VI. GENETIC RELATIONSHIPS IN THE *DROSOPHILA FUNEBRIS* GROUP

G. B. MAINLAND

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

GEOGRAPHIC DISTRIBUTION

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

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

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

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

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

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

Population Densities

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

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

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

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

Stocks Used in These Investigations

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

Macrospina Stocks

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

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

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

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

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

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

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

Limpiensis Stocks

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

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

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

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

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

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

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

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

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

Subfunebris Stock

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

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

Methods Employed

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

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

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

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

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

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

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

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

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

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

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

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

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

Results of Intraspecific Crosses

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

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

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

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

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

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

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

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

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

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

RESULTS OF INTERSPECIFIC CROSSES

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

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

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

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

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

---

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

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

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

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

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

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

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

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

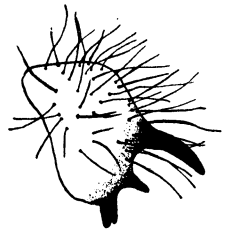
The F_1 interspecific hybrids were examined with regard to morphological differences and similarities to their parental strains. With the exception of *GS* males, the dark red eye color of *subfunebris* was dominant to the light red *macrospina* and *limpiensis*. The *GS* males had an eye color somewhat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

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

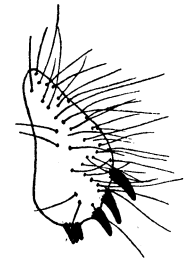
Generally the hybrid males resembled the males of their maternal stock more than those of their paternal. Although larger, males carrying a *subfunebris* X chromosome showed less sexual dimorphism than those carrying a *macrospina* (or *limpiensis*) X chromosome. It should be pointed out that *subfunebris*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

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

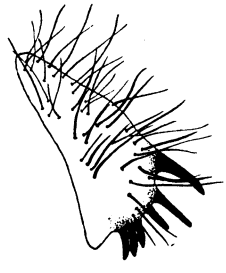
MALE ANAL PLATES



D. MACROSPINA LIMPIENSIS



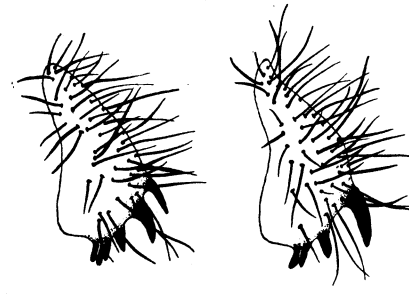
D. SUBFUNEBRIS



D. M. LIMPIENSIS ♂

X

D. SUBFUNEBRIS ♀



D. SUBFUNEBRIS ♂

X

D. M. MACROSPINA ♀

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RELATIONSHIPS IN THE DROSOPHILA FUNEBRIS GROUP

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

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

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

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

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

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

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

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

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

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

Funebris

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

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

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

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

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

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

TAXONOMY BASED ON A GENETIC SCALE

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

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

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

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

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

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

Tentatively this author proposes to define a species as follows:

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

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

SUMMARY

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

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

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

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

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

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

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

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

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

BIBLIOGRAPHY

Crow, J. F., 1941. Studies in *Drosophila* speciation. I. The *Drosophila mulleri* group. Gen., 26:146.

————, 1942. This Bulletin.

Diederich, G. W., 1941. Non random mating between yellow-white and wild type *Drosophila melanogaster*. Gen., 26:148.

Dobzhansky, Th., 1935. A critique of the species concept in biology. Phil. of Sci., 2:344–355.

————, 1936. Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. Gen., 21:113–135.

————, 1937. Further data on *Drosophila miranda* and its hybrids with *Drosophila pseudoobscura*. J. Gen., 34:135–151.

————, 1941. Genetics and the origin of species. Rev. Ed. New York: Columbia Univ. Press, pp. 466.

Dobzhansky, Th., and P. C. Koller, 1938. An experimental study of sexual isolation in *Drosophila*. Biol. Zentral., 58:589–607.

Emerson, H. E., 1935. Thermitophile distribution and quantitative characters as indicators of physiological speciation in British Guiana termites (Isoptera). Ann. Entom. Soc. America, 28:369–395.

Griffen, A. B., 1941. Studies in *Drosophila* speciation. II. The *Drosophila melanica* group. Gen., 26:154.

————, 1942. This bulletin.

Haldane, J. B. S., 1922. Sex ratio and unisexual sterility in hybrid animals. J. Gen., 12:101–109.

Kinsey, A. C., 1930. The gall wasp, genus *Cynips*. A study of the origin of species. Indiana Univ. Studies XVI, pp. 577. (Definition of species, p. 20.)

————, 1937. Supra-specific variation in nature and its classification. A. N., 71:206–222.

Lancefield, D. E., 1929. A genetic study of two races or physiological species in *Drosophila obscura*. Zeit. ind. Abst. Vereb., 52:287–317.

Lotsy, J. P., 1931. On the species of the taxonomist in its relation to evolution. Genetica, 13:1–16.

MacKnight, R. H., 1939. The sex determining mechanism of *Drosophila miranda*. Gen., 24:180–201.

Mainland, G. B., 1941. Studies in *Drosophila* speciation. III. The *Drosophila macrospina* group. Gen., 26:160–161.

————, 1942. The *Drosophila macrospina* group. Gen., 27:155.

Mayr, Ernst, 1940. Speciation phenomena in birds. Amer. Nat., 74:249–278. (Reprinted, 1941, Biol. Symp., 2:59–88.)

Miller, D. D., 1939. Structure and variation of the chromosomes in *Drosophila algonquin*. Gen., 24:699–708.

———, 1941. Interspecific hybrids involving *Drosophila athabasca*. Gen., 26:161.

Muller, H. J., and G. Pontecorvo, 1940a. Recombinants between *Drosophila* species the F_1 hybrids of which are sterile. Nature, 146:199–200.

———, 1940b. The artificial mixing of incompatible germ plasms in *Drosophila*. Science, 92:418.

———, 1942. Recessive genes causing interspecific sterility and other disharmonies between *Drosophila melanogaster* and *simulans*. Gen., 27:157.

Patterson, J. T., 1941. The *virilis* group of *Drosophila* in Texas. Amer. Nat., 75:523–539.

———, 1942a. *Drosophila* and speciation. Science, 95:153–159.

———, 1942b. Isolating mechanisms in the genus *Drosophila*. Biol. Symp., 6:271–287.

Patterson, J. T., and J. F. Crow, 1940. Hybridization in the *mulleri* group of *Drosophila*. Univ. Texas Publ., 4032:251–256.

Patterson, J. T., W. S. Stone, and A. B. Griffen, 1940. Evolution of the *virilis* group in *Drosophila*. Univ. Texas Publ., 4032:218–250.

Patterson, J. T., and M. R. Wheeler, 1942. Description of new species of the subgenera *Hirtodrosophila* and *Drosophila*. Univ. Texas Publ., 4213:70–109.

Spencer, W. P., 1940a. Subspecies, hybrids and speciation in *Drosophila hydei* and *Drosophila virilis*. Amer. Nat., 74:157–179.

———, 1940b. Levels of divergence in *Drosophila* speciation. Amer. Nat., 74:299–311. (Reprinted 1941, Biol. Symp., 2:99–111.)

———, 1942. New species in the *quinaria* group of the subgenus *Drosophila*. Univ. Texas Publ., 4213:55–66.

Spett, G., 1931. Gibt es eine partielle sexuelle Isolation unter den Mutationen und der Grundform von *Drosophila melanogaster?* Zeit. ind. Abst. Vereb., 60:63–83.

Stalker, H. D., 1941. Sexual isolation in the *virilis* complex of *Drosophila*. Gen., 26:170.

———, 1942. Sexual isolation studies in the species complex *Drosophila virilis*. Gen., 27:238–257.

Stalker, H. D., and W. P. Spencer, 1939. Four new species of *Drosophila*, with notes on the *funebris* group. Ann. Ent. Soc. Amer., 32:105–113.

Standfuss, M., 1896. Handbuch der palaarktischen Grossschmetterlinge fur Forscher und Sammler. G. Fischer, Jena.

Sturtevant, A. H., 1921. The North American species of *Drosophila*. Carnegie Inst. Washington, Publ., 301:1–150.

———, 1942. The classification of the genus *Drosophila*, with descriptions of nine new species. Univ. Texas Publ., 4213:27–51.

Sturtevant, A. H., and Th. Dobzhansky, 1936. Observations on the species related to new forms of *Drosophila affinis*, with descriptions of seven. Amer. Nat., 70:574–584.

Sturtevant, A. H., and E. Novitski, 1941. Sterility in crosses of geographical races of *Drosophila micromelanica*. Proc. Nat. Acad. Sci., 27:392–394.

Thorpe, W. H., 1940. Ecology and the future of systematics. Huxley's New Systematics, pp. 341–364.

Timofeff-Ressovsky, N. W., 1940. Mutations and geographical variation. The New Systematics (edited by J. S. Huxley), pp. 73–136. London: Oxford Univ. Press.

Wharton, L. T., 1942. This bulletin.

Wright, S., 1932. The roles of mutation, inbreeding, crossbreeding, and selection in evolution. Proc. Sixth Inter. Gen. Cong., 1:356–366.

———, 1937. The distribution of gene frequences in populations. Proc. Nat. Acad. Sci., 26:307–313.

———, 1940. Breeding structure of populations in relation to speciation. Amer. Nat., 74:232–248.

APPENDIX

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

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

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

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

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

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

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

TABLE 1.

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

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

TABLE 2.

Fertility of Intraspecific *macrospina* Crosses

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

TABLE 2—(Continued)

Fertility of Intraspecific *macrospina* Crosses

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

TABLE 3.

Fertility of Intraspecific F_1 Hybrids Tested by Inbreeding

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

TABLE 3—(Continued)

Fertility of Intraspecific F_1 Hybrids Tested by Inbreeding

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

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

TABLE 4.

Fertility of Intraspecific F_1 Male Tested by Backcrossing.

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

*Data from small mass matings.

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

TABLE 5.

Fertility of Intraspecific F_1 Female Hybrids Tested by Backcrosses.

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

*Data from small mass matings.

TABLE 6.

Fertility of First Backcross Progenies.

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

TABLE 7.

Cross-fertility in Interpecific Matings.

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

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

TABLE 8.

Fertility Interspecific F_1 Hybrids Tested by Inbreeding

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

TABLE 9.

Fertility of Interspecific F_1 Hybrid Males Tested by Backcrossing

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

TABLE 10.

Fertility of Interspecific F_1 Hybrid Females Tested by Backcrossing.

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

TABLE 11

Fertility of First Backcross Female Progenies of Interspecific Hybrids

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

TABLE 12

Fertility of First Backcross Male Progenies of Interspecific Hybrids

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

TABLE 13

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

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