
Yuki Satoh,* Shuichi Yano and Akio Takafuji

Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606–8502, Japan

(Received 9 March 2000; Accepted 1 September 2000)

---

**Abstract**

Precopulatory guarding behavior in males of *Tetranychus urticae* occurs because only the first mating is effective for females. Sufficient copulation duration to fertilize females in the laboratory is shorter than it is in nature. This finding leads to the hypothesis that the copulation duration of *T. urticae* is prolonged by postcopulatory guarding which prevents the female from remating with other males. To test this hypothesis, we manipulated the interval between the first and second copulations of a female. A significant positive correlation was detected between the mating interval and the proportion of daughters fathered by the first male of each brood. Males using postcopulatory guarding are successful when other males disturb the mating pair to take over from the mating male. These results demonstrate that males gain from prolonged copulation that prevents a female from remating until their sperm precedence is assured. Thus, males of *T. urticae* increase their paternity not only by precopulatory guarding but also by the postcopulatory guarding.

**Key words:** *Tetranychus urticae*, copulation duration, postcopulatory guarding, allozyme, double mating

---

**INTRODUCTION**

In many sexually reproducing animals, females often mate with more than one male. In such species, males who can increase the paternity gain more offspring. Consequently, characteristic behaviors of males to prevent mates from remating will evolve as adaptive strategies. Such behaviors may include blocking the female’s reproductive tract (mating plugs), prolonging copulation after completion of sperm transfer (prolonged copulation), and guarding females from other males (postcopulatory guarding) (Parker, 1970). In prolonged copulation, experimental observations are essential to discriminate the boundary between the time needed for sperm transfer and that for guarding females.

Males of the arrhenotokous spider mite, *Tetranychus urticae* Koch, often guard quiescent female deutonymphs (last nymphal stadium). This behavior is precopulatory guarding because only the first mating is effective for females of the mites (Potter et al., 1976a, b; Potter and Wrensch, 1978). However, the minimal copulation duration for fertilizing a female is shorter than that observed without disruptions (Overmeer, 1972; Potter and Wrensch, 1978). Furthermore, the second mating of a female may become effective if the first mating is artificially interrupted (Potter and Wrensch, 1978). These observations suggest that the copulation duration of *T. urticae* is prolonged by a postcopulatory guarding which prevents the female from remating.

In the present study, we determine the minimal copulation duration needed to fertilize a female by artificially manipulating the duration of copulation. We also examined whether the prolonged copulation of *T. urticae* males may function as postcopulatory guarding by manipulating the interval between the first and second copulations of the female.

**MATERIALS AND METHODS**

**Mites.** Strains of *T. urticae* (green form) used in this study were collected from sow thistles (*Sonchus oleraceus* L.) and chickweed (*Stellaria media*) at a chrysanthemum (*Dendranthema grandiflorum* (Ramatuelle) Kitamura) garden in Taima City, Nara Prefecture on May 21, 1998. The mites were mixed and maintained on detached bean (*Phaseolus vulgaris*, one of the most preferred hosts for *T. urticae*) leaves placed on wet cotton in petri-
dishes (90 mm in diameter and 14 mm deep). The dishes were placed in transparent plastic containers to maintain humidity, in a growth chamber controlled at 25°C: 16L 8D.

To examine the paternity of the mite, we used malate dehydrogenase (MDH) allozymes as a genetic marker. Goka and Takafuji (1995) reported a polymorphism for MDH and found that the MDH was a dimeric enzyme regulated by two codominant alleles (we refer to them as “S” and “F”) segregating at one autosomal locus. Allozymes are appropriate genetic markers to test sperm precedence of spider mites because they are generally considered to be neutral traits.

**Electrophoretic methods.** In the following MDH analysis, adult females were individually homogenized in 10 µl of 32% (w/v) sucrose with 0.1% Triton X-100 and 0.002% bromophenol blue. The electrophoresis experiments with polyacrylamide vertical slab gels and the MDH staining recipe followed the method used by Goka et al. (1996). The gels were 1 mm thick and 100 mm× 80 mm in area and the concentration of acrylamide was 6.5% in the separating gels and 2.5% in the stacking gels. Electrophoresis was carried out at a constant current of 20 mA/gel at 4°C. MDH was stained by placing the gels for 1 h in a 0.1M Tris-HCl (pH 8.9) solution containing 80 mg of DL-sodium malate, 10 mg of NAD⁺, 1 ml of 1% (w/v) nitro blue tetrazorium and 1 ml of 0.6% (w/v) phenazine methosulphate per 100 ml of buffer.

**Mite strains and genetic marker.** We detected two alleles (“S” and “F”) at the MDH locus in the mite population. By the following artificial selection over two generations, we obtained two homozygous strains (hereafter “S-strain” and “F-strain”). First, we randomly selected 40 mated females from the population. The females were individually introduced onto bean leaf squares (10×10 mm) pressed on water-saturated cotton in petri-dishes described above. We allowed the females to oviposit for 3 days. The MDH genotypes of all mothers were then analyzed by electrophoresis. The eggs laid by 5 females which had “SS” and five which had “FF” genotypes for MDH were reared to adults. Second, 5 sib-mated F₁ daughters obtained from each of the females were individually allowed to lay eggs for 3 days in the same manner as above. MDH genotypes of the F₁ daughters were then analyzed. We mixed each of the five lineages produced by the F₁ daughters with the respective homozygous genotype for MDH to establish two homozygous strains (“S-strain” and “F-strain”). The two strains were maintained in the growth chamber described above. There was no sexual incompatibility between the two strains.

**Copulation duration for fertilization.** In order to determine the minimal copulation duration necessary to fertilize a female’s eggs (hereafter “threshold time for fertilization”), copulation duration was artificially manipulated. To obtain virgin females, we isolated 20 virgin females on a bean leaf disc, and their sons (haploids) were reared in a growth chamber at 30°C: 16L 8D to accelerate development. Twenty five pairs of virgin females and virgin males were placed on bean leaf squares (5×5 mm). They were all 1- to 2-day-old adults. Each copulation was interrupted at various times between 30–200 s by removing the male with a fine brush. We observed mating behavior by a binocular microscope at room temperature (between 20–28°C) during the daytime (10:00–16:00). The copulation duration was measured by a stopwatch. Mated females were individually transferred onto bean leaf squares (2×2 cm) in petri-dishes placed in transparent plastic containers and were allowed to oviposit for 5 days at 25°C: 16L 8D. We counted the number of eggs laid and reared them to adults at 25°C: 16L 8D. We then recorded the number of daughters to estimate the effectiveness of each copulation assuming that the juvenile mortality was zero.

**Factor determining the copulation duration.** To elucidate whether or not prolonged copulation may act as postcopulatory guarding, double mating experiments were conducted. We used virgin females of S-strain and virgin males of both strains. Virgin females and males were obtained in the same manner as described before. First, we allowed a female to mate with a one-day old male of S-strain. The copulation was artificially interrupted at 60 s, which is sufficient copulation duration to fertilize a female, and the male was removed with a fine brush. After an interval of 40–1,320 s, we allowed the female to mate with a F-strain male without artificial interruption. The interval between the two matings was regarded as artificial post-cop-
ulatory guarding. If a female did not mate with a second male, we excluded the data from analysis. The number of females tested was 27. We observed the copulation under a binocular microscope at room temperature during the daytime. The copulation duration was measured by a stopwatch.

After the double-mating, the females were individually transferred onto bean leaf squares (2×2 cm) in petri-dishes and were allowed to oviposit until death at 25°C: 16L 8D. To avoid the coexisting of different generations, we transferred the females onto new leaf squares every 4th day. We counted the number of eggs and reared them to adults at 25°C: 16L 8D, and counted the number of daughters (i.e. fertilized eggs) from each females. Because spider mites of the family Tetranychidae have a haplodiploid mating system, only daughters (diploids) were preserved as samples to examine their paternity. The mean number of daughters produced by each mother was ca. 42, and the total number of samples analyzed by the electrophoresis was 1,130. Because the males used in the first copulation were S-strain and those used in the second were F-strain, a daughter fathered by the first male should have homozygous genotype for MDH genotype (SS), whereas a daughter fathered by the second male should have a heterozygous genotype (FS). We calculated the proportion of daughters fathered by the first male for each family. The data were transformed by arcsine root transformation, and the correlation between mating interval and sperm precedence of the first males was analyzed.

RESULTS

Threshold time for fertilization

No daughters were produced by a female only when the copulation was interrupted at 30 s. When copulation lasted for more than 40 s, all females produced some daughters. There was no significant correlation between the copulation duration and the number of female offspring (Fig. 1; $r=0.095$, $p=0.6517$, $n=25$). This result showed that the minimal duration of copulation to fertilize females eggs (threshold time for fertilization) was shorter than 40 s, and fertilization rate did not increase when copulation lasted for more than 40 s. In addition, there was no significant correlation between the copulation duration and fecundity (number of eggs per 5 days) of females (Fig. 2; $r=0.021$, $p=0.9220$, $n=25$).

Factor determining the copulation duration

A significant positive correlation was detected between the mating interval and the proportion of daughters fathered by the first male of each brood (Fig. 3; $r=0.433$, $p=0.0347$, $n=24$). Thus, the second mating became less effective as the mating interval increased. We excluded impractical cases in which the mating interval (artificial postcopulatory guarding) exceeded 1,000 s because the maximum length of copulation duration was less than 1,000 s (personal observation).

DISCUSSION

The results showed that the threshold time for
fertilization was shorter than 40 s. By the double-mating experiment, we revealed that the sperm precedence of the first male increased with the interval between the first and second copulation. In other words, the paternity of the first male is assured by a prolonged copulation that prevents a female from remating with other males. When the functional sex ratio becomes skewed towards males, males frequently disturb the mating pair attempting to take over the mating male (Potter and Wrensch, 1978; personal observation). Especially under such conditions, males without postcopulatory guarding behavior would not be favored. The prolonged copulation of *T. urticae* males may be categorized as “postcopulatory guarding”, and the sperm precedence of the first mating in *T. urticae* (Helle, 1967; Potter et al., 1976a) may be partly due to this postcopulatory guarding. Thus, males of *T. urticae* increase their paternity not only by precopulatory guarding (Potter et al., 1976a) but also by postcopulatory guarding.

There are two alternative hypotheses that we should consider to explain the prolonged copulation. Firstly, the longer copulation may allow a male to transfer more sperm or some substance which enhances the fertilization rate. For *T. urticae*, however, this hypothesis is discarded because no positive correlation was found between the copulation duration and the number of female offspring, although there may be a positive correlation under 60 s. Secondly, the longer copulation may permit a male to invest more nutrients which enhance the number of offspring. Since adult females of *T. urticae* convert nutrients obtained by feeding into their eggs within a day (Yano et al., unpublished), nutritional investment by a male, if any, should increase egg production. However, our results showed no positive correlation between copulation duration and egg number. Thus, the second hypothesis can also be rejected.

In general, females benefit from preferring males that supply superior genes and/or resource investment to their offspring (Krebs and Davies, 1993). Females also benefit from multiple matings either because they can supplement the depletion of the sperm stores, and/or gain genetically superior offspring as a result of sperm competition between the multiple males (Walker, 1980; Yasui, 1997, 1998). If so, the decision by females may also be an important factor in determining the copulation duration. But in the case of *T. urticae*, we have never observed virgin females, shortly after eclosion, rejecting the males’ attempt to mate. Therefore the decision by a female is unlikely to be an important factor determining the copulation duration in *T. urticae*.

In *T. urticae*, it had been suggested that the duration of the first copulation determines the effectiveness of the second mating (Potter and Wrensch, 1978). Our results show that the effectiveness of the second mating will depend on the duration of postcopulatory guarding involved in the first mating.

There are two possible mechanisms by which artificial postcopulatory guarding might enhance sperm precedence. First, fertilizing eggs would take some time, which males must gain by prolonging copulation. This mechanism is possible because it has been inferred that the spermatozoa of tetranychid mites leave the spermatheca and travel through the haemolymph to the ovary, where eggs are fertilized (Smith and Boudreaux, 1972; Pijnacker and Drenth-Diephuis, 1973). Secondly, males may form mating plugs or insert some substance into the female’s reproductive tract which would take some time to inactivate the spermatozoa transferred by rematings. Potter and Wrensch (1978) reported that the second matings are ineffective in older females whose sperm stores had been deficient. If the mating plugs are responsible for this result, the second mechanism is also possi-
ble. In either case, more detailed investigations are needed to clarify the mechanism of the sperm precedence of the first male in *T. urticae*.

**ACKNOWLEDGEMENTS**

We are grateful to Dr. M. Inoue, Nara Fruits Promotion Center, for collecting the mites. We appreciate valuable suggestions and encouragement from Dr. J. Takabayashi, Dr. T. Nishida of Kyoto University and Dr. Y. Yasui of Kagawa University. Thanks are also due to Dr. K. Goka, National Institute for Environmental Studies, for helpful advice in the electrophoretic analysis.

**REFERENCES**


