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Is only the first mating effective for females in the Kanzawa spider mite, *Tetranychus kanzawai* (Acari: Tetranychidae)?

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Abstract Although only the first mating is effective for females in *Tetranychus urticae* (Acari: Tetranychidae), it remains unclear whether this is also true for closely related species, such as *T. kanzawai*. To address this question, I analyzed paternity in the progeny of *T. kanzawai* females that had been observed to copulate with two males by using a microsatellite DNA marker. In this study, mating was allowed to take place without experimental interruption. The results show that progenies were sired by both males in only 1 of 14 families, whereas progeny were sired only by the first males in the other families. This result suggests that only the first mating would be, by and large, effective in *T. kanzawai*.

Keywords Paternity · Double mating · Microsatellite marker · *Tetranychus kanzawai*

Introduction

Female receptivity for mating, which includes paternity of offspring, is different among species (Thornhill and Alcock 1983; Ringo 1996). Females of some species mate only once in their lifetime, while females of other species mate more than once. In order to gain an insight into the mating system of organisms, it is useful to examine female receptivity (Thornhill and Alcock 1983).

In tetranychid mites (Acari: Tetranychidae), adult males often engage in precopulatory mate guarding while the female develops in the final quiescence teleiochrysalis developmental

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stage (for example in *Tetranychus urticae*, Potter et al. 1976; Satoh et al. 2001; in *T. kanzawai*, Oku et al. 2003). Copulation lasts 193.51 ± 6.87 s (mean ± SE) ($n = 29$; K. Oku, personal observation) and after mating the males soon leave the female. This behavior suggests that only the first mating event leads to the siring of offspring, otherwise males would benefit more from spending their time and energy in mating as many females as possible, instead of guarding a single one for a long time period. Indeed, only the first mating is effective for females in *T. urticae* (Helle 1967; Potter and Wrensch 1978; Satoh et al. 2001). Although Oku et al. (2003, 2005) assumed that *T. kanzawai* has the same female receptivity characteristics as *T. urticae*, this has not been experimentally confirmed. Recently, molecular biological methods have become a powerful tool for paternity analysis even in mites (Yasui 1988, 1997; Satoh et al. 2001; Kolodziejczyk et al. 2002). Thus, I analyzed paternity in progeny of *T. kanzawai* females that had mated with two males by using one microsatellite locus, *Tk*MS006, isolated and characterized by Nishimura et al. (2003) as a DNA marker.

Materials and methods

Mites

The study population of *T. kanzawai* was collected from a convolvulus plant (*Calystegia japonica*) in Kyoto, Japan, and maintained on expanded primary leaves of kidney bean (*Phaseolus vulgaris*) pressed onto water-saturated cotton in Petri dishes (90 mm diameter, 14 mm depth). All dishes were placed together in a transparent plastic container and kept at 25°C, at 50% relative humidity, with an L16: D8 photoperiod (hereafter called 'laboratory conditions').

To establish homozygous strains for each of the alleles of the microsatellite locus, 60 virgin females and 60 adult males were randomly selected from the stock cultures and transferred onto leaf squares (10 × 10 mm) in pairs ($n = 60$). After mating, females were allowed to lay eggs for 3 days under laboratory conditions. Then, all females and males were collected from each leaf square and their genotypes were analyzed by PCR-based strain identification as described below. The leaf squares where females had laid eggs on were kept under laboratory conditions. As a result of the analysis, six couples and four couples of each homozygous genotype were detected. For each strain separately, the progeny of the selected couples was combined and introduced on bean leaves and kept under laboratory conditions.

Double mating

To elucidate whether insemination by the second males is effective or not, double mating experiments were conducted. For each strain, 10 teleiochrysalis females were transferred onto a leaf disc and kept for 1 day under laboratory conditions. After adult female emergence, they were separately transferred onto leaf squares (5 × 5 mm), and one adult male (1 day old) of the same strain with females was introduced onto each of the leaf squares. In this study, the process from insertion of male genitalia to its extraction was identified as a mating. As soon as the first mating was terminated by the couple, the male was removed from the leaf square, and one male of the other strain was introduced onto the leaf square. I excluded six females from this experiment because they did not remate within 60 min after introduction of the second males. After the double mating, females were individually

transferred onto 20 × 20-mm leaf squares ($n = 14$), and allowed to oviposit for 7 days under laboratory conditions. Subsequently, the adult females were removed from the leaf squares and the leaf squares were kept under laboratory conditions until the progeny developed into the adult stage. Then, the genotypes of progeny were analyzed by electrophoresis as described below. Since spider mites of the family Tetranychidae have a haplodiploid sex-determination system, only daughters (diploids) were preserved as samples to examine their paternity. The mean number of daughters produced by each female was 26.5, and the total number of individual progeny analyzed by the electrophoresis was 371. The males in the first mating have the same genotype as females. Thus, a daughter sired by the first male should have a homozygous genotype for the microsatellite marker, whereas a daughter sired by the second male should have a heterozygous genotype for this marker. The paternity rate of the first and the second males was statistically compared for each family by using a Wicoxon's signed-rank test.

DNA extraction

Each adult mite was homogenized in 20 µl of lysis buffer (10 mM Tris–HCl [pH 8.0], 100 mM EDTA, 0.5% Igepal CA-630 [Sigma], 10 mM NaCl, 1 mg/ml Proteinase K) using a pellet mixer in a sample tube. The homogenate was incubated at 65°C for 15 min and then at 95°C for 10 min. After incubation, the homogenate of mites was diluted with either 380 µl (female) or 180 µl (male) of 0.1 × TE buffer (1 mM Tris–HCl [pH 8.0], 0.1 mM EDTA) and stored at −20°C until use in a polymerase chain reaction (PCR) as a DNA template.

PCR amplification

The primers were forward: 5′-AGCGCGTTTACAGCTTTCAG-3′ and reverse: 5′-ACCA GGCCTATTGCAACTTC-3′ (see Nishimura et al. 2003). PCR amplification was conducted with 1 µl of each DNA template in a total reaction volume of 20 µl buffer containing 0.25 mM dNTPs, 0.5 units of Ex Taq^{TM} polymerase (TaKaRa), 10 pmol of each primer in 2.5 mM $MgCl_2$, and 1 × Ex Taq buffer (Mg^{2+} free) (TaKaRa). The reaction mixture was put into 0.2 ml PCR tubes, and amplification was performed in a thermal cycler (I Cycler, Bio-Rad Laboratories) with the following profile: 96°C for 3 min; 35 cycles of 96°C for 1 min, 52°C for 1 min, and 72°C for 2 min; and a final 10 min at 72°C for last strand elongation. The PCR products were checked by acrylamide gel (10%) electrophoresis using the 1 × TBE buffer system and visualization by ethidium bromide staining under UV light.

Results and discussion

Out of 14 progenies, one had been sired by both males, whereas the others were sired by the first male only ($z = -3.298$, $P = 0.001$; Fig. 1). Although there were several cases where DNA detection failed, this result suggests that only the first mating would be, by and large, effective in *T. kanzawai*. The number of egg production is reduced by density during development in *T. kanzawai* (Oku et al. 2002), which may be one of the reasons why the families differed in number of progeny (Fig. 1). In *T. urticae*, when the first insemination is insufficient, the second mating becomes effective (Helle 1967). This may be the reason why *T. kanzawai* progeny were sired by two males in family 1 (Fig. 1).

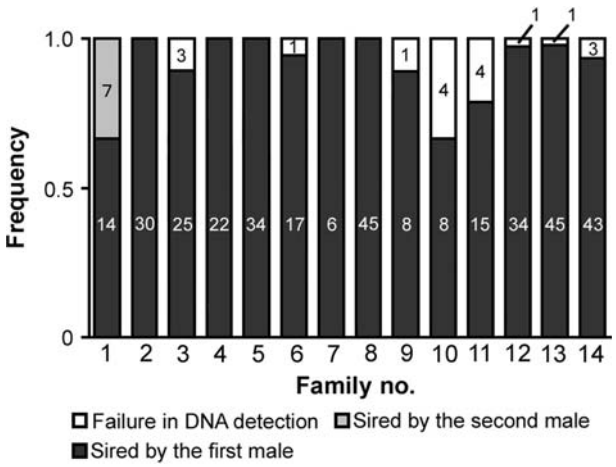

Fig. 1 Paternity in progeny of females that had mated with two males. Numbers inside bars represent the individual number of progeny in each family

In this study, the second mating was conducted after the first mating was completed. When the first mating is interrupted, however, the second mating becomes effective if the interval between the first and the second mating is short (for examples in *T. urticae*, Potter and Wrensch 1978; Satoh et al. 2001). In tetranychid mites, more than one male sometimes guard one teleiochrysalis female (Potter et al. 1976; K. Oku, personal observation). Furthermore, teleiochrysalis females already guarded by a male attract extra conspecific males (Potter et al. 1976; K. Oku, unpublished data). Therefore, it is possible that the first mating is interrupted by conspecifics and then multiple mating is conducted during a short period. Potter and Wrensch (1978) estimated that 14.3% of *T. urticae* females would receive two effective inseminations in crowded population. This point should be paid attention in order to understand their mating behavior. At this moment, it is difficult to say that tetranychid mites, like *T. kanzawai* and *T. urticae*, are not polyandrous. It is necessary to investigate the mating system of tetranychid mites in more detail in the future.

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