

## IV. THE INSEMINATION REACTION IN INTRASPECIFIC MATINGS OF DROSOPHILA

MARSHALL R. WHEELER\*

### INTRODUCTION

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

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

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

---

\*Predoctoral Fellow of the National Research Council.

## MATERIALS AND METHODS

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

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

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

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

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

## DESCRIPTIONS OF THE INSEMINATION REACTIONS

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

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

### GENUS CHYMOMYZA Czerny

#### *Chymomyza amoena* Loew.

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

#### *Chymomyza procnemis* Williston.

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

### GENUS SCAPTOMYZA Hardy

#### *Scaptomyza graminum* Fallén.

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

## GENUS DROSOPHILA Fallén

**Subgenus Hirtodrosophila Duda.***Drosophila duncani* Sturtevant.

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

**Subgenus Pholadoris Sturtevant.***Drosophila victoria* Sturtevant.

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

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

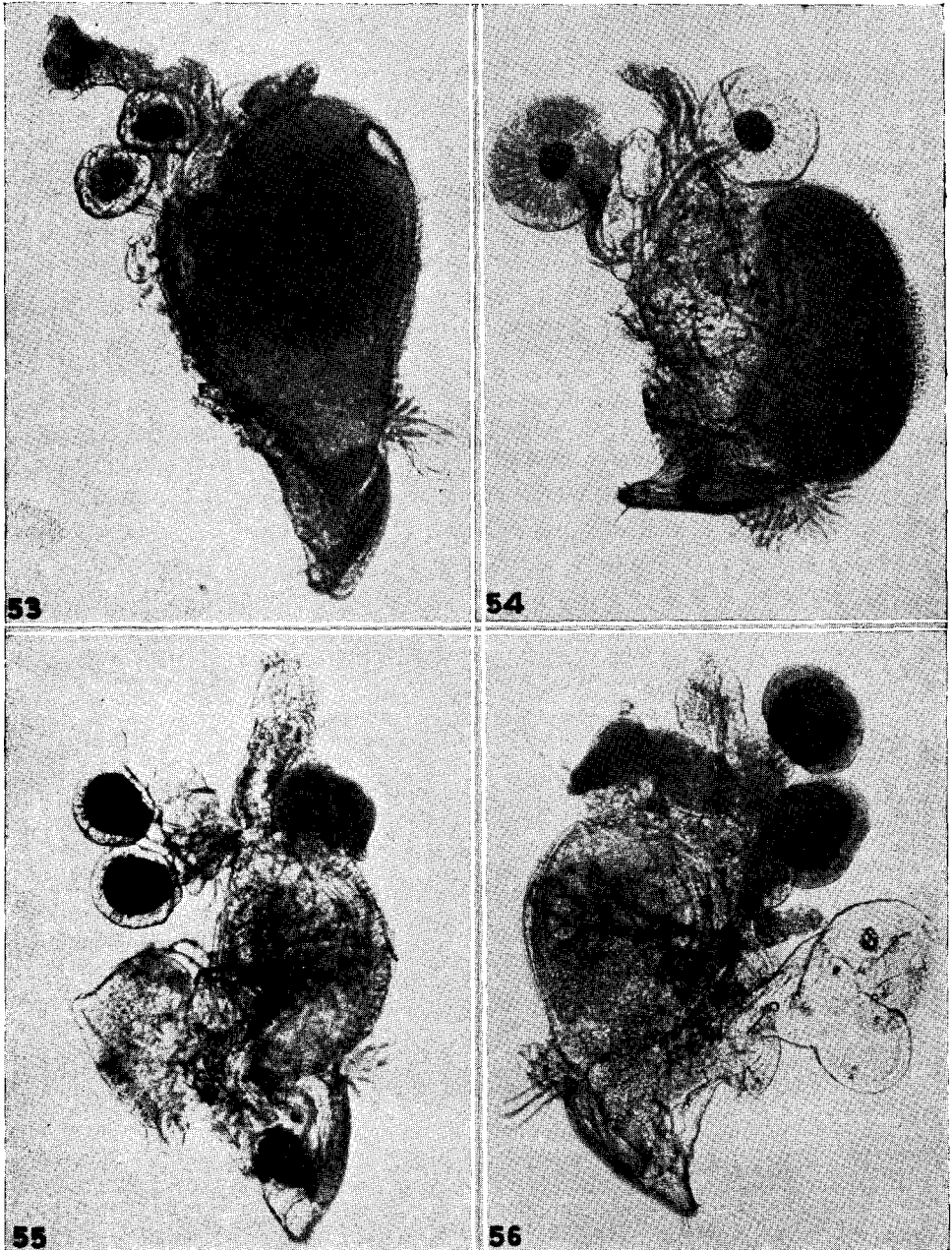


PLATE XIV. Vaginae from homogamic matings of four species of *Drosophila*. Fig. 53, *D. duncani* at thirty minutes. Fig. 54, *D. victoria* at five hours. Fig. 55, *D. sucinza* at thirty minutes. Fig. 56, *D. fumipennis* at one hour.

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

### **Subgenus *Dorsilopha* Sturtevant.**

*Drosophila busckii* Coquillett.

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

### **Subgenus *Sophophora* Sturtevant.**

#### *1. saltans group*

*Drosophila sturtevanti* Duda.

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

*Drosophila rectangularis* Sturtevant.

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

*Drosophila prosaltans* Duda.

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

*Drosophila cordata* Sturtevant.

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

*Drosophila elliptica* Sturtevant.

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

*Drosophila emarginata* Sturtevant.

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

2. *willistoni* group*Drosophila willistoni* Sturtevant.

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

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

*Drosophila equinoxialis* Dobzhansky.

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

*Drosophila nebulosa* Sturtevant.

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

*Drosophila sucinea* Patterson and Mainland.

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

*Drosophila fumipennis* Duda.

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



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

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

### 3. *melanogaster* group

#### *Drosophila melanogaster* Meigen.

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

#### *Drosophila simulans* Sturtevant.

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

*Drosophila ananassae* Doleschall.

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

4. *obscura* group*Drosophila pseudoobscura* Frolova.

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

*Drosophila persimilis* Dobzhansky and Epling.

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

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

*Drosophila affinis* Sturtevant.

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

*Drosophila azteca* Sturtevant and Dobzhansky.

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

*Drosophila tolteca* Patterson and Mainland.

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

### **Subgenus *Drosophila*. Fallen.**

#### *1. quinaria group*

*Drosophila transversa* Fallén.

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

*Drosophila munda* Spencer.

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

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

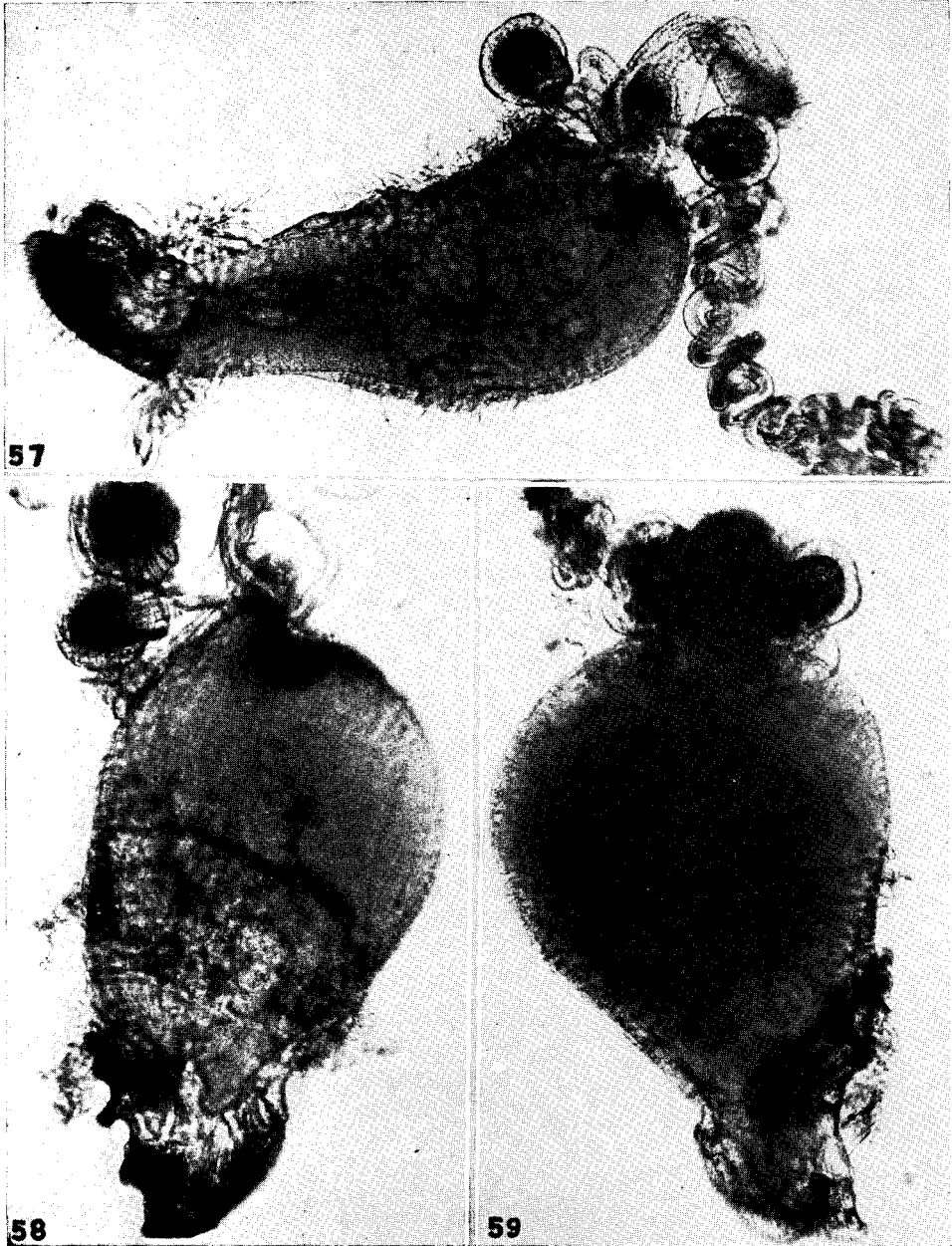


PLATE XV. Vaginae from homogamic matings of three species of *Drosophila*. Fig. 57, *D. munda* at thirty minutes. Fig. 58, *D. transversa* at thirty minutes. Fig. 59, *D. subquinaria* at one hour.

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

*Drosophila quinaria* Loew.

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

*Drosophila subquinaria* Spencer.

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

*Drosophila suboccidentalis* Spencer.

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

*Drosophila innubila* Spencer.

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

*Drosophila subpalustris* Spencer.

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

2. *guttifera* group*Drosophila guttifera* Walker.

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

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

### 3. *pinicola* group

*Drosophila pinicola* Sturtevant.

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

### 4. *virilis* group

*Drosophila virilis* Sturtevant.

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

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

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

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

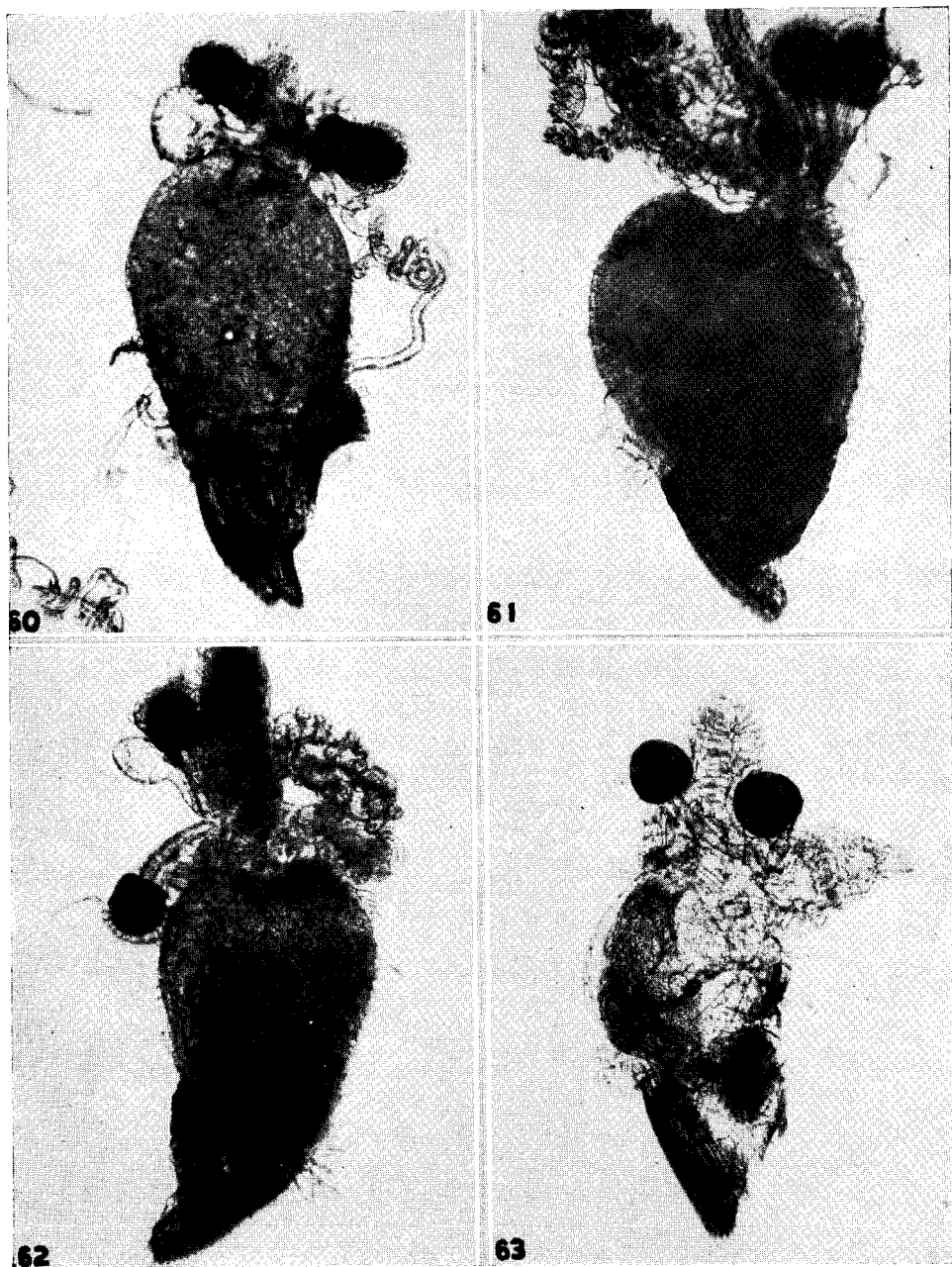


PLATE XVI. Vaginæ from homogamic matings of four species of *Drosophila*. Fig. 60, *D. guttifera* at one hour. Fig. 61, *D. americana* at thirty minutes. Fig. 62, *D. montana* at thirty minutes. Fig. 63, *D. lacicola* at thirty minutes.



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

*Drosophila americana* Spencer.

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

*Drosophila americana texana* Patterson, Stone, and Griffen.

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

*Drosophila montana* Patterson and Wheeler.

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

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

*Drosophila lacicola* Patterson.

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

5. *testacea* group

*Drosophila putrida* Sturtevant.

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

6. *tripunctata* group

*Drosophila tripunctata* Loew.

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

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

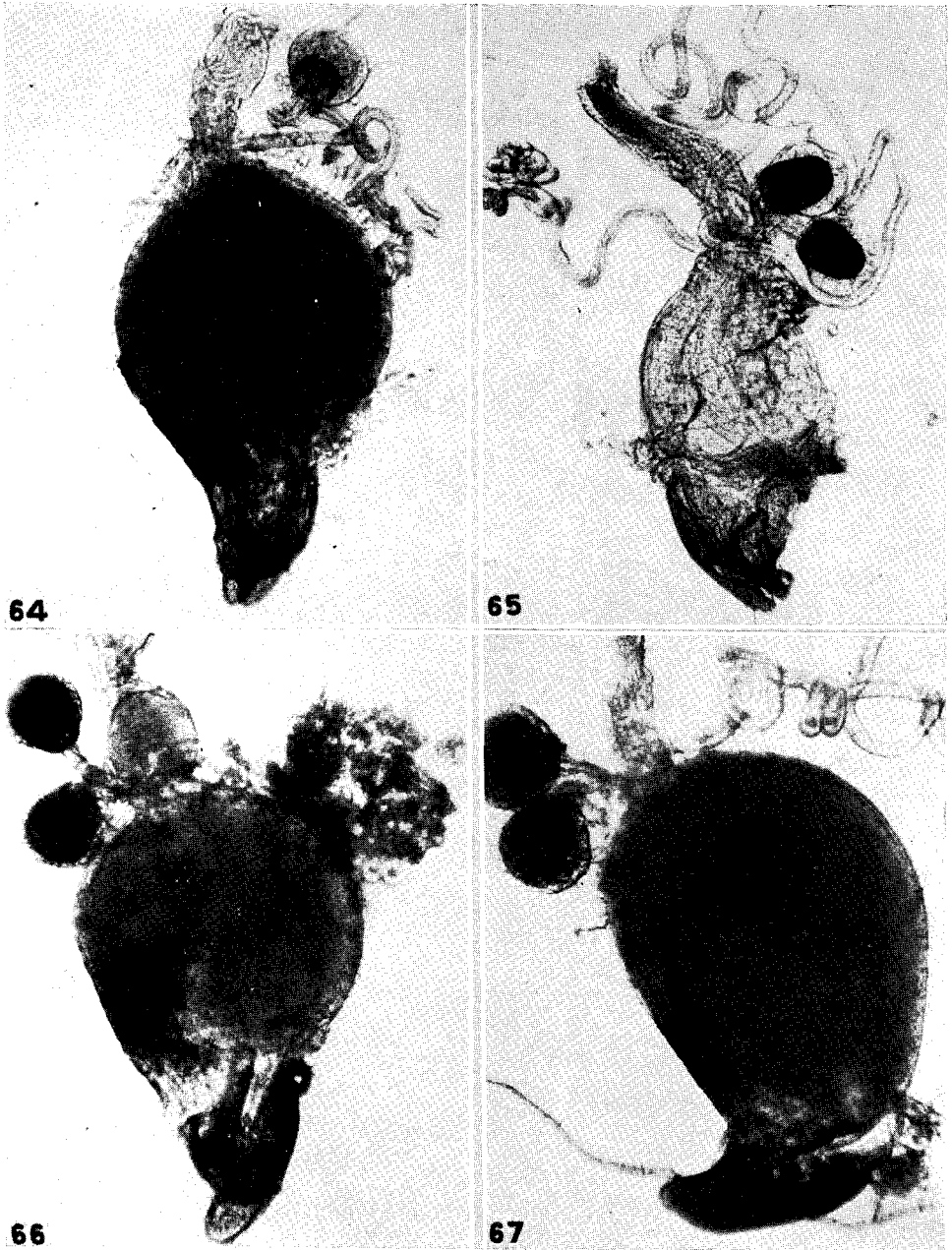


PLATE XVII. Vaginae from homogamic matings of four species of *Drosophila*. Fig. 64, *D. putrida* at one hour. Fig. 65, *D. tripunctata* at thirty minutes. Fig. 66, *D. sub-funebris* at one hour. Fig. 67, *D. macrospina* at thirty minutes.

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

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

*Drosophila crocina* Patterson and Mainland.

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

#### 7. *funnebris* group

*Drosophila funnebris* (Fabricius).

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

*Drosophila subfunnebris* Stalker and Spencer.

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

*Drosophila macrospina* Stalker and Spencer.

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

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

#### 8. *repleta* group

##### *Drosophila repleta* Wollaston.

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

##### *Drosophila neorepleta* Patterson and Wheeler.

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

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

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

##### *Drosophila canapalpa* Patterson and Mainland.

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

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

*Drosophila melanopalpa* Patterson and Wheeler.

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

*Drosophila hydei* Sturtevant.

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

*Drosophila mulleri* Sturtevant.

*Drosophila aldrichi* Patterson and Crow.

*Drosophila arizonensis* Patterson and Wheeler.

*Drosophila mojavensis* Patterson and Crow.

*Drosophila buzzatii* Patterson and Wheeler.

*Drosophila hamatofila* Patterson and Wheeler.

*Drosophila ritae* Patterson and Wheeler.

*Drosophila peninsularis* Patterson and Wheeler.

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

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

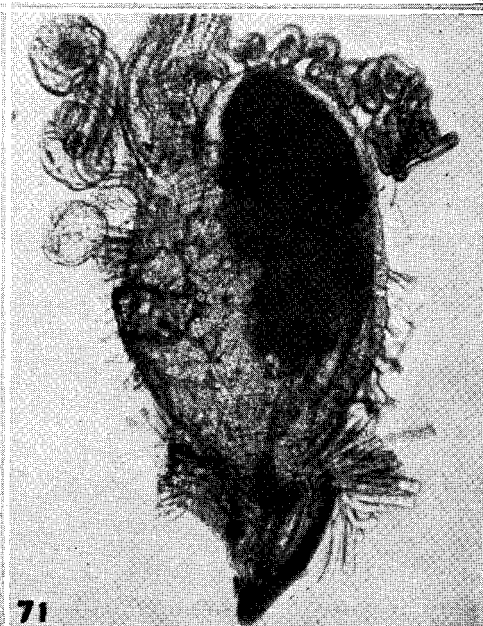
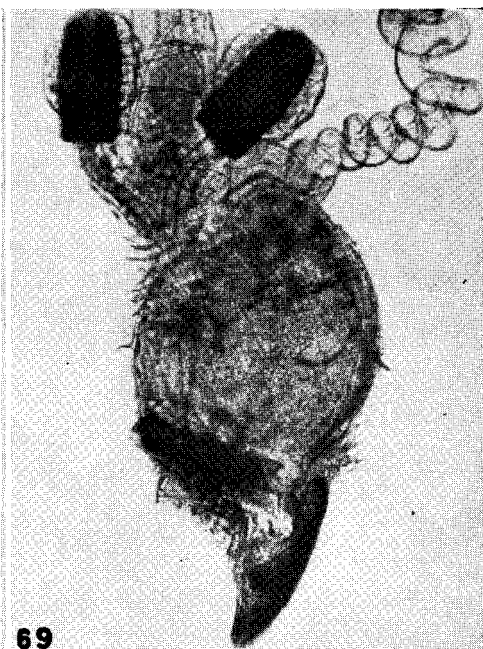
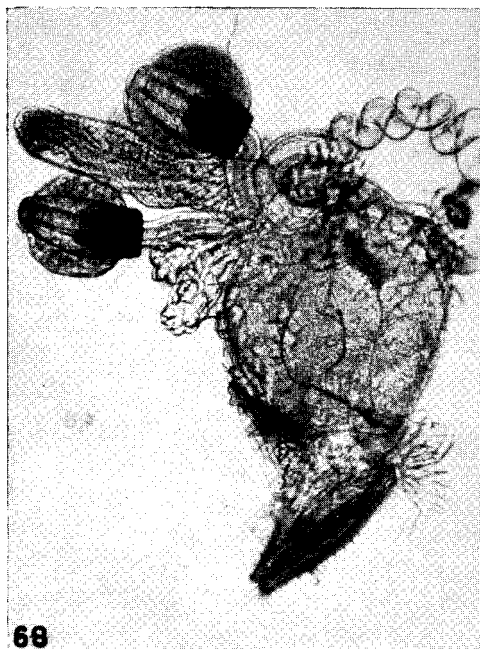


PLATE XVIII. Vaginae from homogamic matings of four species of *Drosophila*. Fig. 68, *D. repleta* at thirty minutes. Fig. 69, *D. neorepleta* at thirty minutes. Fig. 70, *D. mercatorum* at one hour and thirty minutes. Fig. 71, *D. hexastigma* at one hour and fifteen minutes.

*Drosophila mercatorum* Patterson and Wheeler.

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

*Drosophila mercatorum pararepleta* Dobzhansky and Pavan.

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

*Drosophila meridiana* Patterson and Wheeler.

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

*Drosophila anceps* Patterson and Mainland.

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



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

*Drosophila hexastigma* Patterson and Mainland.

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

*Drosophila gibberosa* Patterson and Mainland.

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

#### 9. *robusta* group

*Drosophila robusta* Sturtevant.

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

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

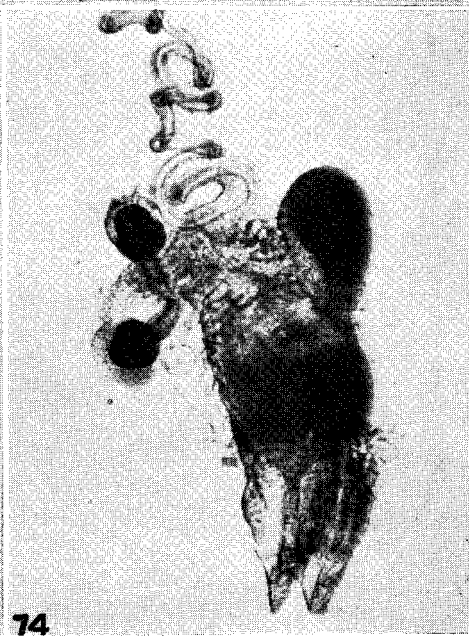
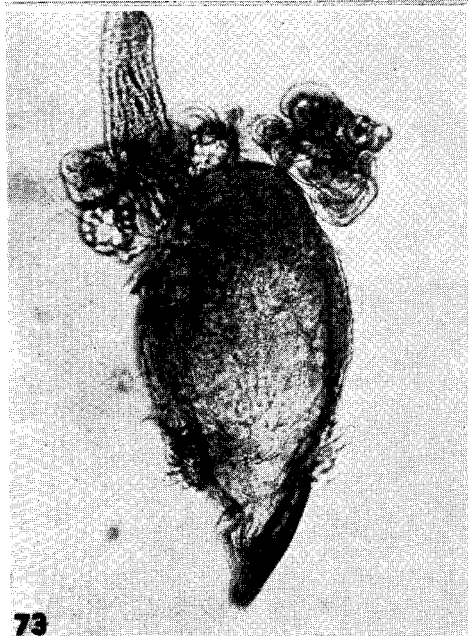
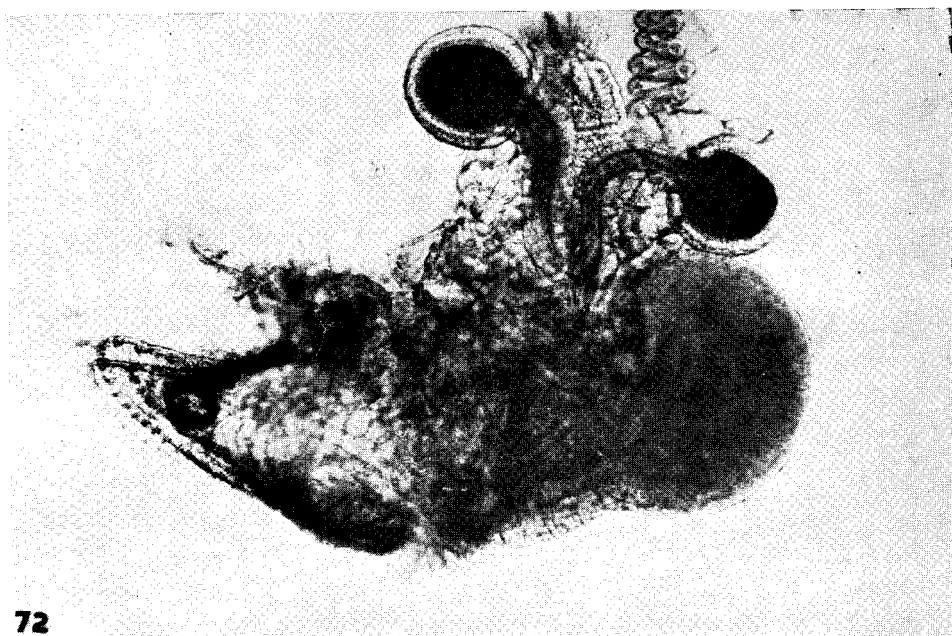


PLATE XIX. Vaginae from homogamic matings of three species of *Drosophila*. Fig. 72, *D. gibberosa* at two hours. Fig. 73, *D. anceps* at thirty minutes. Fig. 74, *D. carbonaria* at thirty minutes.

10. *melanica* group*Drosophila melanica* Sturtevant.

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

*Drosophila melanica paramelanica* Patterson.

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

*Drosophila nigromelanica* Patterson and Wheeler.

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

*Drosophila micromelanica* Patterson.

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

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

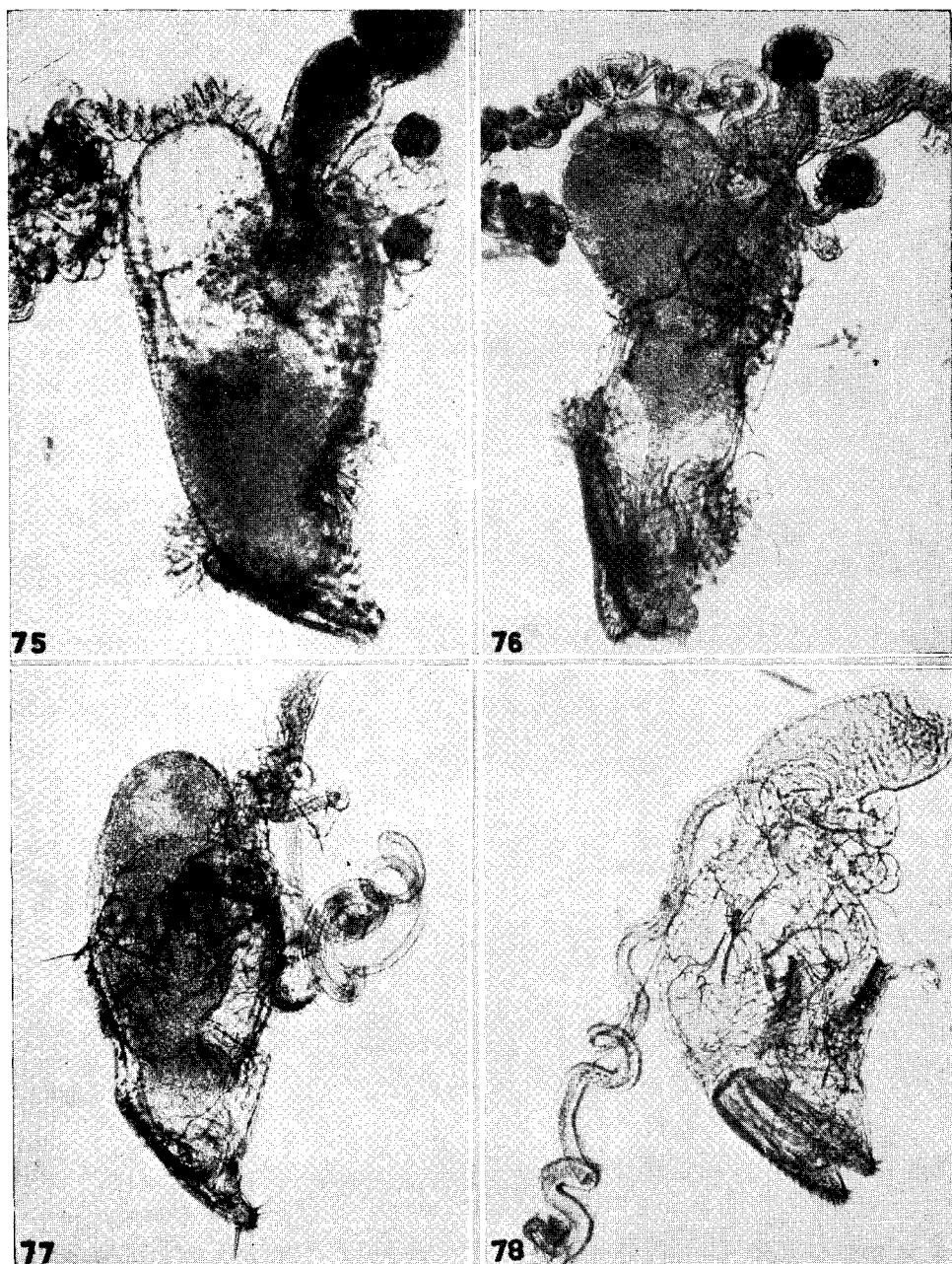


PLATE XX. Vaginae from homogamic matings of four species of *Drosophila*. Fig. 75, *D. melanica* at thirty minutes. Fig. 76, *D. melanica paramelanica* from stock. Fig. 77, *D. micromelanica* at thirty minutes. Fig. 78, *D. polychaeta* at thirty minutes.

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

#### 11. *polychaeta* group

*Drosophila polychaeta* Patterson and Wheeler.

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

#### 12. *carbonaria* group

*Drosophila carbonaria* Patterson and Wheeler.

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

#### 13. *cardini* group

*Drosophila cardini* Sturtevant.

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

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

#### 14. *immigrans* group

*Drosophila immigrans* Sturtevant.

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

#### 15. *macroptera* group

#### 16. *alagitans* group

#### 17. *rubrifrons* group

No members of the above groups were available for study.

#### 18. *guarani* group

*Drosophila guarani* Dobzhansky and Pavan.

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

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

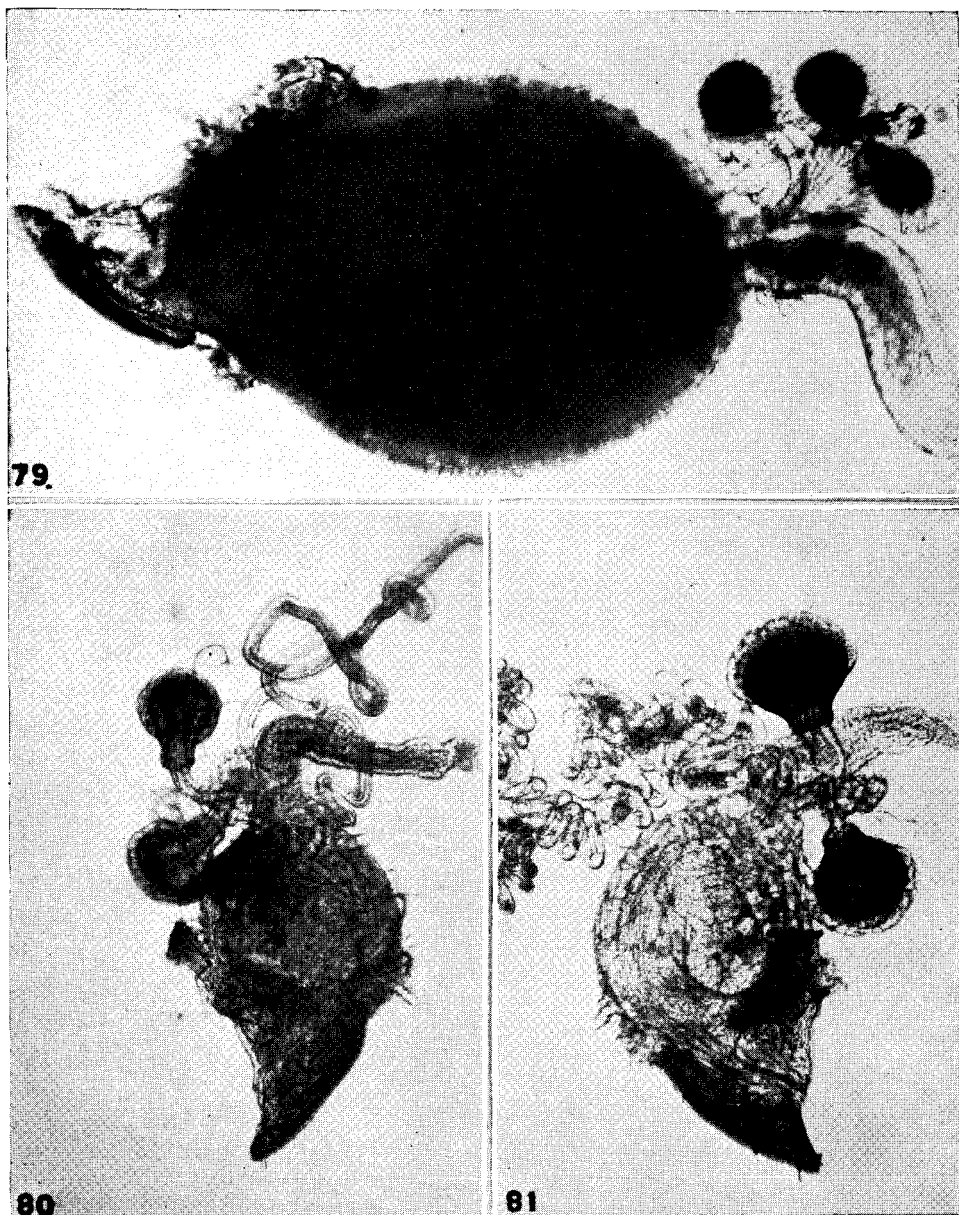


PLATE XXI. Vaginae from homogamic matings of three species of *Drosophila*. Fig. 79, *D. pallidipennis* at one hour. Fig. 80, *D. cardini* at thirty minutes. Fig. 81, *D. subbadia* at thirty minutes.

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

*Drosophila subbadia* Patterson and Mainland.

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

*Species unclassified as to grouping*

*Drosophila parachrogaster* Patterson and Mainland.

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

*Drosophila pallidipennis* Dobzhansky and Pavan.

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

*Drosophila pallidipennis centralis* Patterson and Mainland.

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



## DISCUSSION

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

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

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

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

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

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

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

TABLE 1

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

REFERENCES

- Kaufmann, B. P., and M. Demerec. 1942. Utilization of sperm by the female *Drosophila melanogaster*. *Am. Nat.*, 76:445-469.
- Mayr, E. 1946. Experiments on sexual isolation in *Drosophila*. VII. The nature of the isolating mechanisms between *Drosophila pseudoobscura* and *Drosophila persimilis*. *Proc. Nat. Acad. Sci.*, 32:128-137.
- Nonidez, J. F. 1920. The internal phenomena of reproduction in *Drosophila*. *Biol. Bull.*, 39:207-230.
- Patterson, J. T. 1946. A new type of isolating mechanism in *Drosophila*. *Proc. Nat. Acad. Sci.*, 32:202-208.
- Patterson, J. T. 1947. The insemination reaction and its bearing on the problem of speciation in the mulleri subgroup. *This bulletin*.
- Stalker, H. D. 1942. Sexual isolation studies in the species complex *Drosophila virilis*. *Genetics*, 27:238-257.
- Sturtevant, A. H. 1942. The classification of the genus *Drosophila* with descriptions of nine new species. *Univ. Tex. Publ.*, 4213:6-51.