment in the eye is regularly distributed but is reduced to a rusty brown color. These two pigments give a contrast which together with the altered reflection of light causes an eye color that is as dark as sepia. The flecky color in *lz* seems to be associated with the heavy concentration of brown pigment and the irregular distribution of red in the eye although no area lacks red pigment all together. In lz^{BS} *v* the occasional fly with the wide, dark rim and colorless center of the eye apparently has a concentration of

red pigment through which little if any light can be reflected. The resulting color is that of muddy gumbo.

In part the colors of the eyes as well as facet irregularity seem to be secondary effects of a gene action which causes irregular cell differentiation in the ommatidia as shown by Waddington and Pilkington for lz^s. Another effect of the alleles, reduced fertility, is related to some extent to abnormal genitalia. The degree of effect of the lz^{37} gene on the genitalia is associated with a similar degree of effect on fertility (Anderson 1945). In lz^g, the abnormal genitalia are due to lack of proper cellular differentiation in the genital disc, and this is possibly true for the other alleles. There is thus a suggestion that these four effects are all related to one primary action of the gene, the control of cell differentiation. However, seriation of the alleles for their effects upon one trait is not always the same as that for another. Although the exceptions may be less extreme for color and facet arrangement, all of the alleles except lz^{37} are identical in that they cause total absence of the spermathecae and parovaria. Moreover, some of the alleles that are phenotypically identical do not interact in the same manner when in compounds with other alleles. These facts suggest that some of the alleles differ in their production of the effects and that the gene at this locus produces more than one physiologically independent effect.

SUMMARY

Ten lozenge alleles were studied for their effects upon facet irregularity and pigmentation of the eyes. The two pigments were studied separately by inserting *bw* to remove the red pigment and *st* and also *v* to remove the brown.

The alleles can be seriated in their effects on facet irregularities, ranging from almost normal to total absence of facets. Some duplicate alleles occur. For color of the eyes, one group of the alleles can be graded from normal up through increasing darkness to sepia and another series grades down to a dilute brown. Production of brown and of red pigment can be seriated from normal through decreasing quantity to almost total absence of pigment.

Attempts to fit the several seriations of effects show that in general they are in agreement but that striking exceptions occur. Some alleles which duplicate each other for one or more effects differ greatly for another.

It is suggested that in part the facet irregularities and the production and distribution of brown and red pigments are interrelated and that both are secondary effects of the genic control of the structure of ommatidia. It is also suggested that in some of the alleles the effects on the eyes are results of independent controls by the gene. Color of the eye is caused by quantity and distribution of pigment, but also by contrasts of the two pigments in which one intensifies the other.

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