Copulation Duration and Sperm Transfer
in the Melon Fly, *Dacus cucurbitae*
COQUILLET (Diptera: Tephritidae)^{1,2}

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Prolonged copulation occurs in many orders of insects. Its evolutionary significance has been discussed with particular reference to sperm competition (reviewed by PARKER, 1970; GROMKO et al., 1984; GWYNNE, 1984; TSUBAKI, 1987). In many cases, evidence suggests that it involves post-copulatory mate-guarding by males (e.g., SILLEN-TULLBERG, 1981; McLAIN, 1981; SIVINSKI, 1983).

The melon fly *Dacus cucurbitae* begins copulation at dusk. Copulation normally continues during the night and terminates at dawn (KUBA and SOEMORI, 1988). Copulation duration is usually longer than 10 hr and genital contact is maintained during the entire period. As the copulation is generally initiated during a limited period of time (dusk), it is unlikely that prolonged copulation functions as post-copulatory mate-guarding.

TSUBAKI and SOKEI (1988) showed that the amount of sperm a male transfers increased with the duration of copulation in *D. cucurbitae*. They suggested that a "sperm-loading" hypothesis (DICKINSON, 1986), which suggests that males mating with females for long periods benefit by numerically overwhelming the sperm of competitors, may explain the prolonged copulation of *D. cucurbitae*. To evaluate the amount of sperm a male transfers, they categorized it into 6 groups depending on the relative amount of sperm in the spermathecal duct and the spermatheca. For more precise discussions, however, it is necessary to describe quantitatively the amount of sperm transfer as a function of copulation duration, which TSUBAKI and SOKEI

(1988) could not show due to technical problems.

In this study, we directly examined the amount of sperm stored in the spermathecae of *D. cucurbitae* to clarify the rate of sperm transfer to females in relation to copulation duration. We show that the amount of sperm stored in females increases continuously to a saturated state which occurs about 4 hr after the initiation of copulation, and that the prolonged copulation does not thereafter contribute to the increase in the amount of sperm stored.

Materials

Flies used in this experiment were obtained from a stock which had been reared in the Mass Rearing Facility of the Okinawa Prefectural Fruit Fly Eradication Project Office according to the method described by NAKAMORI and KAKINOHANA (1980), and KAKINOHANA (1982). This strain originated from Itoman, Okinawa in May/June, 1985. About 37 generations had been produced since the beginning of mass-rearing.

Flies that emerged on September 30, 1989 were sexed on the 2nd day of their emergence and maintained in screen cages (30 cm × 30 cm × 45 cm) provided with water and a 4:1 mixture of sugar and autolyzed yeast (Asahi Beer Company Dried Yeast AY-65®). Rearing conditions were kept at 25 ± 1°C and 60 ± 20% RH with natural day length.

Copulation

A twenty-day old virgin male and a virgin female were introduced into a plastic cup (120 ml) and allowed to copulate ($n=112$). Copulated pairs were artificially separated 0.5, 1, 2, 4, 8, and 12 hr after the initiation of copulation ($n=12-16$ for each treatment).

Measurement of sperm number

The method for examining the amount of spermatozoa a female had in her spermathecae was according to TSUBAKI and YAMAGISHI (unpublished) with some modification. Females were dissected 3 to 7 days after copulation. Since the duration before dissection might bias the data due to sperm mortality (TSUBAKI and YAMAGISHI, unpublished), we tried to choose females evenly from all treatments on each day. Spermathecae were detached from

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the bursa copulatrix and placed into a drop of water on a slide glass. They were then crushed and cut into small pieces with fine forceps, releasing the spermatozoa. The resultant fluid was stirred with a needle to evenly distribute the spermatozoa. It was then spread on slide glass (about 18×18 mm) and air-dried. The number of spermatozoa was counted under a $\times 200$ magnification microscope. Since it was difficult to examine the entire area where spermatozoa was distributed, a fixed area of 35 samples was systematically chosen using a grid-type micrometer (in total, ca. 4.8% of the entire area). The average number of spermatozoa in these samples was then multiplied by an appropriate multiplying factor to estimate the total spermatozoa number.

Change in sperm number

The results of sperm counts in relation to copulation duration is shown in Fig. 1. During the first 0.5 hr no sperm was transferred to the female. Then sperm stored increased continuously to about 6,400 throughout the duration of the copulation until 4 hr.

Fig. 1. Number of sperm within spermathecae as a function of copulation duration. The solid line is drawn to connect mean number of sperm. There are not significant differences in mean numbers of sperm between copulation durations of 4, 8 and 12 hr (t -test, $P > 0.05$).

No sperm was transferred during earlier phases of copulation. This coincided with the observation of TSUBAKI and SOKEI (1988). This is also consistent with the results in KUBA and SOEMORI (1988) which showed that a copulation longer than 0.5 hr was needed to produce fertile eggs.

TSUBAKI and SOKEI (1988) reported a continuous increase in the amount of sperm storage throughout copulation by using a categorizing method. They also suggested that the rate of sperm transfer during the first 2 hr is higher than that during later periods. We have obtained direct evidence for this suggestion by counting the sperm number in spermathecae.

Furthermore, Fig. 1 shows that the number of sperm stored do not increase significantly after 4 hr (t -test, $p > 0.05$). This result suggests that the prolonged copulation of *D. cucurbitae* cannot be explained solely by the "sperm-loading" hypothesis.

What is the function of copulation lasting longer than 4 hr? KUBA and SOEMORI (1988) reported that females of *D. cucurbitae* which mated for a short period (shorter than 160 min) usually recopulated within 2 days after the first mating. On the other hand, females that had mated for a long period (longer than 10 hr) remated around the 12th day after the first mating. In the housefly *Musca domestica*, MURVOSH et al. (1964) found that almost all sperm were transferred within the first 10–15 min of copulation, although copulation lasts about 1 hr. Females which copulate once usually become unreceptive to a further copulation attempt by males, however, RIEMANN et al. (1967) reported that females which interrupted copulation after the first 15 min are as receptive as virgin females. PARKER (1970) suggested that extra copulating time might be of value in the transfer of agents necessary to initiate unreceptivity in the female. This may be the case in *D. cucurbitae*.

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Agglutination Test Using Protein-A-Coated Latex Particles for Detecting the Nuclear Polyhedrosis Virus of the Silkworm, *Bombyx mori* (Lepidoptera: Bombycidae)¹

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We developed latex agglutination tests for the detection of the infectious flacherie virus (IFV), the cytoplasmic polyhedrosis virus (CPV), the denso-nucleosis virus (DNV), and the nuclear polyhedrosis virus (NPV) of the silkworm, *Bombyx mori* (SHIMIZU et al., 1983; SHIMIZU and ARAKAWA, 1986; ARAKAWA, 1989). However, the test for NPV was not as sensitive as those for other viruses because of the low titer of the anti-virus IgG (ARAKAWA, 1989).

In this paper, we modified the latex agglutination test by coating the latex particles with protein A

before coupling with IgG according to QUERFURTH and PAUL (1979) and SOMOWIYARJO et al. (1987) for the detection of the NPV.

Polyhedra were collected from the hemolymph of NPV-infected silkworm larvae, and dissolved in carbonate solution (80 mM Na₂CO₃ + 25 mM NaCl). Liberated virions were purified by sucrose density-gradient centrifugation, as in KAWARABATA and FUNAKOSHI (1980).

Anti-NPV rabbit IgG was prepared as in ARAKAWA (1989). This IgG was coupled with latex particles (Sekisui Chemical Co., Ltd.) previously coated with protein A (E. Y. Labs. Inc.). Protein A was dissolved in distilled water containing 0.05% NaN₃ at a concentration of 1.0 mg/ml. Before use, it was further diluted with 0.1 M glycine buffer, pH 8.2, containing 0.5 M NaCl. The procedures of SHIMIZU and ARAKAWA (1986) were employed for agglutination tests.

In the preliminary agglutination tests, in which 0.3 µm, 0.4 µm, 0.5 µm, 0.6 µm, 0.7 µm, 0.8 µm, and 1.0 µm diameters of latex particles were compared, the best sensitivity was obtained with 0.5 µm-dia. particles. This latex particle size was therefore used throughout the rest of the experiments.

Latex particles (0.5 µm dia.) were coated with protein A by mixing with an equal volume of diluted protein A solution and incubating for 4 hr

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