# SEXUAL ISOLATION STUDIES IN THE SPECIES COMPLEX DROSOPHILA VIRILIS[1]

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Received August 15, 1941

## INTRODUCTION

SEXUAL isolation is a phenomenon frequently observed in the genus Drosophila. STURTEVANT (1921) reports that *D. melanogaster* males, while able to hybridize with *D. simulans* females, are more apt to mate with the females of their own species. LANCEFIELD (1929) found similar results in races A and B of *D. pseudoobscura*. DOBZHANSKY and KOLLER (1938) report cases of sexual isolation between *D. pseudoobscura* races A and B, *D. pseudoobscura* and *D. miranda*, and *D. azteca* and *D. athabasca*. MILLER (1939, 1941) finds that *D. athabasca* will form hybrids with both *D. affinis* and *D. algonquin*. His unpublished data suggest that sexual isolation exists between *D. athabasca* and both the other two species. Similar observations have also been made with forms of the *virilis* complex.

This complex at present includes three subspecies, or as some workers prefer to consider them, species. They are: *Drosophila virilis virilis* Sturtevant (STURTEVANT 1916), *Drosophila virilis americana* Spencer (SPENCER 1938), and *Drosophila virilis texana* Patterson (PATTERSON unpublished manuscript).

In order to study the sexual isolation in *virilis* and *americana*, SPENCER (1938) placed males with equal numbers of females of their own and the other subspecies and found that in both cases the males inseminated more of their own females than those of the other subspecies. He also found that the *americana* males more frequently inseminated *virilis* females than did the males in the reciprocal cross. PATTERSON, STONE, and GRIFFEN (1940b) made up series of single pair matings within and between the two subspecies. Their results showed that the cross-matings in which *virilis* was the female were more often successful than the reciprocal matings, thus corroborating SPENCER'S results. Here, however, in most cases the success of the mating was not measured directly, but by the offspring obtained, so that many factors besides sexual isolation were involved.

The results of SPENCER and PATTERSON, STONE and GRIFFEN suggest the existence of sexual isolation in the *virilis* complex similar to that found by DOBZHANSKY and KOLLER in *D. miranda* and *D. pseudoobscura*. In order to get more critical data on sexual isolation between *virilis* and *americana*, and in order to ascertain whether there is any sexual isolation among

[1] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the UNIVERSITY OF ROCHESTER.

the different strains of *americana*, further experiments were carried out. It is the purpose of this paper to report these experiments.

The author wishes to express his appreciation to DR. CURT STERN for his helpful advice and guidance throughout the problem. Appreciation is also due to DR. W. P. SPENCER for his friendly suggestions and unfailing cooperation in supplying many of the *americana* strains used. To DR. DONALD CHARLES the author is grateful for his criticism and help in regard to the mathematical treatment of the data.

Acknowledgement is due to MARION L. STALKER and MR. RAY MAAS for their assistance in the preparation of the figure.

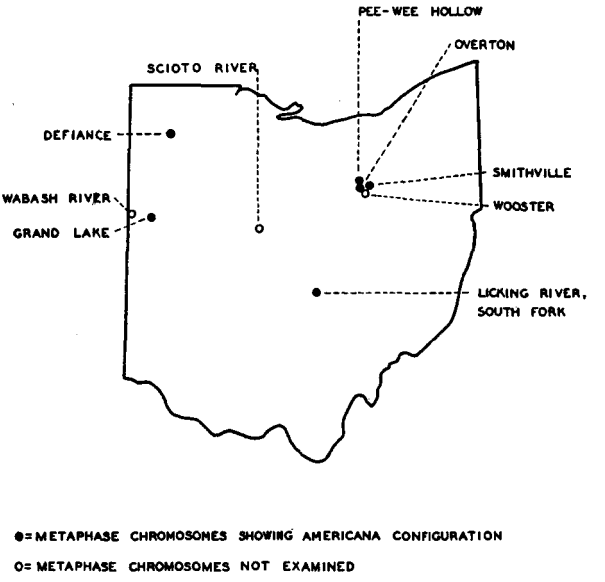


FIGURE 1. Localities in Ohio where *D. virilis americana* has been collected.

## STRAINS OF VIRILIS AND AMERICANA USED

The wild strains of *virilis* and *americana* used are listed below, together with the sources from which they were obtained. Figure 1 indicates the localities in Ohio from which the *americana* strains were collected.

*D. virilis virilis* Sturtevant. This is the so-called Pasadena strain of *D. virilis*. It originated from a single pair which were bred from fruit exposed by DR. A. H. STURTEVANT at COLUMBIA UNIVERSITY in 1913. DR. STURTEVANT believes that it may have been crossed with some other *virilis* strain some time in the past. This strain will be referred to as Pasadena *virilis*, or simply as V.

*D. virilis americana* Spencer, from Smithville, Ohio. This is the type strain of *americana*. It is descended from a single fertilized female taken by

DR. W. P. SPENCER in the vicinity of Smithville, Ohio, June 25, 1936. This is one of the strains used in the early work on the subspecies (SPENCER 1938, 1940; HUGHES 1939; STALKER 1940; PATTERSON, STONE, and GRIFFEN 1940b). It will be referred to as Smithville *americana*, or As.

*D. virilis americana* Spencer from Overton, Ohio. This strain originated from a single fertilized female collected at Overton, Ohio, July 12, 1939. It will be referred to as Ao.

*D. virilis americana* Spencer, from Independence State Park, Defiance, Ohio. This strain is descended from a single fertilized female collected July 4, 1940. (The configuration of the metaphase chromosomes was determined through the kindness of DR. D. D. MILLER). It will be referred to as Ad.

*D. virilis americana* Spencer, from Licking River, south fork, near Buckeye Lake, Ohio. This strain originated from a single fertilized female collected June 28, 1940. It will be referred to as Al.

*Americana* is one of the rarest of the *Drosophila* species collected in Ohio. At many collecting stations it has not been taken at all, even after daily collections extending over a month or more. In a short collecting trip made during the summer of 1940 only 17 specimens of *americana* were taken among 15,171 specimens of other *Drosophila* species. Thus *americana* formed little more than one-tenth of one percent of the population which came to the traps. Little is known concerning the food of Drosophila in nature. However, judging from the rapidity with which *americana* comes to traps containing fermenting food in the laboratory, its rarity in such traps in nature probably indicates relatively small populations rather than a predilection for other food.

*Americana* has also been collected in Texas, and specimens which appear to be *americana*, but which have not been cytologically examined, are recorded from Tennessee.

SPENCER (1940) reports that *virilis* has been taken three times in the United States: in New York City; Terre Haute, Indiana; and Los Angeles, California. PATTERSON, STONE, and GRIFFEN (1940b) report this species from Texas and Louisiana. KIKKAWA and PENG (1938) report that it is common in Asia.

The collection records of PATTERSON, STONE, and GRIFFEN (1940b) give an average of one *virilis* in 20,000 specimens of the other *Drosophila* species, and their figures for the "red group," which contains both *texana* and *americana*, is one in 100,000. Since *americana* is apparently much rarer in Texas than is *texana*, this would indicate that *americana* is present in much smaller numbers in Texas than in Ohio.

SPENCER (1938, 1940) has listed many of the morphological and physiological characteristics by which *americana* and *virilis* differ. Table 1 gives the differences mentioned by SPENCER together with some additional ones discovered by the author which are marked by asterisks.

TABLE 1

*Morphological and Physiological Differences between virilis and americana.*

| | VIRILIS | AMERICANA |
|---|---|---|
| | **Morphological Differences** | |
| | 1. abdomen squattier | abdomen more fusiform |
| | 2. body color lighter | body color darker |
| | 3. eye smaller and lighter in color, eye pile coarse | eye larger and darker colored, very fine pile |
| | 4. cheek wider | cheek narrower |
| | 5. carina narrower | carina wider |
| | 6. narrow cloud on post. cross-vein of wing | broad, lens-shaped cloud on post. cross-vein of wing |
| | 7. fewer branches on arista | more branches on arista |
| * | 8. testis red | testis orange-red |
| * | 9. spermatheca longer and more rounded at tip | spermatheca shorter and squarer at tip |
| * | 10. ventral sperm receptacle longer and thicker | ventral sperm receptacle shorter and thinner |
| | 11. viscera tough, not easily ruptured | viscera rupture easily in dissection |
| | 12. pupa case gray or black | pupa case reddish-brown |
| | **Physiological Differences** | |
| | 1. etherizes slowly, recovers slowly | etherizes quickly, recovers quickly |
| | 2. undisturbed adults occupy isolated positions in culture bottle | undisturbed adults form closely grouped aggregates |
| * | 3. young adults more active when disturbed | young adults more sluggish when disturbed |
| | 4. puparia tend to form on sides of bottle | puparia tend to form in or on food |
| * | 5. females begin to lay eggs three to six days after eclosion | females begin to lay eggs four to nine days after eclosion |
| * | 6. period from fertilization of egg to eclosion shorter | period from fertilization of egg to eclosion longer |

The chromosome configurations of *virilis* and *americana* have been investigated cytologically by HUGHES (1939), PATTERSON, STONE, and GRIFFEN (1940b) and genetically by STALKER (1940). These workers have shown that while both sexes of *virilis* have five pairs of rod-shaped chromosomes and a pair of dot-like microchromosomes, *americana* females have one pair of rods, two pairs of V-shaped chromosomes, and a pair of microchromosomes. *Americana* males have a pair of rods, a pair of V-shaped chromosomes, a pair of microchromosomes, and one V-shaped chromosome typically showing somatic pairing with two rod-shaped chromosomes. The pair of V-shaped chromosomes present in both sexes are autosomes, corresponding to chromosomes 2 and 3 of *virilis*. The V-shaped chromosome present once in the male and twice in the female corresponds to chromosomes X and 4 of *virilis*. The rod-shaped chromosomes pairing with this V-shaped chromosome in the *americana* male correspond to chromosomes Y and 4 of *virilis*. *Virilis* chromosome 5 corresponds to the rod-shaped chromosomes found paired in both sexes of *americana*.

## METHODS

Two types of investigations were made. The first consisted of measurements of the sexual isolation as determined by the insemination frequencies. The second type consisted of a number of observations of matings between and within the two subspecies. These observations were made with the purpose of breaking down the phenomenon of sexual isolation into some of its component parts.

All flies used in these experiments were raised on cornmeal-molasses-Moldex food medium, made up with one-half the usual amount of molasses. It was found that while this decrease in molasses did not appreciably increase the length of the life cycle, it helped in keeping the cultures from becoming infected with bacteria, etc. This factor is more important in *virilis* and *americana* than in species such as *melanogaster*, since the former species require several days ageing before the adults lay fertilized eggs.

The flies to be tested were raised at $25 \pm 0.75$°C. When the adults in any one culture bottle began to emerge, the bottle was removed from the incubator and kept at room temperature until discarded. The room temperature averaged from one to four degrees lower than that of the incubator, and since adults age more slowly at a low temperature than they do at 25°C, the individuals emerging over a 24 hour period and ageing at room temperature were more nearly of the same physiological age than they would have been had they aged at 25°C.

Adults were collected every day between 10 and 12 a.m., just following the period of the day when most individuals seemed to emerge. Flies collected at this time were for convenience considered as 0 days old, although they were actually from 0 to 24 hours old.

Newly emerged flies were sorted for sex and aged at $25 \pm 0.3$°C. for a number of days. After ageing, the males and females were placed together without etherization in a culture bottle with fresh, heavily yeasted food. This culture bottle was kept at the same temperature for 24 hours, after which the females were dissected and the sperm receptacles examined for sperm. When it was inconvenient to dissect the females at the end of the 24 hour period, the bottles containing the males and females were placed in an electric refrigerator which ran at about 7°C, and kept there until the dissections could be carried out. Tests showed that neither *virilis* nor *americana* males inseminated their females at this temperature.

Dissections of females were carried out on a flat slide in physiological salt solution, and the sperm receptacles were examined under a compound microscope. It was found that contrary to DOBZHANSKY and KOLLER'S (1938) experience with *D. pseudoobscura* and *D. miranda*, inseminated *virilis and americana* females often contained sperm in one or both spermathecae, even when the ventral receptacles were empty. Thus a female was not considered uninseminated unless both spermathecae and ventral re-

ceptacle were seen to contain no sperm. As in *D. pseudoobscura* and *D. miranda*, the ventral receptacles of both *virilis* and *americana* virgin females contained long thread-like bodies which had a superficial resemblance to sperm, but which could be easily recognized with practice.

*Virilis* and *americana* arrive at sexual maturity sooner if aged under optimum food conditions than if partially starved during the ageing period. For this reason special care was taken to supply plenty of fresh food to maturing adults. In all cases where the ageing period extended for over three days, the culture bottles were changed every third day. Since the bottles were always heavily yeasted, such frequent changes did not entail any yeast starvation.

Sexual maturity of Drosophila males may be considered as that stage in their development in which they are able to inseminate females of their own species. However females of some Drosophila species pass through two distinct stages in their adult development. In the first stage they will mate, and can be inseminated, but are unable to lay any eggs due to the fact that their ovaries are undeveloped. In the second stage they can both mate and oviposit. In *virilis* and *americana* females these two stages are quite distinct. The term "sexual maturity" as used in this paper will indicate the first of these two stages of female development.

In order to determine the age at which the different strains used became sexually mature, equal numbers of males and females were aged separately for various periods, from one to six days, placed together for 24 hours, and the insemination frequencies of the females determined. In these experiments the males and females were always of the same age. Table 2 gives the results obtained.

TABLE 2

*Insemination frequencies of females of different ages, exposed to males of their own strain and age for 24 hours. The numbers in parentheses give the number of females examined in each case.*

| STRAIN | AGE IN DAYS AT TIME OF MATING | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| V | 0% (75) | 0% (102) | 1.4% (145) | 89.5% (293) | 97.8% (355) | 100% (101) | |
| As | 0% (56) | 0% (83) | 1.7% (116) | 93.7% (300) | 93.8% (397) | | 100% (55) |
| Ao | | 0% (47) | 1.8% (57) | 81.8% (77) | 97.6% (281) | 98.5% (65) | |
| Ad | 0% (20) | 0% (46) | 11.9% (84) | 85.0% (139) | 96.8% (282) | 94.9% (102) | |
| Al | | 0% (127) | 6.6% (151) | 72.1% (197) | 95.0% (322) | 95.8% (119) | |

From the table it can be seen that there was no insemination in any strain up to the third day, with some insemination following the third day, and a good deal following the fourth. Since there was little increase in the percentage of females inseminated following the fifth day, five-day-old males and females were used in all subsequent sexual isolation experiments.

## MATINGS WITHOUT CHOICE OF FEMALES

These experiments involved matings within the various strains of *virilis* and *americana*, between *virilis* and *americana*, and between different strains of *americana*.

TABLE 3

*Insemination frequencies in matings within and between different strains of virilis and americana. Ten each of five-day-old males and females were placed in each bottle.*

| ♀ ♀ | ♂ ♂ | FEMALES INSEMINATED | FEMALES NOT INSEMINATED | PERCENT INSEMINATED | ISOLATION |
|---|---|---|---|---|---|
| V | V | 347 | 8 | 97.7 | |
| As | As | 372 | 25 | 93.7 | |
| Ao | Ao | 274 | 7 | 97.5 | |
| Ad | Ad | 273 | 9 | 96.8 | |
| Al | Al | 306 | 16 | 95.0 | |
| V | As | 258 | 89 | 74.4 | † |
| V | Ao | 320 | 36 | 89.9 | † |
| V | Ad | 358 | 21 | 94.5 | † |
| V | Al | 282 | 52 | 84.4 | † |
| As | V | 8 | 316 | 2.5 | † |
| Ao | V | 44 | 315 | 12.3 | † |
| Ad | V | 40 | 334 | 10.7 | † |
| Al | V | 51 | 261 | 16.3 | † |
| As | Ao | 257 | 26 | 90.8 | * |
| As | Ad | 255 | 15 | 94.4 | |
| Ao | As | 290 | 20 | 93.5 | * |
| Ao | Ad | 297 | 20 | 93.7 | * |
| Ad | As | 277 | 18 | 93.9 | |
| Ad | Ao | 294 | 20 | 93.6 | * |

* Insemination frequency significantly lower than that of *one* of the intra-strain matings.
† Insemination frequency significantly lower than that of *both* of the intra-strain matings.

Males were placed with only one type of female and thus were unable to exercise any choice in mating. Bottles were made up of ten five-day-old males and ten five-day-old females of their own or another strain. At the end of 24 hours the females were dissected and examined for sperm. The insemination frequencies obtained are shown in table 3.

In experiments of this type, isolation between any two strains is shown only to the extent that the insemination frequency of a mating between them is lower than frequencies obtained in matings within each of them.

For example, table 3 shows that the insemination frequency for the mating between V females and As males is 74.4 percent. Before a comparison can be made between this figure and the frequencies of the inseminations within V and As, tests must be made to show that all three matings, V×V, As×As, and V×As, possess sufficient internal homogeneity to be treated as units. As an example of how these tests were made, let us consider the mating V×V. Here the distribution of inseminated females in all the bottles making up the mating was compared with the distribution that would be expected on a purely chance basis. The $\chi^2$ test was applied to the observed and expected distributions, and the P value obtained in the case of V×V was .28, indicating that no significant difference existed between observed and expected distributions. Thus the mating V×V may be considered sufficiently homogeneous to be treated as a unit, and the $\chi^2$ test may now be used to compare it with other matings. None of the P values obtained in the homogeneity tests was lower than .14, thus indicating that the $\chi^2$ test may be used to measure differences between the matings shown in table 3. A P value of .05 or less was considered to indicate a real difference between any two matings.

The last column of table 3 indicates whether a given mating shows an insemination frequency lower than that obtained in one, (*); in both, (†), or in neither of the matings within the two strains involved. Only the matings marked (†) clearly indicate sexual isolation.

With these criteria of sexual isolation in mind, let us consider the data summarized in table 3. All matings between *virilis* and *americana* show sexual isolation. Also in all four *americana* strains tested, the matings with *virilis* in which *americana* was used as females showed much more complete isolation than the reciprocals. This is in agreement with the findings of SPENCER (1940).

The degree of sexual isolation shown in any of the interspecific matings depends on the *americana* strain used. Considering first those matings between *virilis* females and *americana* males, we see that the insemination frequencies range from 74.4 percent to 94.5 percent. Since none of the matings within any of the *americana* strains differ significantly from each other (with the exception of As×As and Ao×Ao, whose difference gives a P value of .035), we may conclude that the four *americana* strains have about the same inherent insemination ability. Thus significant differences shown in the V×A series of matings are probably not due to differences in the inherent insemination ability of the A strains, but actually represent differences in sexual isolation.

By applying the $\chi^2$ test, it is found that each of the four V×A matings shows significant differences from all the others. In the reciprocal A×V series, there are fewer significant differences shown, and in two cases,

Al×V and Ao×V, and Ao×V and Ad×V, no significant difference in the insemination frequencies is found.

The lower part of table 3 shows the insemination frequencies obtained in matings among the different A strains. As the asterisks indicate, there were no clear cases of sexual isolation in intra-specific matings of this type.

A comparison of the V×A matings with the reciprocal series indicates that strains showing strong isolation in one cross may be relatively incompletely isolated in the reciprocal one. For example, on the basis of the cross V×Al, Al would be considered as showing relatively complete isolation, but in the reciprocal cross, Al×V, Al shows less isolation than any of the other three A strains.

DOBZHANSKY and KOLLER (1938) obtained similar results in matings between *D. pseudoobscura* and *D. miranda*. These workers found that although as a general rule matings between *D. miranda* females and *D. pseudoobscura* males gave the highest insemination frequencies, the outcome of one cross could not be predicted by a knowledge of the reciprocal one.

A comparison of the results obtained here with the geographical origin of the strains tested (see fig. 1) shows that while strains from different localities behave differently in crosses to *virilis*, a knowledge of the behavior of one strain does not allow one to predict the behavior of a strain from the same or an adjacent locality. For instance, As and Ao come from collecting stations eight miles apart, yet they differ more when crossed to V than do Ao and Ad, which come from stations separated by 134 miles.

## MATING WITH CHOICE OF FEMALES

Where the sexual isolation between any two strains of Drosophila is relatively incomplete,—that is, when mating can take place between them almost as readily as within either of them,—then this very slight isolation may perhaps be most readily detected by experiments in which the males are given a choice of two types of females, one type being of their own strain, the other of an alien strain.

The above work involving matings in which the males were given no choice has shown that there exist many such cases of very slight isolation in matings within the *virilis* complex. In the experiments to be reported below, culture bottles were made up with two five-day-old males, ten five-day-old females of their own strain, and ten of an alien strain. The females were dissected and examined for sperm after a 24-hour exposure. The males were aged 20 in a bottle, the food being changed the third day. They were lightly etherized preliminary to being placed with the females. The females of the two strains, which were distinguished by small nicks on the costal vein of the right or left wing, were aged together under the same conditions as the males. Both sexes were kept at $25 \pm .3$°C throughout the age-

ing and mating periods. Since in these experiments the experimental (alien) and control (conspecific) females were aged together in the same bottle, the environmental conditions during the ageing and mating periods should not affect the degree of isolation observed.

In order to express more easily the degree of sexual isolation, an Isolation Index, suggested by DR. D. CHARLES, was calculated. This Isolation Index consists of the percentage of conspecific females inseminated minus the percentage of alien females inseminated, this number being divided by the percentage of conspecific females inseminated plus the percentage of alien females inseminated.

$$\text{Isolation Index} = \frac{\%\ \text{conspecific}\ ♀♀\ \text{insem.} - \%\ \text{alien}\ ♀♀\ \text{insem.}}{\%\ \text{conspecific}\ ♀♀\ \text{insem.} + \%\ \text{alien}\ ♀♀\ \text{insem}}.$$

If the males inseminate equal percentages of their own and the alien females, the index will be equal to zero, denoting no sexual isolation. If they inseminate only their own females, the index will be equal to +1, denoting complete sexual isolation. If, on the other hand, the males inseminated more of the alien females than the conspecific ones, the index would have a negative value.

Table 4 summarizes the results of matings between *virilis* and various strains of *americana*.

TABLE 4

*Frequencies of intraspecific and interspecific inseminations in mixed cultures of virilis and americana.*

| ♂♂ | ♀♀ | % OWN ♀♀ INSEMINATED | % ALIEN ♀♀ INSEMINATED | $\chi^2$ OF THE DIFFERENCE | P | ISOLATION INDEX* | N† |
|---|---|---|---|---|---|---|---|
| V | V & As | 87.2 | 0 | 402.78 | <.01 | 1.00 | 523 |
| V | V & Ao | 80.6 | 1.6 | 322.65 | <.01 | .96 | 518 |
| V | V & Ad | 63.6 | 19.2 | 144.44 | <.01 | .62 | 508 |
| V | V & Al | 90.0 | 1.2 | 411.59 | <.01 | .97 | 521 |
| As | As & V | 75.8 | 16.0 | 183.87 | <.01 | .65 | 510 |
| Ao | Ao & V | 75.1 | 16.9 | 178.03 | <.01 | .63 | 523 |
| Ad | Ad & V | 73.1 | 36.5 | 69.51 | <.01 | .33 | 515 |
| Al | Al & V | 64.1 | 39.5 | 32.12 | <.01 | .24 | 528 |

* See text.

† Total number of females examined. The alien and conspecific females were present in approximately equal numbers. Accidental deaths during the course of the experiment caused inequality in some bottles.

The $\chi^2$ values in tables 4 and 5 were obtained by use of a formula derived from the four-fold table by DR. D. CHARLES.[1]

[1] $\chi^2 = NC^2N_F/N_U(4N_SN_D)/[N+C(N_S-N_D)]^2$. Where N = total number of females, both conspecific and alien. $N_F$ = total number of conspecific and alien females inseminated. $N_U$ = total number of conspecific and alien females not inseminated. $N_S$ = total number of conspecific females. $N_D$ = total number of alien females. C = isolation index.

From the data in table 4 it can be seen that there is in every cross distinct and significant sexual isolation between *virilis* and the *americana* strain involved.

DOBZHANSKY and KOLLER (1938) carried out similar experiments and found that in matings between *D. miranda* and *D. pseudoobscura*, in which the males were given a choice of both types of females, they began to fertilize the alien females after most of the conspecific females were fertilized. They explain their results as follows: "Two interpretations of the above results seem about equally plausible. First, the males may copulate with females of their own species so long as any of the latter remain unfertilized, and may turn toward females of the foreign species only after the supply of conspecific virgins is exhausted. The occasional females that are unfertilized by males of their own species even after two weeks' exposure may be those that have already used up the supply of sperm received during the first copulation, and have not yet remated. Second, our results may be described in terms of unequal probabilities of fertilization. Suppose that the probability of copulation taking place when males of the species A meet females of their own species equals a, and when they meet females of the species B equals b, $(a > b)$. This being granted, the number of homogamic as well as heterogamic matings must increase as time goes on; some females of either species may remain unfertilized and others may be impregnated once, twice, three, and more times. Our data are not critical for a discrimination between the above alternatives."

The first of these alternatives—namely, the males inseminating alien females after the supply of conspecific virgins is used up—may apply to the matings reported here. If this is true not only for matings between, but within the two subspecies, then the measurements of sexual isolation obtained would vary with the inseminating ability of the males used. For example some males might inseminate not only all of their own, but likewise all of the alien females, in which case there would be no isolation shown. On the other hand, other males with less fertility, might inseminate only some of the conspecific and none of the alien females. In this case the isolation shown would be complete. Although no cases of the former type, where all 20 females were fertilized, were found, even an approach to this condition might have considerable effect on the results obtained. It is obvious that although in bottles where all or most of the females were inseminated the sexual isolation may be hidden, in no case will there be an apparent isolation shown where none actually exists. With these facts in mind, we may make comparisons between the different crosses reported in table 4, although all such comparisons must obviously be made with some reservations.

In all crosses between the two subspecies, those in which the male was *virilis* show a much more complete isolation than the reciprocal ones. This is in agreement with the results obtained in the crosses involving no choice of mate.

In these matings, as in those above, knowledge of the insemination frequency in any one cross does not allow one to predict the results of the reciprocal cross.

### MATINGS BETWEEN DIFFERENT AMERICANA STRAINS

Table 5 summarizes the results of the experiments similar to those reported above, but involving only *americana* strains.

TABLE 5

*Frequency of insemination in mixed cultures of americana, containing two types of females, one type from the same strain as the males, the other from an alien strain.*

| ♂♂ | ♀♀ | % OWN ♀♀ INSEMINATED | % ALIEN ♀♀ INSEMINATED | $\chi^2$ OF THE DIFFERENCE | P | ISOLATION INDEX* | N† |
|---|---|---|---|---|---|---|---|
| As | As & Ao | 70.2 | 67.5 | .44 | .51 | .02 | 507 |
| As | As & Ad | 68.5 | 51.0 | 16.36 | <.01 | .15 | 509 |
| As | As & Al | 60.7 | 57.1 | .71 | .44 | .03 | 551 |
| Ao | Ao & As | 64.7 | 49.8 | 11.63 | <.01 | .13 | 514 |
| Ao | Ao & Ad | 67.9 | 46.3 | 24.03 | <.01 | .19 | 547 |
| Ao | Ao & Al | 65.2 | 49.1 | 5.32 | .02 | .14 | 534 |
| Ad | Ad & As | 60.4 | 47.5 | 16.91 | <.01 | .17 | 477 |
| Ad | Ad & Ao | 55.3 | 51.0 | .88 | .36 | .04 | 489 |
| Ad | Ad & Al | 62.6 | 57.3 | 1.58 | .21 | .04 | 538 |
| Al | Al & As | 72.0 | 59.6 | 9.27 | <.01 | .09 | 548 |
| Al | Al & Ao | 66.1 | 63.8 | .29 | .61 | .02 | 522 |
| Al | Al & Ad | 60.3 | 52.4 | 4.10 | .06 | .07 | 552 |

* See text.

† Total number of females examined. The alien and conspecific females were present in approximately equal numbers. Accidental deaths during the course of the experiment caused inequality in some bottles.

In these matings the isolation indices are in no cases so large as those in the matings between the two subspecies. However, there appears to be significant isolation in six of the 12 matings, and with one exception every strain shows significant isolation from every other strain in at least one of the two reciprocal crosses. In the case of the exception, Al and Ao, in one of the reciprocal crosses (Al♂♂ × Al♀♀ & Ao♀♀), the P value is .06, which is on the borderline of significance. DOBZHANSKY and KOLLER (1938) report similar intraspecific sexual isolation in geographically separated strains of *D. miranda*.

It will be recalled that sexual isolation was not found in matings between *americana* strains in which the males were given no choice of mates. This indicates that where only slight isolations exist between two strains, the males show a preference for their own females if a choice is possible, but if they are given no choice, will mate with the alien females almost as readily as with their own.

## MATING BEHAVIOR IN THE VIRILIS COMPLEX

In an attempt to gain some insight into the factors hindering insemination in crosses between *virilis* and *americana*, a series of observations was made on the copulations between and within the two subspecies. The Pasadena strain of *virilis* (V) and the Smithville strain of *americana* (As) were used throughout. Adult *americana* and *virilis* raised under comparable conditions are approximately the same size, and since individuals used came from well fed larvae, there was little if any size difference between the members of the two strains used.

Observations were made on single pairs, which had been aged for a number of days, on fresh food, heavily yeasted. The flies were etherized lightly, placed singly in creamers with fresh food for 24 hours; then each was transferred without etherization into a creamer with an individual of the opposite sex. The creamers were placed on a white sheet of paper, under a bright electric light, and kept at about 25°C throughout the period of observation. A hand lens was used in the course of the observations, which were made one at a time.

### MATINGS OF VIRILIS FEMALES × VIRILIS MALES

Copulations were observed in 18 pairs of six-day-old flies. The typical mating behavior was as follows:

As soon as the male noticed the female he usually began to court vigorously. This courting consisted of licking her abdomen, especially the ovipositor, with his proboscis, and rubbing her abdomen with his fore-tarsi. At the same time he would frequently give quick sidewise flicks with one wing.

If the female ran away, the male was likely to lose her temporarily. Occasionally she would stand still, but would kick at the male with her hind legs. The male seemed to parry these kicks with his fore-tarsi. The female often gave flicks with her wings similar to those of the male, even while kicking. Finally, if the courtship was successful, the female would stand still, stop kicking, and spread her wings. The male then mounted almost immediately, and copulation occurred.

The average length of the intensive courting immediately preceding copulation was 9.3 seconds. The average length of copulation was two

minutes and 12 seconds, the shortest time being one minute and 15 seconds, the longest two minutes and 45 seconds. The range of duration of copulation is shown in table 6.

In 16 out of the 18 cases, mounting preceded copulation; in two cases copulation took place just before, or at the same time as mounting. STURTEVANT (1921) points out that in the genus Drosophila, copulation precedes mounting. Both subspecies of *virilis* studied seem to be clear exceptions to this rule. In the present writer's material no scissors movements of the wings was observed in either subspecies, although STURTEVANT reports it in *virilis*. Occasionally there were circling movements of the male around the female, but these were usually noticeable only in cases of prolonged courting.

TABLE 6

*Frequencies of different copulation durations in matings within and between americana and virilis.*

| TYPE OF MATING | DURATION OF COPULATIONS IN SECONDS | | | | |
|---|---|---|---|---|---|
| | 40–60 | 61–90 | 91–120 | 121–150 | 151–190 |
| V×V | | 1 | 3 | 11 | 3 |
| As×As | | 3 | 3 | 4 | 1 |
| V×As | | 2 | 4 | 9 | 5 |
| As×V | 2 | | | 1 | |

Copulation was usually terminated by the male withdrawing his penis but remaining mounted. The female then flicked her wings and kicked with her hind legs until he dismounted. In about half the cases the male began to court following copulation. This second courtship was less vigorous than the first and brought no response from the female other than kicking or running.

Dissections of 27 females, including the 18 mentioned above, showed that following a single copulation only 23 of the 27 were inseminated. There was no clear correlation between the length of copulation and the presence of sperm in the female. For instance the shortest copulation observed, 75 seconds in length, resulted in insemination of the female, while copulations of 115, 140, and 164 seconds did not.

### *Matings of americana females × americana males*

Copulation was carefully observed in 11 six-day-old pairs, and 19 additional females were dissected after it was seen that they had copulated once. Of these 19, 14 contained sperm, five did not. Again there was no apparent correlation between length of copulation and presence of sperm. For example, two copulations of 95 and 155 seconds' duration, respectively,

resulted in insemination; on the other hand, two of 97 and 160 seconds' duration did not.

The behavior of the *americana* pairs was very similar to that of the *virilis* except in the following respects:

The courtship was distinctly longer than in *virilis*, the average time being 56 seconds. The average length of copulation was two minutes, the longest copulation being three minutes and 45 seconds, the shortest one minute and 30 seconds. The range of the duration of copulations is shown in table 6. The males seemed to have more difficulty in getting properly mounted than in *virilis*. If they mounted and could not copulate within two or three seconds, they were promptly dislodged by vigorous kicking and wing-flicking of the female. In most cases the females apparently attempted to dislodge the males 15 or 20 seconds before the end of copulation by means of kicking and wing-flicking. This rarely occurred in *virilis*.

### *Matings of virilis females × americana males*

Copulation between *virilis* females and *americana* males was observed in 20 pairs which were six days old. Sperm was transferred in 13 of the 20 copulations. There seemed to be no clear correlation between presence of sperm and length of copulation. For example, copulations of 100 and 173 seconds' duration resulted in insemination, those of 90 and 173 seconds did not.

The courtship and copulation showed a number of characteristics not seen within either subspecies. The *americana* males paid much less attention to the *virilis* females than they normally did to their own females. Often they stood motionless, paying no attention to the females, even when the latter passed close by. In four cases the females courted the males first. They came up alongside the males and flicked their wings, and in two cases the females even rubbed the males' abdomens with their fore-tarsi. The reactions of the courted males varied considerably. In two cases they extended their fore or middle legs (a typical reaction of Drosophila when keeping other individuals away while they are feeding or resting). In no case did they immediately begin courting the females.

Courtship was often long and frequently interrupted. The average time for active courtship immediately preceding copulation was two minutes and nine seconds, much longer than in conspecific matings of either *virilis* or *americana*. The males followed the females much less vigorously than in conspecific matings, and if they lost them often stopped moving, and only began courting again when the females ran by. The *virilis* females apparently resisted the courting of the *americana* males much more than they did that of the males of their own subspecies. This resistance took the form of prolonged and vigorous kicking with the hind legs. In five cases the

males did not mount when the females spread their wings, but continued courting. The females closed their wings again in three to five seconds, but opened them repeatedly for short periods. In several cases the males attempted to mount after the females had partially closed their wings. In one case when the female spread her wings the male stopped licking, turned away, and began to feed.

When the males mounted they appeared to have difficulty in copulating, apparently because the females kept the tips of their abdomens close to the surface on which they were standing. If the males did not copulate within two or three seconds after mounting, the females kicked them off. There was an average of 2.9 mountings per copulation. One male mounted twenty-two times before copulating, and afterwards, being unable to separate from the female, was dragged, and part of the genitalia torn away. The average length of copulation was two minutes, the longest being three minutes and five seconds, the shortest one minute and ten seconds. The range is shown in table 6.

*Matings of americana females × virilis males*

Seventeen pairs of nine-day-old flies were observed for two or more hours each. The males in this cross courted rather sporadically and usually followed the females for only a centimeter or so. An approaching female was usually met by an avoiding reaction, such as an extended middle or hind leg, or a quick side-step. In some cases the males courted vigorously for twenty or thirty seconds and then stopped suddenly and ran off. The females usually ran and kicked vigorously, even after persistent courting.

In two of the three cases where copulation took place, the females spread their wings only slightly, and the males when in copula were too far back and had difficulty in staying mounted. The females kicked them off, but they copulated again almost immediately; the second time being further forward, they were not dislodged for forty-two and fifty-three seconds, respectively, although the females were kicking most of the time. The males did not court again, although subsequent dissection showed that there had been no insemination.

In the third case, although again the female did not spread her wings widely and kicked all during copulation, the pair was in copula for one minute and twenty-two seconds, and insemination took place.

The above factors which hinder the transfer of sperm between *virilis* and *americana* may be divided into two main groups: those mechanisms which are normally operative in intraspecific matings and those which are operative only in interspecific matings.

Among the first group may be mentioned such factors as copulation without insemination and resistance in the form of running and kicking by

which the females delay copulation. These factors are operative in both intra- and interspecific matings.

The second group includes such factors as the lack of coordination in copulation shown in the matings of *virilis* females to *americana* males. Here the males, although apparently less interested in the *virilis* females than those of their own subspecies, yet court fairly persistently. The females seem ready to mate, as is shown by their occasional courting of the males. Yet when the males actually begin to court, the females run and kick vigorously. On the other hand, when the females spread their wings, the males frequently fail to mount, an omission which was never observed in intraspecific matings. But the males may attempt to mount when the wings of the females are partly closed. When the males do mount, copulation is made difficult by the fact that they are frequently unable to reach the ovipositor plates of the female.

Likewise in the matings of *americana* females by *virilis* males, the sudden cessation of courting by the male and the kicking by the female not only during the courtship but also all during copulation have not been observed in either of the intraspecific matings.

Thus in crosses between *virilis* and *americana*, incomplete sexual isolation is produced partly by exaggeration of the normal mating behavior found in conspecific matings and partly by behavioristic peculiarities found only in interspecific crossses. These latter peculiarities seem to be in part associated with a lack of coordination between the male and female of the two subspecies.

## DISCUSSION

Geographic isolation of two originally identical groups of animals may lead to such evolutionary divergence that if they come together again, any hybrids formed will be weak or sterile. Thus those members of the two groups which will interbreed to form such hybrids are at a selective disadvantage, and genes preventing such interbreeding would have a positive selective value.

DOBZHANSKY (1937a, b, 1940) suggests that "occurrence of hybridization between races and species constitutes a challenge to which they may respond by developing or strengthening isolating mechanisms that would make hybridization impossible." He believes that the genes producing these isolating mechanisms, having a positive selective value at the border zone between the two races and a neutral value within each race, would gradually diffuse from the border zone throughout. If we accept this hypothesis as of general validity, the different degrees of isolation shown by strains from different parts of Ohio might be explained as follows: since

*virilis* is either very rare in Ohio or absent altogether and is known to occur in Texas, the zone of hybridization between *americana* and *virilis* would presumably be somewhere south of Ohio. The isolation genes would then diffuse northwards, and by the time they reached Ohio they might become variously fixed in the small apparently discontinuous *americana* populations. These populations would be expected to differ by chance as to the number and kind of such isolating genes which they contained.

Another hypothesis might likewise postulate no genetic isolation at the start of divergent evolution and its acquisition in the course of time. However, according to this hypothesis the genetic isolating mechanisms would be acquired, not by the challenge of hybridization, but independently during evolutionary changes which result in increasing differences between sub-groups. "The evolutionary origin of interspecific sterility lies not at the beginning of divergent evolution but occurs in the course of it as a by-product" (STERN 1936). The data presented in this paper might equally well be interpreted according to this hypothsis. Even in the absence of *virilis*, insular *americana* populations might be expected to acquire isolating mechanisms by the chance fixation of certain genotypes. The different *americana* populations would then be expected to vary considerably in the strength of the isolating mechanisms which they acquired.

Since the different localities in Ohio are represented by only one *americana* strain each, the picture of the distribution of the isolation genes that is obtained is far from complete. Accordingly, these data are not critical for a discrimination between either of the above alternatives, or a combination of both.

There are many mechanisms which keep two groups of related organisms isolated. Among them DOBZHANSKY (1937a, b) mentions: geographical isolation, ecological isolation, seasonal or temporal isolation, sexual isolation, mechanical isolation, failure of sperm to reach or penetrate eggs, inviability of the zygote, and sterility of the hybrid. Thus sexual isolation is only one of the many ways the mixing of the *americana* and *virilis* genotypes may be inhibited. In addition to it, mechanical isolation has been observed in one case, V ♀ × As ♂ (see above). Finally SPENCER (1940) and PATTERSON, STONE, and GRIFFEN (1940b) report partial sterility of the hybrids and low egg hatchability in crosses between the two subspecies. Whether this low hatchability of the eggs is due to zygote mortality or to a large number of eggs remaining unfertilized is not known.

## SUMMARY

The Pasadena strain of *Drosophila virilis virilis* was tested for sexual isolation with strains of *D. v. americana* from four localities in Ohio. In experi-

ments in which males of one subspecies are placed with equal numbers of females of the other, *virilis* shows sexual isolation in matings with all *americana* strains. The matings in which *virilis* enters as the male show significantly greater isolation than the reciprocal ones. The *americana* strains vary in regard to the sexual isolation they show when mated to *virilis*.

Similar "no choice" experiments show no sexual isolation among any of the four *americana* strains.

In experiments in which males are placed with equal numbers of conspecific and alien females, matings between the two subspecies show that the males exercise choice and inseminate more of the females of the conspecific than the alien strain.

Similar matings involving a choice of females demonstrate sexual isolation among all *americana* strains. This isolation is much less complete than that found between *americana* and *virilis*.

Strains of *americana* of the same geographical origin may behave differently in crosses to *virilis*. The degree of isolation existing among different *americana* strains in intraspecific matings does not appear to be correlated with the distance separating the localities from which they were collected.

Observations on pair-matings between and within the two subspecies show that the sexual isolation mechanisms in interspecific crosses depend on two types of behavioristic peculiarities. One type is normally found in intraspecific matings; the other type, found only in interspecific matings, is associated with lack of coordination between the male and female.

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