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Some Aspects of Courtship Behavior in *Drosophila nigromaculata*, with Regard to Sperm Storage¹⁾²⁾

By

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(With 1 Text-figure and 3 Tables)

Drosophila nigromaculata belongs to the *quinaria* species group of the subgenus *Drosophila* (Kikkawa and Peng, 1938). It is remarkable for its wide geographical distribution in Hokkaido (Momma 1957; Shima 1960; Wakahama *et al.* 1963; Kaneko and Shima 1965; Makino *et al.* 1964, 1965, and some others).

Since 1958, the present author has undertaken a study on some sexual activity of *D. nigromaculata*. Insemination reaction and sperm storage in once-mating specimens were published by Shima (1966a, b). This paper reports the results of some observations, regarding courtship behavior, remating and sperm storage of this species in homogamic matings.

Here the author's sincere gratitude is expressed to Professor Sajiro Makino for his keen interest in this subject, and also to Dr. Eizi Momma for his expert guidance and encouragement through out this work. He is obliged to Dr. Shôichi F. Sakagami for being given important suggestion. Cordial thanks are also due to Dr. Akasi Kaneko for his valuable advice and friendly assistance.

Material and method: Two strains of flies coming from Sapporo and Matsumae (southern part of Hokkaido) were used for study. These strains have been maintained in the laboratory for two or three years. After emergence, males and females were kept separately at a constant temperature of 20°C for 8 days, at the time when sexual activity reached its maximum. Flies of uniform ages were employed for the study of courtship behavior. Two females were placed together with one male in a clean vial of 1.5×7.5 cm, and they were observed through a period of 30 minutes. The male choice method was adopted for the observation (Dobzhansky and Mayr 1944). Courtship behavior was analyzed and recorded according to the nomenclature described by Spieth (1952).

1) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, June 21, 1966.

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The remating experiments were carried out in 120 small vials as follows: the first mated female was placed individually per a vial, into with two virgin males. Virgin males were always used as the second mate. After been placed with the

Table 1 Number of females remated on various days after the first mating

Days after first mating (8 days after emergence)	Number of females		Total females examined
	second mating	non remating	
2-7	0	20	20
8	13	7	20
9	15	5	20
10	18	2	20
11	18	2	20
12	20	0	20

second males, the females were kept under observation for a period of 2 hours. If the second mating did not ensue during the observation period, the female was separated from the second males. On the next day the same female was paired again with the second male. When the second matings occurred, the second males were removed, and the remated females were individually subcultured. Further, the mating more than twice was also tested in a few females. Some of females used in the second mating test were dissected at varying intervals ranging from 2-5 minutes to about 4 days after copulation. The preparation was covered with a coverglass, with special care. The reproductive tract was observed under a binocular microscope.

The test of male potential in successive insemination was carried out as follows: A virgin male was placed together with four females in a vial. Observations were made for 4 hours.

Generally the observations were made in the morning at room temperatures ranging from 23 to 26°C. All the flies were cultured at a constant temperature of 20°C, use being made of the standard medium.

Results

Courtship behavior of *D. nigromaculata* was described as below:

1) Shortly after a pair of flies were placed in a vial, the male oriented himself toward the passing female. He quickly followed her, either with rapid vibration of his wings (usually, more than 10 strokes), or directing his wings slightly upward, and keeping this angle.

2) The female reacted towards the courting male as follows: either she faced the male directly, or moved her abdomen when the male displayed his wings. Finally she either stood still to receive the male or left him.

3) The male tapped the female first with one or both of his fore legs on her wing of one side, and finally on her genital region and then with the proboscis.

4) He circled her and made a dance head to head with her, sometimes touching her antennae with his own, or he remained at a small distance from her. When the female remained stationarily he could make circling completely around her several times (either clockwise or counter-clockwise).

5) Then the male licked her genitalia with his proboscis, while in most cases the female spread her wings in a sharp angle upwards.

6) The male mounted and grasped the posterior part of her thorax, unless she did not refuse him by kicking, fluttering her wings, running or flying away, closing her wings and twisting her abdomen towards inside.

Immediately after copulation, the female usually made a movement of circle from left to right or inversely in the state of mounts and sometimes took a short walk, and finally stood still.

Shortly after copulation, most males reassumed courtship behavior to other females in the vicinity.

The males courted continuously up from 5 seconds to 20 minutes. Courtship duration of the male was in general prolonged when the female was not ready to mating, but a long courtship sometimes seemed to be effective to put unreceptive females in mating condition. Copulation usually took place after courtship of 10 to 60 second. Average time of copulation was about 6 minutes. It was sometimes reduced by female's refusal during copulation. No attempt was made to examine whether these short time copulations included ejaculation, or not. Generally, courtship patterns of this species were not different between the first and the second matings.

The results of remating were given in the accompanying Table 1 and 2. The duration until occurrence of the second mating after the first mating varied from 8 days to 12 days, except a few females (Table 1). When females were dissected soon after copulation, the ventral receptacle and the spermathecae were completely filled with sperm, and reaction mass reached its maximum size at an hour as shown in Fig. 1 C, in similar manner to the first mated females. A great number of sperm were retained in the storage organs for several days (Fig. 1 and Table 2).

The uniform aged virgin males (8 days after emergence) were used, as test of a male potential in successive insemination, to mate in succession with several 8-days-aged virgin females. It was evident that a male was capable of mating with three different females within 3 or 4 hours. The dissection of females confirmed that some males were especially able to inseminate as many as six different females within a period of 3 days.

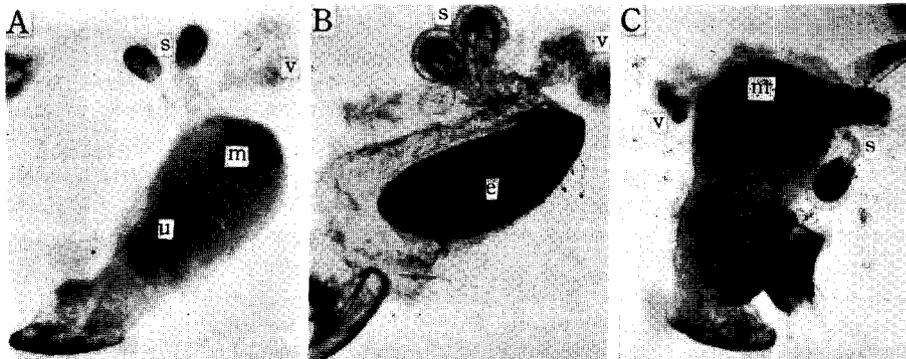
Discussion

Courtship is the ritual which precedes mating, and the components of this ritual are so specific that they may be useful for taxonomic characteristics

Table 2 Insemination reaction, and survival of sperm within the ventral receptacle and spermathecae, of females dissected at regular intervals after the second mating

Time of dissection	Number of dissected females	Insemination reaction	Sperm			Remarks
			uterus	ventral recep.	spermathecae	
before remating	15	none	—	+	+	8 days after first mating
2 minutes	15	reaction mass in uterus	‡‡	‡	+	few sperm in both organs
5 minutes	20	small mass	‡	‡	‡	highly motile sperm in both organs
30 minutes	15	mass enlarge	+	‡	‡	same condition
60 minutes	15	mass maximum	+	‡‡	‡	ventral receptacle full of sperm
2 hours	15	densely mass	+	‡‡	‡‡	both organs full of sperm
12 hours	15	same condition	—	‡	‡‡	same condition
24 hours	15	clearing	—	‡	‡‡	sperm reduced in both organs
48 hours	15	uterus normal	—	‡	‡	sperm more reduced
96 hours	15	no change	—	+	‡	sperm more reduced in ventral receptacle

‡‡ large amount of sperm ‡ less sperm + a few sperm — no sperm

Fig. 1. Preparations of the reproductive and sperm storage organs. $\times 80$.

- A The reaction mass grew into maximum size at an hour after the first mating.
 B Motile sperm remained alive in the sperm storage organs, just before the second mating (8 days after the first mating).
 C The reaction mass repeatedly grew into maximum size at an hour after the second mating.
- e, egg; m, reaction mass; s, spermathecae; u, uterus; v, ventral receptacle.

(Spieth, 1952). Courtship behavior is also a part of the sexual isolating mechanisms (Koref-Santibañez and Solar, 1961, 1963). The discriminating ability of both sexes of *D. nigromaculata* does not always correspond to their courtship behavior. A general mating pattern of *D. nigromaculata* resembles in many aspects other species belonging to the subgenus *Drosophila* group as described by Spieth (1952), but differs in a few points: they are 1) the males may court continuously up from 5 seconds to 20 minutes, 2) a copulation takes place usually after courtship of 10 to 60 seconds, and 3) most females make movement of circle from left to right or inversely being as mounts and they occasionally take a short walk.

The reproductive organs were dissected at different times from immediately to about 40 days after the first copulation. In the dissection of 2 minutes after copulation, it became evident that the uterus was enlarged with an appearance of opaque reaction mass containing many sperm and granular-like substances. Fifteen minutes after copulation a bigger mass was found in the uterus and reached its maximum size at an hour (Fig. 1 A). Some motile sperm were observed in the ventral receptacle and spermathecae. Motile sperm maintained alive in the ventral receptacle from 2 minutes to 360 hours, and in spermathecae from 5 minutes to 480 hours after first copulation (Shima 1966b). Lefevre and Jonsson (1962) showed that in *D. melanogaster* one insemination was normally enough to fill those organs to its capacity, leaving no space for storage of sperm from second mating, and that although fecundity of twice-mated females did not significantly exceed once-mated females, the majority of their progeny derived from sperm of second mating. Gugler *et al.* (1965) concluded that within 20-minutes sperm from the second mating could replace sperm in the first mating in both storage organs of *D. melanogaster*.

In *D. nigromaculata*, a female preserved some sperm within her storage organs when she remated. The anatomy of the receptacle and spermathecae seems to indicate that no mechanism on preservation of a double volume of sperm exists in a well-inseminated female. The sperm storage organs are non-elastic and do not bulge even in repletion with sperm. The second mating is generally the same as the first one, in an insemination reaction and survival of sperm within the ventral receptacle and spermathecae at regular time intervals after mating (Shima 1966b). On the 8th day after the first mating, the female (just before the second mating) preserved a few survived sperm in the storage organs (Fig. 1B). As far as the scope of the present observations is concerned, it is not evident whether these survived sperm were displaced or diluted by the sperm from the second male. The preliminary experiments for remating more than twice resulted in that they could mate a few times in their lifetime (Table 3).

In conclusion, sperm displacements, sperm mixing, and the sequence of sperm utilization have remained as questions for further studies.

Summary

The courtship behavior in *D. nigromaculata* differs in some aspect from other species of the same subgenus *Drosophila*, showing generally no difference between the first and the second pairings. It required 8–12 days that a female

Table 3 Variability in number of eggs laid during lapses since the time of mating (8 days after emergence) in an example out of the data on remating more than twice

Copulation time	6'22''																
Days after mating	1	2	3	4	5	6	7	8	9	10	11	12	13	14	total		
No. of eggs laid	5	2	4	15	14	2	8	5	4	0	5	5	11	25	105		
No. of hatched eggs	5	2	4	13	9	2	8	3	3	0	5	2	0	0	56		
Hatchability															53.3%		
Second mating Copulation time	5'50''																
Days after first mating	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	total	
No. of eggs laid	1	11	24	10	0	9	11	46	30	15	30	30	26	0	28	271	
No. of hatched eggs	0	9	19	6	0	6	3	10	7	0	12	16	0	0	0	88	
Hatchability															32.5%		
Third mating Copulation time	6'09''																
Days after first mating	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	total	Grand total
No. of eggs laid	4	3	1	0	6	4	4	2	5	4	9	4	1	2	0	49	425
No. of hatched eggs	4	3	0	0	4	0	2	0	0	0	0	0	0	0	0	13	157
Hatchability															26.5%	36.9%	

happened to mate with the second male after mating with the first male. When the females were dissected at regular intervals after remating, many mortile sperm were observed in the storage organs for several days, as reported in once-mated.

A virgin male was capable of inseminating in three successive different females within 4 hours.

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