

Males of the two-spotted spider mite attempt to copulate with mated females: effects of double mating on fitness of either sex

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Abstract In *Tetranychus urticae* (Acari: Tetranychidae), when the intervals between first and second copulation are more than 24 h, only the first copulation is effective for females. Therefore, adult males should copulate only with virgin females, but not with females that copulated more than 1 day ago. Indeed, *T. urticae* males preferred virgin females to mated females under dual choice conditions. In the absence of virgin females, however, 60% of males copulated with mated females ($n = 30$). Therefore, the effects of male copulation behaviour on male and mated-female fitness were examined, respectively. Since *T. urticae* is arrhenotokous (i.e., only daughters have genes derived from their father), the proportion of females among the offspring was used as an index of male fitness. After males had lived with/without a mated female, the males were allowed to copulate with a virgin female. The proportion of females among the offspring did not differ between males with and without a female. On the other hand, when mated females lived with an adult male, their egg production was lower than mated females without a male. These results suggest that males do not seem to obtain fitness benefit from the copulation behaviour and that mated females incur a fitness cost due to the male behaviour.

Keywords Copulation · Fitness · Male harassment · Mated female · Tetranychid mite

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Introduction

Natural selection should favour individuals who have traits to maximize their own fitness (Krebs and Davies 1993). Therefore, it is argued whether traits, such as behaviour and morphology, are an adaptation in terms of costs and benefits (e.g., Soler and Martínez 2000; Tompkins et al. 2004; Ohba et al. 2008).

In *Tetranychus urticae* (Acari: Tetranychidae), when the intervals between first and second copulation were more than 24 h, only the first copulation results in fertilization (Helle 1967). Moreover, mating ability of males is limited (Krainer and Carey 1989). Therefore, it is predicted that *T. urticae* males copulate only with virgin females. In *T. urticae* and the related species, however, males often attempt to copulate with mated females (for example in *T. ludeni*, Puttaswamy and Channabasavanna 1979; in *T. urticae* and *Tetranychus kanzawai*, K. Oku, personal observation; see “Results”). Males of *T. urticae* do not benefit from copulation with mated females, but may affect subsequent copulation with virgin females. By contrast, male sexual coercion imposes fitness cost on females. For example, male sexual harassment reduces female feeding time, which results in low reproductive success of females (e.g., Magurran and Seghers 1994; Liana 2005; Sakurai and Kasuya 2008). In this study, I first examined whether *T. urticae* males prefer virgin females to mated females and whether males attempt to copulate with mated females. Second, I tested whether *T. urticae* males benefit from copulation with mated females and whether females incur fitness cost by multiple mating. Here, the proportion of females among offspring was used as an index of male fitness, whereas the number of eggs laid during a certain period was used as a female fitness index.

Materials and methods

Mites

Tetranychus urticae (Acari: Tetranychidae) was reared on Lima bean plants (*Phaseolus lunatus* L. cv. Sieva) in a greenhouse ($25 \pm 5^\circ\text{C}$, 50–70% relative humidity, L16:D8) at Wageningen University, The Netherlands.

The life cycle of *T. urticae* consists of egg, larva, quiescent larva, protonymph, quiescent protonymph, deutonymph, quiescent deutonymph and adult stages. Since *T. urticae* is arrhenotokous, virgin females can produce only sons, while mated females can produce both daughters and sons. There are some behavioural differences between males arising from virgin females and males arising from mated females in *T. urticae* (Ohzora and Yano 2008). Therefore, only males arising from virgin females were used in this study. To obtain adult *T. urticae* males arising from virgin females, quiescent deutonymph females were randomly selected from stock cultures. They were transferred onto leaf discs (ca 40 cm²) placed on water-saturated cotton wool, and after adult emergence, allowed to oviposit under laboratory conditions. When eggs had developed to the quiescent deutonymph stage, they were transferred onto new leaf discs and kept under laboratory conditions until adult emergence. All adult males were obtained by this procedure. Furthermore, the performance of *T. urticae* adult females is affected by the density during development (Oku et al. 2002). Therefore, one generation prior to the one used in the experiments was reared separately from the stock cultures. To reduce density effects, ten adult females randomly selected from stock cultures were transferred onto each of a set of leaf discs and allowed to oviposit at $25 \pm 5^\circ\text{C}$ and 50–70% relative humidity, with a L16:D8 photoperiod (hereafter called

'laboratory conditions'). Individuals of the next generation were used for the following experiments.

Perception of female mating status by males

Tetranychus kanzawai males prefer virgin females to mated females (Oku et al. 2005). To examine whether *T. urticae* adult males also recognize the mating status of females, 102 quiescent deutonymph females were randomly selected from the isolated leaf discs described above and divided into two groups on separate leaf discs. Twenty-five adult males were introduced into one group to obtain mated females, whereas the other group was not allowed to mate (virgin female). After 3 days, one virgin female and one mated female were transferred in pairs onto leaf squares (10×10 mm, $n = 51$) on water-saturated cotton wool. One female in each pair was randomly marked with ink to discriminate their mating status and allowed to acclimate for 2 h under laboratory conditions. Then, one unmated male (1 day old) was transferred onto each leaf square, and I recorded the mating status of the female that was first touched by the male. The data were analyzed using a binomial test to determine whether the result differed significantly from a 1:1 ratio.

Do males initiate copulation with mated females?

Since only the first copulation results in fertilization in *T. urticae* when the intervals between the first and second copulation were more than 24 h (Helle 1967), it is predicted that *T. urticae* males do not copulate with mated females even in the absence of virgin females. To test this prediction, 30 mated females were prepared in the same manner as described above. Females (1 day old) were separately transferred onto leaf squares (10×10 mm) on water-saturated cotton wool and kept for 1 day under laboratory conditions. By using eggs laid during the 1 day that the female was on the disc, it was afterwards confirmed that all females had been already mated before this experiment. Then, one unmated male (1 day old) was introduced onto each of leaf squares, and the behaviour of the males was observed for 1 h. In tetranychid mites, when males attempt to copulate with a female, the male slips under the female from behind and holds her legs with his front legs. Then, the male opisthosoma is bent upward to bring the extruded aedeagus in contract with the female's genital opening (Cone 1985). Here, I considered that males which caught the back of a female and bent their opisthosoma intended to copulate with the female. The data were analyzed using a Fisher's exact probability test to determine whether the result differed significantly from the prediction (i.e., copulation:non-copulation = 0:30).

Effects of living with mated females on subsequent male fitness

Since only daughters have genes derived from their fathers, the proportion of females among the offspring was used as an index of male fitness. To examine whether the copulation with mated females affects subsequent male fitness, 78 leaf squares (10×10 mm) were prepared on water-saturated cotton wool. One mated female (2 day old) was transferred onto half of leaf squares ($n = 38$, with females), while the rest of leaf squares remained free of mites ($n = 40$, without females). After 1 day, one adult male (1 day old) was introduced onto each of the leaf squares and kept for 2 days under laboratory conditions. During this treatment, about half of the males found dead on the

leaves in each group, though the cause of this remained unknown. Therefore, only live males were used (with females: $n = 19$, without females: $n = 22$). At the same time, one virgin female immediately after adult emergence was introduced onto each of 41 leaf squares (5×5 mm). After 1 h, the male was transferred onto each of the leaf squares to copulate with the virgin female. The cases where males did not copulate with females within 1 h were excluded from this experiment. Then, the females were transferred onto leaf squares (30×30 mm) and allowed to lay eggs under laboratory conditions. After 5 days, the females were removed, and the leaf squares were kept under laboratory conditions until the eggs had developed to the deutonymph stage. The proportion of females among the offspring was measured on each of the leaf squares, and the data was compared between treatment groups using a t -test on arcsine-transformed data.

Effects of male presence on egg production by mated females

The number of eggs laid by *T. urticae* females within a certain period after maturation is often used as the fitness index (e.g., Yano et al. 1998; Oku et al. 2002). To examine whether male presence affects egg production by mated females of *T. urticae*, 70 mated females (1 day old) were prepared in the same manner as described above. Adult females of spider mites produce eggs by using nutrition from resources eaten within a day (e.g., Yano et al. 1998). To eliminate the effect of feeding prior to the experiment, the females were put into microtubes (0.5 ml) and starved for 1 day under laboratory conditions. Then, females were separately transferred onto leaf squares (10×10 mm) on water-saturated cotton wool. After 1 h, eggs laid by females were counted on each leaf square. One unmated male (1 day old) was introduced onto half of the leaf squares (male presence, $n = 35$), while the rest of leaf squares were served as controls (male absence, $n = 35$). They were kept for 1 day under laboratory conditions, and eggs laid by females were counted on each leaf square. The replicates where either females or males drowned during the 1 day of the experiment were excluded from the data. The number of eggs laid by females during the 1-day experimental period was counted and compared between treatments groups using a t test.

Although the egg production by *T. urticae* females was reduced in the presence of males (see “Results”), it is uncertain whether this was caused by male interference. Thus, to examine whether the presence of other individuals affect egg production by *T. urticae* females, 99 mated females that had been starved for 1 day were prepared in the same manner as described above. Sixty-six leaf squares were prepared on water-saturated cotton wool. Two females were transferred onto half of the leaf squares ($n = 33$), while one female was transferred onto the rest ($n = 33$). They were kept for 1 day under laboratory conditions, and eggs laid by the females were counted on each leaf square. The replicates where females drowned during the 1 day were excluded from the data. The number of eggs laid per female was calculated and the data was compared between treatments groups using a t test.

Results

Perception of female mating status by males

The number of adult males that approached virgin females first was significantly greater than the number of males that approached mated females (37:14, $P < 0.001$), suggesting that *T. urticae* males can recognize the mating status of females.

Do males initiate copulation with mated females?

Twenty-four males (80%) caught mated females, and 18 males out of the males (75%) copulated with mated females ($P < 0.0001$). This result was contrary to my prediction, indicating that males initiate copulation with mated females in the absence of virgin females.

Effects of living with mated females on subsequent male fitness

The proportion of females among their offspring did not differ between males that had previously lived with a mated female and males that had previously lived without females ($t = -1.468$, $df = 37$, $P = 0.151$; Fig. 1).

Effects of male presence on egg production by mated females

In the presence of males, egg production by *T. urticae* females was reduced as compared with the absence of males ($t = -2.25$, $P = 0.028$; Fig. 2a). On the other hand, the presence of other females did not affect egg production by *T. urticae* females ($t = 0.02$, $P = 0.984$; Fig. 2b).

Fig. 1 Effects of living with mated females on subsequent male fitness. The proportion of females among offspring was used as an index of male fitness

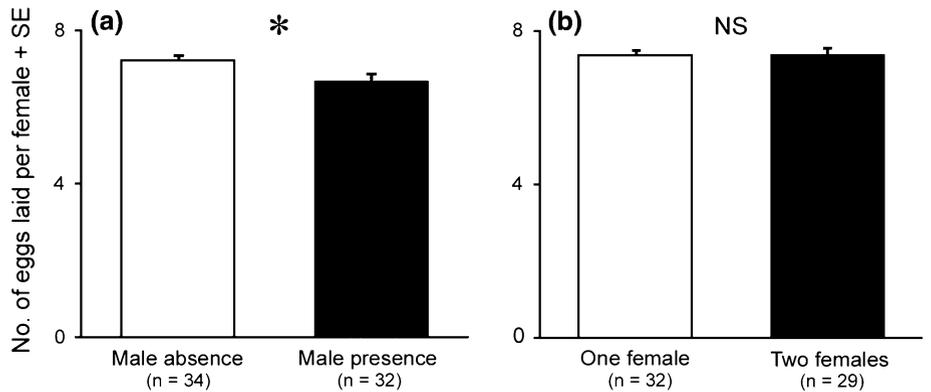
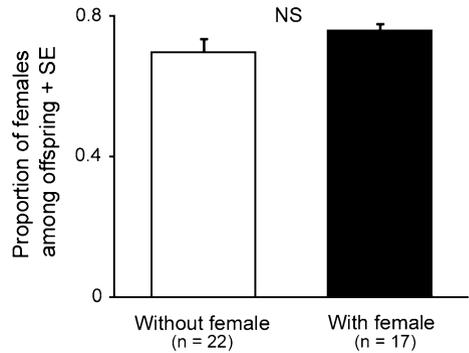


Fig. 2 Effects of the presence of **a** a male and **b** a female on egg production by mated females. * $P < 0.05$ (t -test)

Discussion

Tetranychus urticae males appear to be able to recognize the mating status of females, just like *T. kanzawai* (Oku et al. 2005). In the absence of virgin females, however, more than half of males copulated with mated females. In *T. urticae*, when the intervals between first and second copulation were more than 24 h, only the first copulation results in fertilization (Helle 1967). Since mated females used in this study would have copulated more than 1 day before experiments, the male copulation behaviour does not contribute to male fitness directly. There may be some possibility of enhancing male fitness indirectly. Thus, after a male lived with/without a mated female, the males were allowed to copulate with a virgin female. However, the proportion of females among their offspring did not differ between males with and without a mated female. From this result, there is no evidence to support the male copulation behaviour is adaptive. There may be an alternative explanation for the male copulation behaviour. When the intervals between first and second copulation were shorter than 24 h or when the first copulation was disturbed, the second copulation is effective in some cases (Helle 1967; Potter and Wrensch 1978; Satoh et al. 2001). Possibly, *T. urticae* males may not be able to discriminate between females that have recently copulated and those that have copulated earlier, which may motivate the male copulation behaviour.

On the other hand, egg production by mated females was lower in the presence of a male than in the absence of a male. However, egg production was not reduced in the presence of a female conspecific. These results suggest that mated females were disturbed by males and subsequently spent less time feeding or ovipositing resulting in a lower egg production. Thus, mated females appear to incur cost due to the presence of males. In *T. urticae*, mated females disperse from the natal colony and found a new colony (Hussey and Parr 1963) to avoid food deterioration (Kondo and Takafuji 1985; Oku et al. 2002). Mated females of *T. urticae* may also disperse to avoid costs of the disturbance by males.

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