動物学雑誌 ZOOLOGICAL MAGAZINE 90: 307-316 (1981)

Effects of the Male Accessory Gland Secretion on Oviposition and Remating in Females of *Drosophila melanogaster*

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Received January 26, 1981

ABSTRACT Inseminated *Drosophila* females show two newly released behaviors; a high rate of oviposition and mating reluctance. Using X/O sterile males, factors affecting these behaviors were confirmed to be contained in the secretion of the male accessory gland. Sterile females showed a remating rate similar to that of fertile females. This suggests that the remating of females is related neither to the number of eggs ovulated, nor to sperm stored in the female reproductive organs. The number of eggs laid and the remating rate of females can be determined by the quantity of secretion from the male accessory gland, which was supposed to have been introduced along with the sperm into the female reproductive organs. Sperm transfer and fertility of the sperm were not found to correlate with the quantity of secretion from the male accessory gland. (*Zool. Mag. 90: 307-316, 1981*)

It has been well known that two major behavioral changes, oviposition and mating reluctance, occur in females of *Drosphila* after the completion of copulation. This means that some substances produced in the male reproductive organs are discharged into the female reproductive organs, giving stimuli to release these behaviors. Fox (1956) found the "sex peptide", ninhydrin positive male specific substances, in males of *D. funebris*. Chen and Diem (1961) demonstrated that the "sex peptides" were contained in the male accessory gland.

A number of biochemical studies on the nature of substances of the male accessory gland have been performed (Leahy and Lowe, 1967; Chen and Bühler, 1970; Terranova *et al.*, 1972; Baumann, 1974a, b; Baumann *et al.*, 1975; Wyl, 1976; Wyl and Steiner, 1977). Baumann *et al.* (1975) intended to determine the amino acid sequence of PS-1, which is one of the two sex specific substances of *D. funebris*. Wyl (1976) indicated with the polyacrylamide gel systems that the secretion of the male accessory gland of *D. melanogaster* consists of various proteins, these molecular weights ranging from 12,000 to 122,000 daltons. However, the nature and function of these separated proteins are still unknown.

Reproductive effects of the "sex peptides" or the secretion of the male accessory gland have been examined by transplantation (Garcia-Bellido, 1964; Merle, 1968) or by injection (Leahy, 1967; Leahy and Lowe, 1967; Chen and Bühler, 1970; Burnet *et al.*, 1973; Baumann, 1974a, b). These results strongly suggested that the "sex peptides" or the secretion of the male accessory gland have stimulating effects on oviposition and mating reluctance in females. Effects of the accessory gland secretion can be examined by matings with sterile males which the nature of male sterility is considered. However, there are few studies of this kind. Cook (1970) reported that the fecundity of females mated with sterile males was significantly increased.

The present paper will show reproductive effects of the secretion of the male accessory gland on oviposition and mating reluctance in females tested by the usual mating method including sterile males and serially mated fertile

males.

Materials and Methods

Strains used in the study were as follows; (1) Oregon-R, (2) f-17: males are of the $\widehat{XY}/O$ sex chromosome constitution, $Y^{s}\cdot In(1)EN\ y\cdot Y^{L}\ sc^{8}y+/O$. X/O sterile males can be obtained by crossing these males to Oregon-R females. (3) fs(OZ)-3: females are homozygous for a recessive female sterile gene located on the second chromosome, isolated by the Cy/Pm method from the Ohzu (Ehime, Japan) natural population in 1977.

Serial matings of males were carried out in the following manner. First, 10 virgin females, 5 days-old, were allowed to mate with 15 virgin males, 5 days-old, for 15 min in each of 20 replicate mating vials. Females completing copulation were aspirated out and introduced into oviposition vials, which contained yeasted *Drsophila* medium. These females are referred to as the First-mated females. Flies unmated within 15 min were discarded. Secondly, another 10 virgin females were introduced into each of the mating vials, where they were allowed to mate with 10 males having once mated. Females mated this time are referred to as the Second-mated females. Such serial matings were continued up to the fifth, finally giving the Fifth-mated females. The intervals between matings were limited to 5 min at most. Thus, the quantity of the sperm and the secretion of the male accessory gland transferred into the female reproductive organs might be gradually decreased from the First-mated females to the Fifth-mated females; this will evidently be shown later by the microscopic observations.

To examine the change of fecundity of females after copulation, five females were placed together in each of the oviposition vials and transferred daily for 13 days. Ten replicates were run, testing a total of 50 females for each of the serial mating groups.

The rate of remating of females was represented by the percentage of females remated within 15 min after introduction of virgin males; in each of the four replicates, five mated females and eight males were introduced into a mating vial. Flies were reared and aged on the ordinary cornmeal-agar-molasses medium with yeast at 25°C, in a 12L : 12D light condition.

Results

Oviposition and mating reluctance in females mated with XO males

From crosses between Or-R females and f-17 ($\widehat{XY}/O$) males, $\widehat{XY}/X$ females and X/O males were obtained. $\widehat{XY}/X$ females (denoted OR-17 females) are fully fertile and have a high level of mating activity. X/O males (denoted OR-17 males) are completely sterile, whereas they have a high level of mating activity. Their accessory glands were the same size as much as these of normal males.

Changes of egg production during the first 13 days after the mating were compared between OR-17 females mated with Or-R males and those mated with OR-17 sterile males (Fig. 1). The egg production of OR-17 virgin females was also measured as a control. Each of females once mated with Or-R males produced more than 50 eggs per day on the average over a period of 9 days. By 11 days after the mating, however, the level of the egg production had virtually decreased to that of virgin females. On the other hand, when females mated with OR-17 males (see OR-17♀ × OR-17♂ (1) in Fig. 1), the number of eggs produced exceeded the virgin level only for one day after the mating.

Females remaining as virgins for further 7 days (12 days of age) were crossed with OR-17 sterile males (see OR-17♀ × OR-17♂ (2) in Fig. 1). In this case, an increase in egg production was also found only for one day after the mating. Thus, it is clear that sterile males can stimulate oviposition in females mated with them. However, its effects do not remain for more than one day, in contrast with the effects of copulation with fertile males which last at

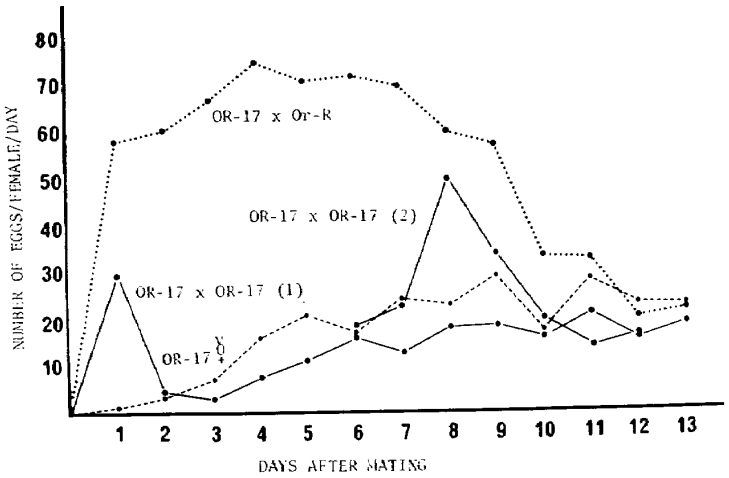

Fig. 1. Change of egg production during the first 13 days after mating. The dotted line; OR-17 females mated with Or-R fertile males. The dashed line; OR-17 virgin females. The solid line; OR-17 females mated with OR-17 sterile males. Each point is the mean of 10 replicate vials which contained 5 females in each.

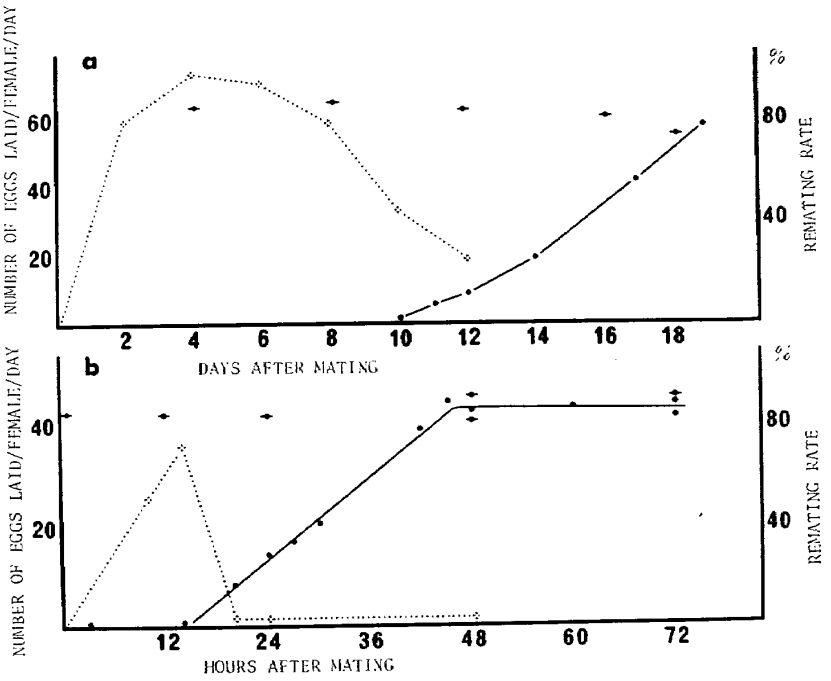

Fig. 2. The remating rate of females once mated with fertile males (a), and sterile males (b). The dotted line; the number of eggs laid. The solid line; the remating rate. —●—; the mating rate of virgin females. Each point is the mean of 20 females.

least 9 days.

The remating rate of females once mated with fertile males was compared with that of females mated with sterile males. The rate of females mated for the first 15 min after mixing both sexes was measured at the interval of day (Fig. 2a) or hour (Fig. 2b) after the first mating. In the fertile cross, OR-17♀

×Or-R♂, the second matings occurred on the 11th day, when oviposition was dropped to the virgin level. Then, they gradually recovered to the virgin level by the 19th day (Fig. 2a). On the contrary, in the sterile cross, OR-17♀ ×OR-17♂, the second matings occurred within 14 hr after the first matings, and they recovered to the virgin level within 48 hr (Fig. 2b). In this case, the second matings occurred when fecundity of females was reduced to the virgin level. However, the period requirred for the recovery of female receptivity was apparently shorter than for the fertile matings.

Remating of sterile females

The results described above suggest that the completion of oviposition may determine a turning point to remate. To ascertain this, the remating rate of fs(OZ)-3 sterile females once mated was measured. These females have ovaries which are completely in a state of atrophy. Oviposition was never observed through the period of 15 days after mating, although they carried many actively motile sperm in their reproductive organs. The remating rate of fs(OZ)-3 females mated with Or-R males is shown in Fig. 3. This result indicates that the remating of strile females occurred with nearly the same rate as for fertile females. Thus, it is likely that remating might be determined neither by oviposition nor by sperm utilization.

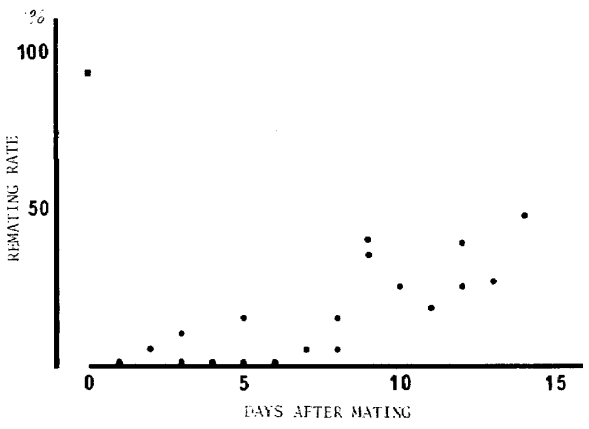

Fig. 3. The remating rate of fs(OZ)-3 sterile females. Each point is the mean of 20 females.

Serial matings of males and its effect on oviposition and receptivity of females

Figure 4 shows morphological changes of the male accessory gland and the vas deference of serially mated Or-R males at the age of 5 days. It was clearly shown that the volume of the accessory gland decreased as the number of successive matings increased. After the 4th mating, the secretion in the lumen of the accessory gland seemed to be actually exhausted.

The decrease in the volume of the vas deference was slower than that of the accessory gland. Dissection of the vas deference after the 4th mating revealed that a great number of mature sperm still remained in it. It was also observed that the Fourth-mated and the Fifth-mated females had recieved plenty of sperm in their ventral receptacles (Fig. 5).

From the facts described above, it can be concluded that the secretion stored in the lumen of the accessory gland was exhausted faster than mature sperm of the vas deference, and that sperm transfer from the male to the female could occur without the secretion of the male accessory gland.

Effects of the serial matings on changes in the rate of oviposition were examined (Fig. 6). Both the First- and the Second-mated females produced more than 50 eggs every day for the first 9 days after matings. Then, the daily egg production gradually decreased and finally reached the virgin level on the 13th day. The Third-mated females produced the same number of the eggs in the 1st day. After the 2nd day, however, their egg production became significantly lower than that of the First- and the Second-mated females, although more than 30 eggs were produced every day until the 8th day.

The Fourth-mated females produced eggs at a high rate only on the 1st day. After the 6th day, the number of eggs laid slightly increased and reached a plateau of daily production of about 15 eggs. The Fifth-mated females did not show a significant increase in egg produc-

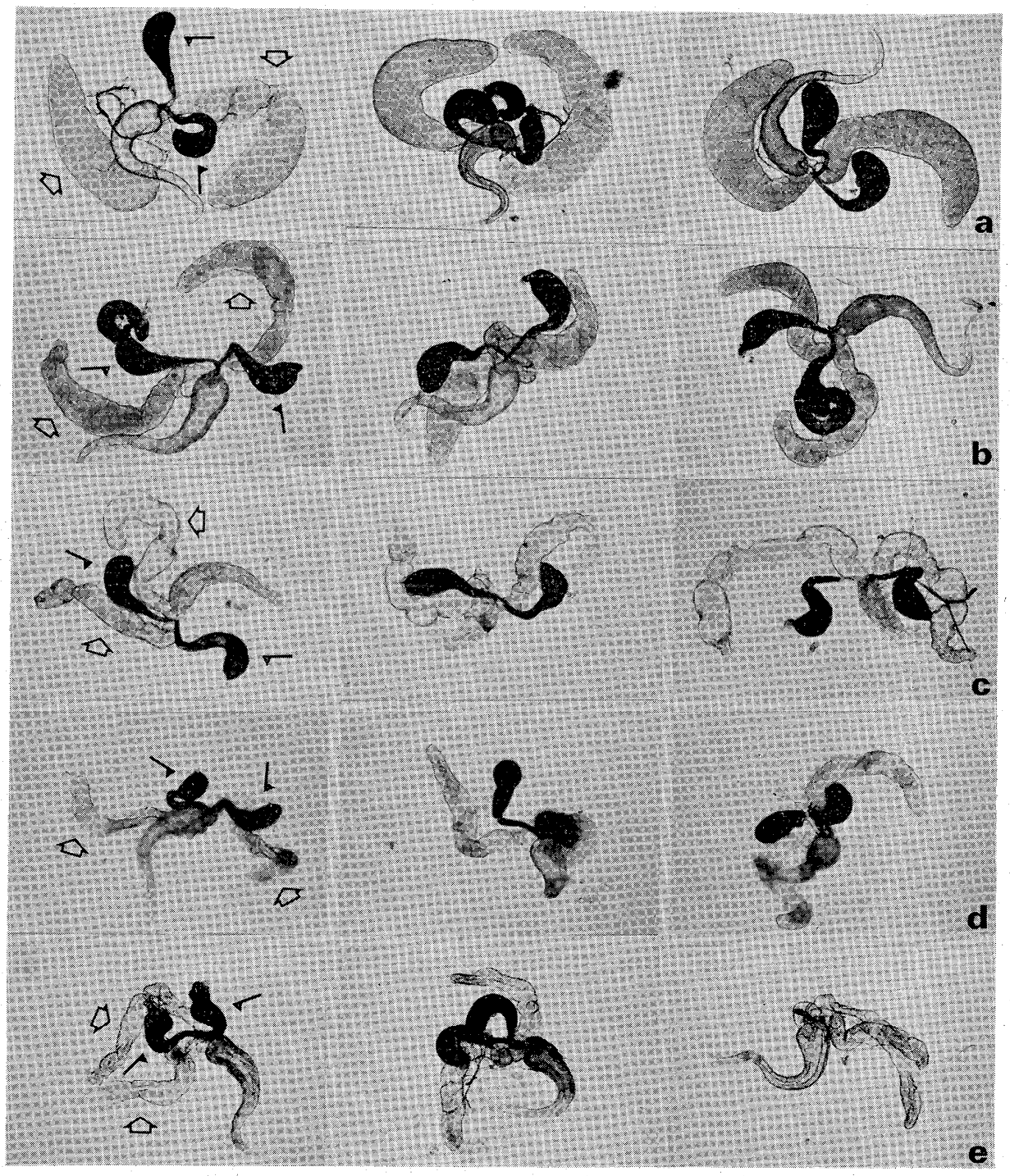

Fig. 4. Change of the male accessory gland and vas deference of serially mated males. a; virgin males, b; the first mated males, c; the second mated males, d; the third mated males, and e; the fourth mated males. ⇦; the accessory gland, ←; vas deference.

tion, about 15 eggs being laid every day after the 2nd day.

These results are consistent with the morphological observations of the male reproductive organs as shown in Fig. 4. Thus, it is clear that the number of eggs laid is closely associated with quantity of the secretion of the male accessory gland received by females. Furthermore, reduction of the egg production would not be caused by reduction in the num-

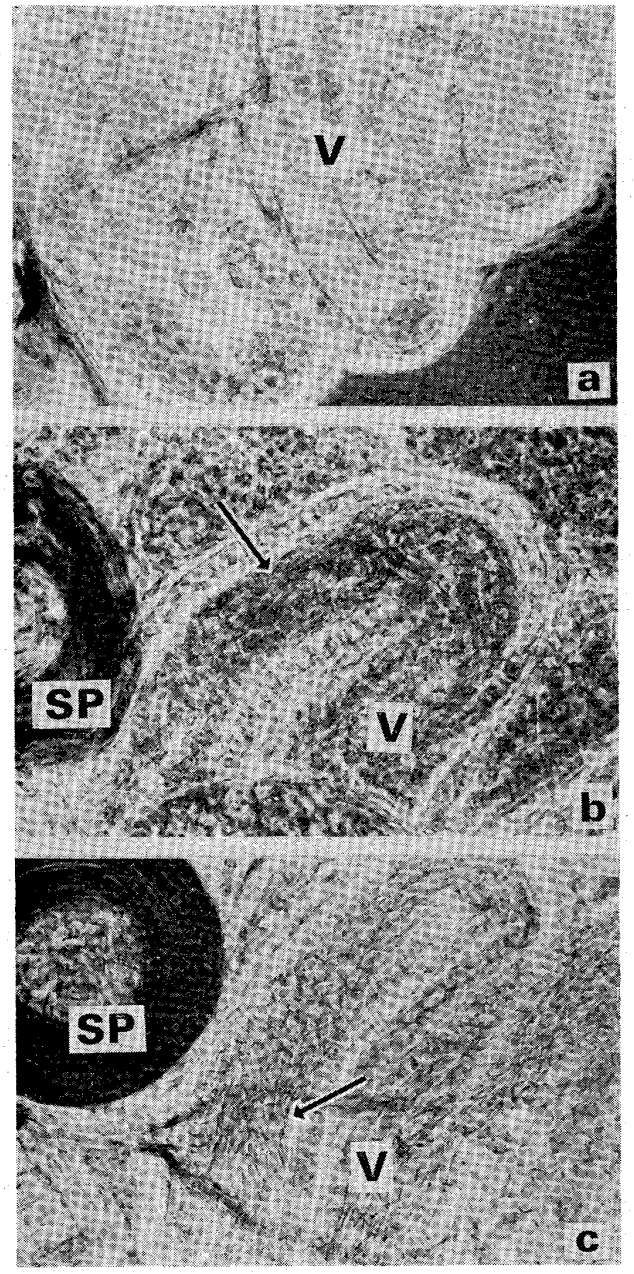

Fig. 5. A part of the female reproductive organs. a; the virgin female, b; the Fourth-mated female, c; the Fifth-mated female. V; the ventral receptacle, SP; the spermathecae, ←; sperm.

ber of the sperm received by females, because the Fourth- or the Fifth-mated females have received a great number of sperm as shown in Fig. 5. No significant difference in the duration of copulation was found between females mated with fresh virgin males and those mated with males having copulated more than once before, consistently giving 17.5 min on the average. This is suggestive that the reduction in egg production as the number of serial matings increased can not be attributable to stimuli derived from copulation itself.

Figure 7 shows that the rate of rematings of a female once inseminated was dependent on the number of times of copulation of the male with which the female copulated. The First-mated females began to remate on the 10th day after the first mating. For the Third-mated females, the remating rate was 10–35% on the 3rd day and about 70% on the 10th day. The Fourth-mated females showed a remating rate of 80% on the 5th or the 6th day, and the Fifth-mated females gave the same remating rate on the 3rd day. These results suggest that the length of the unreceptive period of once mated females may be determined by the quantity of the accessory gland secretion introduced into the female reproductive organs.

Fertility of the sperm and quantity of the secretion of the male accessory gland

When the male repeatedly mated, the secretion of the male accessory gland transferred with the sperm into the female reproductive organs decreased as the number of times of copulation increased. To test whether the sperm stored in the female reproductive organs can maintain fertility over a certain period, the proportion of the fertilized eggs, which was given by the proportion of the adults emerging from the eggs laid, was compared among the five classes of females from the First- to the Fifth-mated ones (Fig. 8).

In Fig. 8, the relative proportion of the adults emerging from the eggs laid is shown on the basis of assigning a 1.0 to the First-mated females. It was clear that the proportion of emerged adults did not significantly decrease from the First- to the Fourth-mated females. The Fifth-mated females gave a lower proportion of emerging adults than those of the former ones. This might be caused by the food conditions which was deteriorated by the smal-

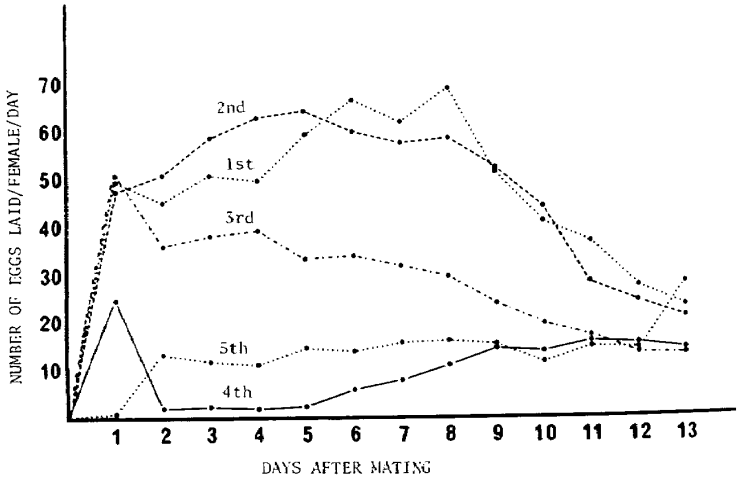

Fig. 6. Change of the number of eggs laid by females mated with serially mated males. Each point is the mean of 10 replicate vials which contained 5 females in each.

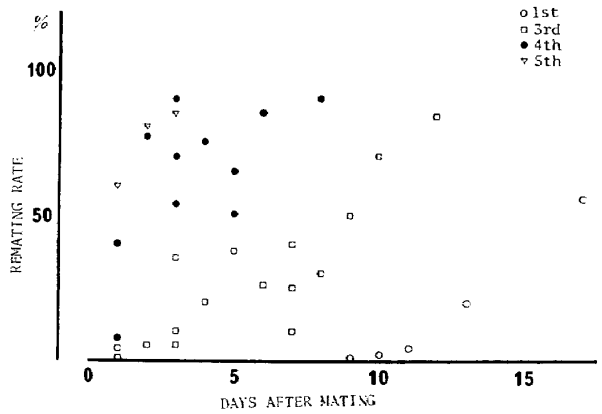

Fig. 7. The remating rate of females once mated with serially mated males. Each point is the mean of 20 females.
○; the First-mated females,
□; the Third-mated females,
●; the Fourth-mated females.
▽; the Fifth-mated females.

ler number of larvae; the number of the eggs laid by the Fifth-mated females was about one tenth as much as that of the Third-mated females.

Thus, it is clear that there was no significant decrease in the proportion of adults emerging from eggs laid from the First- to the Fourth-mated females. This was in marked contrast to the reduction of the number of eggs laid as shown in Fig. 6. It can be concluded that the reduction of the accessory gland secretion may not affect fertility of the sperm but oviposition in the female.

This conclusion can be supported by the following fact. The Fourth-mated females which oviposited only for one day after the first mating were remated twice with X/O sterile males at intervals of 5 days (Fig. 9).

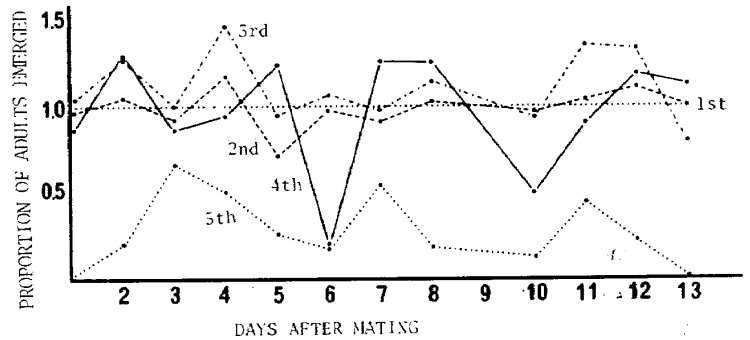

Fig. 8. Proportion of the adults emerging from the eggs laid, relative to that of the First-mated females which are assigned the value of 1.0.

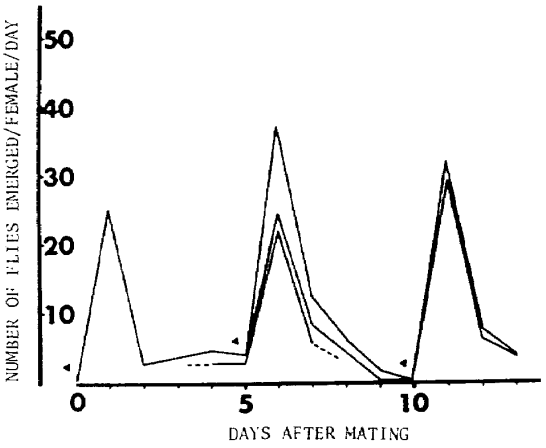

Fig. 9. Number of adult flies emerging from the Fourth-mated females which remated with X/O sterile males at intervals of 5 days. ▼; time of the sterile matings.

These females laid fertilized eggs only for one day after each of the sterile matings. It is clear that the sperm can survive and maintain fertility for at least 10 days under the conditions in which the amount of the secretion of the male accessory gland are not sufficient.

Discussion

Studies on structure and function of the male accessory gland were relevantly reviewed by Fowler (1973) on species of *Drosophila* and by Leopold (1976) on insect species in general including *Drosophila*. The author intended to confirm the role of the male accessory gland on oviposition and mating reluctance in the mated females, using sterile males who transmit their secretion of the accessory gland but not sperm to females. Serially mated males were also employed, since they were expected to transmit their sperm but not the secretion to their mates.

One of the main characteristics of females inseminated by sterile males was that they did not oviposit for more than one day (Figs. 1, 2a). The number of the eggs laid was about 40 per female, which was nearly equivalent to the number of the ovarioles. Results shown in Fig. 1 indicate that the rate of further vittelogenesis in females inseminated by sterile males was the same as that of virgin females. These facts suggest that sterile males can stimulate ovulation of the mature eggs of the ovarioles but not accelerate vittelogenesis.

One of the consitituent of the secretion of the male accessory gland is substances with filamentous structures. The filaments are thought to be generated in the grandular cells which constitute the wall of the apical half of the accessory gland (Acton, 1966; Bairati, 1966, 1968; Tandler *et al.*, 1968; Beaulton and Perrin-Waldemer, 1975; Perrin-Waldemer, 1977). If these filaments are responsible for the vittelogenesis, a short durability of the effects on reproductive behaviors of females inseminated by sterile males can be considered as follows. The filaments were not able to enter into the ventral receptacles or spermathecae by themselves in the case of sterile copulation, whereas they were successfully introduced into these target organs with sperm when fertile copulation occurred.

Another feature in females mated with sterile males was that they recovered their receptivity in a short time after copulation (Fig. 2b). The cause of this can also be explained by the point of view mentioned above; the filaments may contain a substance(s) affecting female receptivity. At any rate, temporal observations on the behavior of the filaments after copulation seem to be important to know better the role of the secretion of the male accessory gland.

The purpose of the male serial matings was to observe the response of females who actually received only the sperm in the later times of the serial matings. Results obtained here were as follows. (1) Duration both of the oviposition and unreceptive state became shorter in females as her mates increased the number of times of serial matings (Figs. 6, 7). This was suggestive that the secretion from the male was consumed at a uniform rate in the female reproductive organs. This assumption may not be in accordance with the ideas

of the "copulation effect" and the "sperm effect", both proposed by Manning (1967). (2) Sperm transfer occurred without the secretion of the accessory gland (Fig. 5), however these sperm maintained fertility for a long time in the female reproductive organs (Figs. 8, 9).

Thus, it is likely that neither sperm transfer nor sperm fertility is dependent upon the secretion of the male accessory gland. The result concerning sperm transfer was not consistent with that of Lefevre and Jonsson (1962), in which the secretion was indispensable for sperm transfer.

Chen (1976) reported that the electrophoretic pattern of the secretion of the accessory gland was highly species-specific among *Drosophila* species. This topic arouses us to examine if the species differences are in the biochemical nature of the secretion, especially of the filaments, and if the species diffrences are effective as one of the post mating isolation mechanisms; females cannot effectively oviposit even if interspecific copulations did occur.

Acknowledgments

I wish to express my thanks to Dr. Hiroshi Ikeda of Ehime University for his invaluable advice. Thanks are also due to Dr. Alan R. Templeton of Washington University for critical reading of the manuscript.

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