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ARTICLE

Effects of density experience on mate guarding behavior by adult male Kanzawa spider mites

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Abstract In the Kanzawa spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae), adult males guard pre-reproductive quiescent females. I experimentally examined the effects of density experience during development and/or after adult emergence on precopulatory mate guarding behavior by *T. kanzawai* males. Mate guarding behavior was modified by density experience after adult emergence. When males had previously experienced high density after adult emergence ($n = 71$), 73.2% of them engaged in precopulatory mate guarding. In contrast, when males had previously experienced low density after adult emergence ($n = 82$), 61.0% of them did not guard females. Mate guarding with physical contact occurred more frequently when males had previously experienced a high density of potential rivals than when they had not, but the difference in behavior between the two groups of males was marginally not significant. Nevertheless, these results suggest overall that *T. kanzawai* males change mate guarding behavior in response to previously experienced density.

Keywords Adult male · Density · Precopulatory mate guarding · Quiescent deutonymph female · Tetranychid mite

Introduction

In general, male reproductive success increases with mate number (Bateman 1948). Therefore, sexual selection favors the evolution of various traits that enhance the reproductive success of males, including the ability to compete with conspecific males for fertilization (Krebs and Davies 1993). Mate guarding is one of several male mating strategies to prevent conspecific males from copulating with mates and has been observed in many taxa (e.g., insects: Alcock 1994; crustaceans: Conlan 1991; Jivoff and Hines 1998; arachnids: Potter et al. 1976; Yasui 1988; Dodson and Beck 1993).

Mate guarding behavior can be divided into two types: contact mate guarding (physical contact) and non-contact mate guarding (attending in close proximity to females without physical contact). Contact mate guarding is more effective in ensuring the reproductive success of males than non-contact mate guarding (e.g., Singer 1987; Convey 1989). However, contact mate guarding is sometimes energetically costly to males (Plaistow et al. 2003; Saeki et al. 2005; Sparkes et al. 1996) and conspicuous to predators (Zeiss et al. 1999; Cothran 2004; Oku and Yano 2008). Therefore, in species which use both contact and non-contact mate guarding, it is expected that males should change mate guarding behavior in response to their circumstances.

In tetranychid mites (Acari: Tetranychidae), adult males guard quiescent deutonymph females, the quiescent stage immediately before adult emergence (Potter et al. 1976).

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Quiescent deutonymph females take about 1 day to develop into the adult stage at 25°C (K. Oku, personal observation). Males mate with the female immediately after adult emergence (Potter et al. 1976; Cone 1985) for 193.51 ± 6.87 (mean \pm SE) s ($n = 29$; K. Oku, personal observation), and after mating the males soon leave the female. During precopulatory mate guarding, males mount or attend a quiescent female (Potter et al. 1976). According to the scenario described above, it has been predicted that males of tetranychid mites mount quiescent deutonymph females in the presence of rivals, while males attend quiescent females in the absence of rivals. However, in a previous study, most *Tetranychus kanzawai* males mounted quiescent females even when rivals were absent (Oku and Yano 2008).

The behavior of organisms may be modified by past experience (e.g., McCart et al. 1997; Carsten and Papaj 2005; Álvarez and Nicieza 2006; Turner et al. 2006; Frost et al. 2007). Flexible behavior allows an organism to adapt to its circumstances (e.g., Álvarez and Nicieza 2006; Frost et al. 2007). Thus, I hypothesized that previous experience would affect mate guarding behavior by *T. kanzawai* adult males. Density experience during development affects the fecundity of *T. kanzawai* females after adult emergence (Oku et al. 2002). I have examined the effects of density experience during development and/or after adult emergence on mate guarding behavior by *T. kanzawai* males. I report here the results and discuss mate guarding tactics in the light of these results.

Materials and methods

Mites

The study population of *T. kanzawai* was collected from convolvulus plants (*Calystegia japonica*) in Kyoto, Japan, and maintained on expanded primary leaves of kidney bean (*Phaseolus vulgaris*) pressed onto water-saturated cotton in petri dishes (diameter 90 mm, depth 20 mm). All of the dishes were placed together in a transparent plastic container and kept at $25 \pm 3^\circ\text{C}$, at $50 \pm 3\%$ relative humidity, under a long-day (16/8 h, light/dark) photoperiod (hereafter called ‘laboratory conditions’).

The life cycle of *T. kanzawai* consists of egg, larva, quiescent larva, protonymph, quiescent protonymph, deutonymph, quiescent deutonymph, and adult. To obtain *T. kanzawai* male eggs, I randomly selected 20 quiescent deutonymph females from the stock culture. I transferred these onto leaf discs in petri dishes, and after adult emergence, allowed them to oviposit under laboratory conditions. Since *T. kanzawai* is arrhenotokous, virgin females can produce only haploid sons. All male eggs used in this study were obtained by this procedure.

Effects of density experience on mate guarding behavior by *T. kanzawai* adult males

To examine whether density experience during development and/or immediately after adult emergence affects mate guarding behavior by *T. kanzawai* adult males, I prepared 110 leaf squares (10 \times 10 mm) pressed onto water-saturated cotton in petri dishes. I transferred one *T. kanzawai* male egg onto each of 100 leaf squares (low density) or ten male eggs onto each of the remaining leaf squares (high density). Since the density of adult males in the field is unknown, I considered ten individuals per leaf square to be a ‘high-density’ condition. The *T. kanzawai* individuals were kept under laboratory conditions until they developed to the quiescent deutonymph stage. At this point, the males from each of the two rearing densities were reorganized on new leaf squares into two groups of either low or high density. This resulted in four groups of males characterized by different combinations of rearing and adult density: (low–low density, $n = 47$; low–high density, $n = 4$; high–low density, $n = 35$; and high–high density, $n = 4$). The four groups of males were reared for 2 days and emerged as adults over this time. At the same time, to obtain quiescent deutonymph females on other leaf squares, I transferred one quiescent protonymph female from stock cultures onto each of 240 new leaf squares and kept them for 2 days under laboratory conditions. I then introduced one male onto each leaf square with one quiescent deutonymph female, alternating males from the four groups, and kept them under laboratory conditions. I used only the leaf squares on which quiescent deutonymph females had settled along veins because *T. kanzawai* males find more quiescent deutonymph females along veins than on the rest of the leaf area (Oku and Yano 2008). After 3 h, I recorded the behavioral situation of the males. I coined the case where males mounted quiescent deutonymph females as ‘mounting’ (Fig. 1a), that in which males were in close proximity (<0.3 mm) to females without physical contact as ‘attending’ (Fig. 1b), and that in which males were far from females (>0.3 mm) as ‘non-guarding’ (Fig. 1c). I observed each male for 5 s and then determined the behavioral situation. The data were analyzed using the

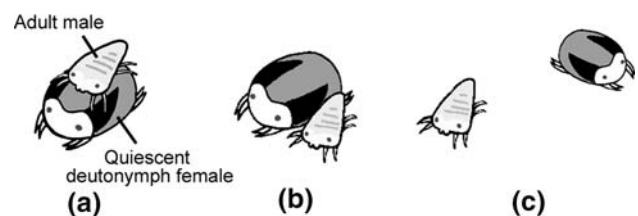


Fig. 1 Illustrations of mounting (a), attending (b), and non-guarding (c) by *Tetranychus kanzawai* adult males

non-parametric Wald test (SAS Institute 2006) to examine whether the density during development and/or after adult emergence has a significant relationship with mate guarding behavior by *T. kanzawai* males.

Effects of previous exposure to rivals on mate guarding behavior by adult males

The guarding behavior by *T. kanzawai* males was enhanced by a high-density experience after adult emergence (see Results). To examine whether this high-density experience with rivals affects mate guarding behavior by *T. kanzawai* males, I transferred one egg onto each of 90 leaf squares pressed onto water-saturated cotton in petri dishes and kept them under laboratory conditions until individuals developed to the quiescent deutonymph stage. I then transferred ten quiescent deutonymph males onto each of four leaf squares (with rivals, $n = 4$), and one quiescent deutonymph male onto each of 43 leaf squares. I added nine quiescent protonymph males from stock cultures onto each of the leaf squares with one quiescent deutonymph male (without rivals, $n = 43$) and kept them for 2 days under laboratory conditions during which the quiescent deutonymph males emerged as adults. Since quiescent protonymph males had just developed to the quiescent deutonymph stage during the 2 days, they were unable to be rivals of adult males (K. Oku, personal observation). At the same time, I prepared one quiescent deutonymph female on each of 160 leaf squares in the same manner as described above. I then introduced one male from either group onto each leaf square with one quiescent deutonymph female and kept them under laboratory conditions for 3 h. The behavior of the males was recorded in the same manner as described above, and the frequencies of behavioral categories between treatments were compared using the G-test.

Results

Effects of density experience on mate guarding behavior by adult males

Mate guarding behavior by *T. kanzawai* adult males was affected by their density experience after adult emergence ($P < 0.0001$, Table 1), while it was not affected by density experience during development ($P = 0.351$). Regardless of density experience during development, the proportion of mounting behavior by males that had previously experienced high density after adult emergence ('high-density male') was higher (23.7 and 15.2%, respectively) than that of males that had previously experienced low density after adult emergence ('low-density male') (8.5 and 0%, respectively; Fig. 2). Furthermore, the proportion of non-guarding

Table 1 Results of a Wald test on the effects of density experience during development and after adult emergence on mate guarding behavior by *Tetranychus kanzawai* males

Timing of density experience	df	Wald χ^2	P
Density during development	1	0.871	0.351
Density after adult emergence	1	19.099	<0.0001
Development \times adult	1	1.482	0.224

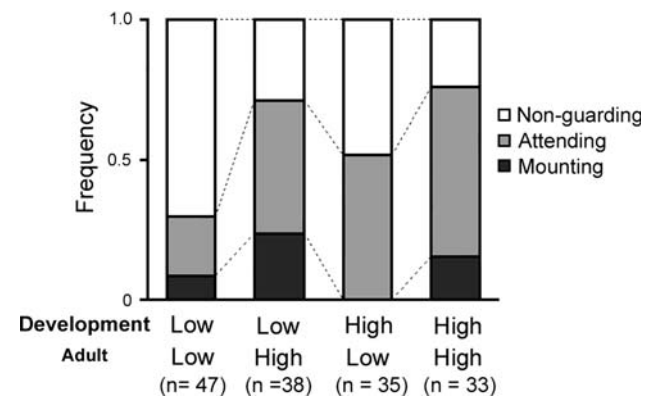


Fig. 2 Effects of density experience during development and/or after adult emergence on mate guarding behavior by *T. kanzawai* males. *Low* denotes low density and *high* denotes high density in terms of the previous experience of *T. kanzawai* males during development and/or after adult emergence

behavior was less in high-density males than in low-density males. In contrast, when males had experienced low density during development, the proportion of attending behavior was greater in high-density males than in low-density males. However, the frequency of attending behavior did not seem to differ between high-density and low-density males when they had experienced high density during development.

Effects of previous exposure to rivals on mate guarding behavior by adult males

A similar proportion of males guarded females whether they had been exposed to rivals as adults or not (with rivals 62.1%, without rivals 54.8%; Fig. 3). In terms of only males that guarded, the proportion that mounted females was higher among those that had been exposed to rivals (21.6%) than among those that had not (3.2%). However, the difference in guarding behavior between the two groups of males was marginally not significant ($G = 5.6998$, $P = 0.058$).

Discussion

Regardless of the density experience during development, *T. kanzawai* males that had previously experienced high

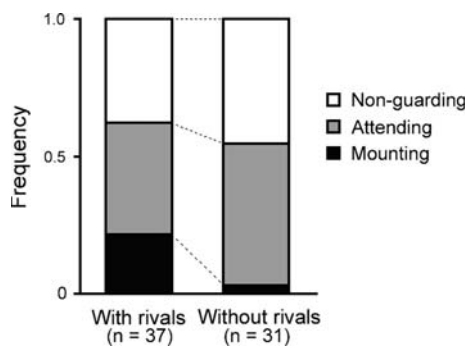


Fig. 3 Effects of rival presence on mate guarding behavior by *T. kanzawai* males

density after adult emergence (high-density male) guarded quiescent deutonymph females more frequently than those that had experienced low density after adult emergence (low-density male). This result indicates that the density experience after adult emergence affects mate guarding behavior by *T. kanzawai* adult males. A low-density experience during development could be counteracted by a high-density experience after adult emergence. In contrast, a high-density experience during development may linger on, such that low-density males previously exposed to high-density rearing conditions still attend quiescent deutonymph females.

Since spider mites have limited visible capacity, a density experience after adult emergence may be one of the important cues that determines subsequent behavior. A low-density experience may suggest that there are few rivals around the males. Males of tetranychid mites prefer to guard quiescent deutonymph females close to adult emergence (Potter et al. 1976; Everson and Addicott 1982; Royalty et al. 1993). Thus, low-density males may spend a great deal of time searching for older quiescent deutonymph females. On the other hand, a high-density experience implies that there are (some) male rivals around. Once high-density males have found a quiescent deutonymph female, they would therefore guard her. Indeed, a high-density experience with rivals seemed to enhance mounting behavior by *T. kanzawai* males compared to males not exposed to rivals—although the difference was not significant in this study. One reason for this may be that males were unable to definitively distinguish between rivals and non-rivals under high-density conditions. Also, in this study, male behavior was observed 3 h after the experiment had started. It has been reported for other animals, including some mites, that the duration of pre- and post-copulatory mate guarding is affected by the operational sex ratio (e.g., Radwan and Siva-Jothy 1996; Rondeau and Sainte-Marie 2001). Thus, if the past density experience of male *T. kanzawai* individuals modify their guarding time, then my findings might have been

somewhat different had I observed leaves at an earlier or later time after male introduction.

Contact mate guarding is usually more effective, but also more costly, than non-contact mate guarding for ensuring male reproductive success (e.g., Convey 1989; Singer 1987). In this study, the proportion of mounting behavior was higher among high-density males than among low-density males, suggesting that *T. kanzawai* males switch from an attending to a mounting strategy only when the benefits exceed the costs. *Tetranychus kanzawai* males may change mate guarding behavior in response to previous or current rival density. To clarify this possibility, future studies should examine whether mounting is more costly to *T. kanzawai* males than attending behavior.

Oku and Yano (2008) observed only mounting behavior as precopulatory mate guarding; however, most of *T. kanzawai* adult males guarded quiescent females. One reason for this result may be that in their experiments, Oku and Yano (2008) kept more than 50 males on the same leaf disc until they were used. Although the results of my study suggest that a high-density experience with rivals enhanced mounting behavior by *T. kanzawai* males, the proportion of mounting males was not very high. It is possible that my criterion of ‘high density’, ten individuals per leaf square, was not high enough to incite all males to mount.

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