Female mate-receptivity behavior in multiple matings of a predacious mite, *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae)

Takashi Tsunoda and Hiroshi Amano†,*

Public Health Laboratory of Chiba Prefecture, Chiba 260–8715, Japan
† Laboratory of Applied Entomology and Zoology, Faculty of Horticulture, Chiba University, Matsudo 271–8510, Japan

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**Abstract**

The mate-receptivity behavior and reproductive capacity of single- and multiple-mated females of a predacious mite, *Amblyseius womersleyi*, were examined. Although most females rejected a second mating within one day after the first mating (1 day later), they accepted it towards the end of the oviposition period (10 days later). Females that had experienced full copulation twice produced significantly more eggs than those that had mated only once. All females that had copulated with a sterile male which had lost its ability to fertilize a female following a series of multiple matings, showed a positive response toward the second copulation on the next day. These results suggest that acquisition of sperm or seminal fluids is probably a critical factor for mating receptivity and that multiple matings are required in order to reach the full reproductive capacity for *A. womersleyi* females.

**Key words:** Phytoseiid mites, multiple matings, mate-receptivity behavior, reproductive capacity, spermathecae

**INTRODUCTION**

Phytoseiid mites include species known to be important natural enemies of spider mites and rust mites and are used as biological control agents throughout the world. The reproductive potential of phytoseiid mites needs to be maximized in order to keep the intrinsic rate of natural increase high. Multiple matings or re-mating are common among many phytoseiid mites studied (Dosse, 1955; Putman, 1962; McMurtry and Scriven, 1964; Prasad, 1967; Swirski et al., 1967a, b; Elbadry and Elbenhawy, 1968; Laing, 1969; Knisley and Swift, 1971; Zaher and Shehata, 1971; Ma and Laing, 1973; Takafuji and Chant, 1976; Amano and Chant, 1978a, 1986). Based on the effect of multiple matings on maximal egg production, phytoseiid mites can be categorized into two types (Schulten, 1985): one can reach maximal egg production in a single mating, and the other requires multiple matings. However, little information is available on the details of multiple mating in phytoseiid mites, and most studies thus far have focused on their pre-mating behavior (Amano and Chant, 1978b; Hoy and Cave, 1985; Tsunoda, 1994). We therefore examined the details of female post-mating behavior to evaluate the implications of multiple matings for phytoseiid mites.

The predacious mite, *Amblyseius womersleyi* Schicha, has a wide distribution throughout Japan, Korea, China, Taiwan, and Australia (Ehara and Amano, 1993). This species is regarded as one of the most important biological control agents for spider mites on greenhouse and field horticultural crops, due to its high performance under warm temperatures (Lee and Ahn, 2000). In this study, we specifically examined the following aspects in *A. womersleyi*: (1) the relationship between the duration of copulation and female fecundity; (2) mate-receptive behavior of the female in multiple matings; and (3) factors affecting female mate-receptive behavior.

**MATERIALS AND METHODS**

**Rearing of mites.** The population of *A. womersleyi* used in the present study was originally collected on leaves of the kudzu-vine, *Pueraria lobata* (Wild.) Ohwi, in a field in Chiba, central Japan, in June 1990. The population was maintained on a polyvinyl plastic sheet placed on a polyurethane sponge with water in a metal tray. The tray was kept in a chamber controlled at 27°C and a 14L10D photoperiod. The green form of the two-
spotted spider mite, *Tetranychus urticae* Koch, which was reared on a kidney plant, *Phaseolus vulgaris* L., was provided as prey. Eggs of *A. womersleyi* were taken from the polyvinyl plastic sheets and individually placed on bean leaflets (1 × 1.5 cm each) with abundant prey eggs (30–40/day). This system guaranteed a constant supply of males and females for the experiments.

For observation of experimental mites, the leaflet was placed on a filter paper that had been cut into strips (1 × 3 cm each). The strips were dipped in water to keep the leaflets fresh, and each strip was laid in a small transparent plastic vial (2.5 cm in diameter and 4.0 cm in height). The top of the vial was wrapped with a polyvinyl sheet and tied with a rubber band to prevent the escape of mites. All experiments were conducted in a chamber controlled at 27°C and a 14L10D photoperiod.

**Duration of copulation, spermathecal state and egg production.** Mating behavior was continuously observed under a microscope for a pair of phytoseiid mites on a leaflet with an ample supply of prey eggs. The leaflet was placed on a pad of cotton wool with water in a glass petri dish. Copulation was either allowed to complete or interrupted before completion with the aid of a fine paintbrush. The male was removed after copulation. The female was either transferred into a vial to observe its egg production or mounted on Hoyer’s medium to classify the spermathecal stage by adopting the criteria proposed by Amano and Chant (1978b). The diameter of the vesicles of the spermatheca was also measured.

The relationship between the duration of copulation (either completed or interrupted) and fecundity was examined. The number of eggs produced by the female was counted every day and after the count the female was transferred to a new vial. Thirty eggs of *T. urticae* were provided daily to each phytoseiid mite as prey.

**Acceptance of multiple matings by females.** We conducted two experiments to examine the females’ propensity to re-mate. First, we compared the behavior of females between one day and ten days after completion of the first mating. Second, we compared the behavior of females that had mated fully and those restricted to 1 h one day after the copulation. Total egg production was compared among three groups of females: those that had copulated fully one time; those that had copulated partially for 1 h at the first mating and copulated fully the next day; and those that had copulated fully at the first mating, rejected copulation the next day and re-mated fully on the tenth day.

**Key factors causing the rejection of re-mating.** To investigate whether mated females could recognize the absence of fertile sperm, we observed the re-mating behavior of females which had completed copulation with sterile males. Sterile males were produced by continuous multiple matings with virgin females. If the male failed to fertilize females for two successive days, as determined by a lack of egg deposition by females, we considered the male to be sterile. The rationale of sterility was based on observations by Amano and Chant (1978a), in which male phytoseiids never recovered insemination ability under similar experimental conditions.

**RESULTS**

**Duration of copulation, spermathecal state and egg production**

Regardless of differences in female age, most of the 25 fully mated *A. womersleyi* females had a spermathecal category (1, 1) as proposed by Amano and Chant (1978b), i.e. there was one sperm mass in one of two spermathecae, after a single mating (Table 1). The duration of copulation for these 25 females was 138.5 ± 23.9 (mean ± SD) min (range, 61–170 min), and the vesicle diameter of spermathecae was 0.019 ± 0.001 (mean ± SD) mm (range, 0.015–0.021 mm). There was a tendency for the duration of copulation to be positively correlated with the diameter of the vesicle (Table 1).

<table>
<thead>
<tr>
<th>Duration of Copulation (min)</th>
<th>Spermathecal Category</th>
<th>Egg Production (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>(1, 1)</td>
<td>38.0 ± 8.5</td>
</tr>
<tr>
<td>170</td>
<td>(1, 1)</td>
<td>23.9 ± 2.6</td>
</tr>
</tbody>
</table>

Table 2 shows that in eight females that experienced a single full mating, the fecundity averaged 38.0 ± 8.5 (mean ± SD) eggs and oviposition period 12.6 ± 2.6 (mean ± SD) days. When copulation was disrupted at 30 min, the females produced no eggs. The longer the duration of the copulation, the larger the number of eggs produced by the female (*p* < 0.001, Mann-Whitney’s *U*-test).

**Acceptance of multiple matings**

Most of the females that had copulated fully rejected the male’s approach to re-mate one day after the first mating and only 3 out of 42 females (7.1%) copulated at the second mating (Table 3).
On the other hand, 10 out of 13 females (76.9%) accepted re-mating ten days after the first mating. The acceptance rate of re-mating was significantly lower the day after mating than that of the first mating, but it increased on the tenth day ($p < 0.05$, Fisher’s exact test).

Among the females that copulated for only 60 min at the first mating, 83.3% re-mated on the following day. These females showed the same positive response towards males as virgin females.

Table 1. Relationship between the duration of copulation and the condition of the spermathecae in *Amblyseius womersleyi* females

<table>
<thead>
<tr>
<th>Duration of copulation (min)</th>
<th>No. of females examined</th>
<th>Diameter of vesicle* (mm)</th>
<th>No. of females in each category*</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>7</td>
<td>0.013±0.003</td>
<td>0-0</td>
</tr>
<tr>
<td>45</td>
<td>9</td>
<td>0.013±0.002</td>
<td>0-0</td>
</tr>
<tr>
<td>60</td>
<td>7</td>
<td>0.014±0.003</td>
<td>1-0</td>
</tr>
<tr>
<td>75</td>
<td>9</td>
<td>0.014±0.006</td>
<td>1-1</td>
</tr>
<tr>
<td>90</td>
<td>7</td>
<td>0.019±0.002</td>
<td>2-1</td>
</tr>
<tr>
<td>Complete</td>
<td>25</td>
<td>0.019±0.001</td>
<td>2-2</td>
</tr>
</tbody>
</table>

* Mean±SD; $n=$(No. of females)×2.

<table>
<thead>
<tr>
<th>Duration of copulation (min)</th>
<th>No. of females</th>
<th>Total number of eggs laid per female*</th>
<th>Oviposition period (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>7</td>
<td>0.013±0.003</td>
<td>0 a</td>
</tr>
<tr>
<td>60</td>
<td>7</td>
<td>12.4±4.4 b</td>
<td>4.6±0.5 b</td>
</tr>
<tr>
<td>90</td>
<td>8</td>
<td>21.6±3.8 c</td>
<td>6.6±0.7 c</td>
</tr>
<tr>
<td>Complete</td>
<td>8</td>
<td>38.0±8.5 d</td>
<td>12.6±2.6 d</td>
</tr>
</tbody>
</table>

* The results are shown as mean±SD. Means followed by different letters in a column are significantly different at $p<0.05$ by Mann-Whitney $U$-test.

Table 2. Relationship between the duration of copulation and egg production in *Amblyseius womersleyi* females

On the other hand, 10 out of 13 females (76.9%) accepted re-mating ten days after the first mating. The acceptance rate of re-mating was significantly lower the day after mating than that of the first mating, but it increased on the tenth day ($p < 0.05$, Fisher’s exact test).

Among the females that copulated for only 60 min at the first mating, 83.3% re-mated on the following day. These females showed the same positive response towards males as virgin females.

Table 4 shows that fecundity of females that re-mated fully on the tenth day was significantly higher than that of females which experienced either a single full mating or a full re-mating on the day following an incomplete first mating ($p < 0.05$, Mann-Whitney’s $U$-test).

All females that copulated with a sterile male at the first mating also accepted a normal male on the following day (Table 3). The duration of copulation with a sterile male was 120.6±45.8 min (mean±SD; $n=8$), and that with a normal male on the next day was 148.8±36.3 min ($n=8$). These values showed no statistical difference. All females that had copulated with sterile males had spermathecae categorized as (1, 1) with one sperm mass existing in one spermatheca ($n=7$).

**DISCUSSION**

Most phytoseiid mites have one or more sperm masses in each of the two spermathecae. These mites include: *A. andersoni* (Amano and Chant, 1978a), *A. potentillae* (Overmeer et al., 1982), *T. pyri* (Overmeer et al., 1982), *A. finlandicus* (Amano and Chant, 1986), *A. cucumeris* (Castagnoli and Liguori, 1991), and *T. exhilaratus* (Castagnoli and Liguori, 1991). Other phytoseiid mites have one sperm mass in only one of the two spermathecae, and these mites include *Phytoseiulus persimilis*, *A. bibens* (Amano and Chant, 1978b; Schulten et al., 1978) and *A. womersleyi*, the subject of this study.

When copulation was interrupted and the duration of copulation was shortened, the diameter of the vesicle in a spermatheca decreased in *P. persimilis* and *A. bibens* (Schulten et al., 1978). As the diameter of the vesicle reflected the sperm mass which could fertilize eggs, a reduction of copulation duration also caused the decrease of total egg deposition in *P. persimilis, A. bibens, A. andersoni, A. potentillae* and *T. pyri* (Amano and Chant, 1978a; Schulten et al., 1978; Overmeer et al., 1982). In the case of *A. womersleyi* in this study, although the relationship between the duration of
copulation and the diameter of the vesicle was not clear, a reduction in fecundity was observed as the duration of the copulation decreased.

In Drosophila females, for example, the degree of male acceptance increased as the spermathecae became depleted (Thornhill and Alcock, 1983). Most female flies mated again after about seven days, if given the opportunity to lay eggs freely after the first copulation and if males were provided at the appropriate time. However, the female regained receptivity relatively quickly if she received only mechanical stimulation from a copulatory partner but no sperm or associated fluids. The receipt of accessory gland fluids prolonged the period of female nonreceptivity. The combination of mechanical stimulation, accessory-gland materials, and sperm was speculated to be a key for slow recovery of female receptivity.

Most A. womersleyi females that copulated for 1 h and all of those that copulated with a sterile male, accepted re-mating on the following day in the present study. Stimuli from the male before and during copulation do not seem to influence the receptivity of females, since sterile males are considered to be normal in copulation judging from the mating duration. These results suggest that acquisition of sperm itself and/or succeeding fertilization of eggs is an important factor for mating acceptance in A. womersleyi females as well as Drosophila females.

In regard to structures surrounding sperm masses in spermathecae, Amano and Chant (1978b) proposed the following two possibilities: (1) the wall structure enclosing the sperm mass was the liquid injected by the male, or (2) the wall structure was formed as a result of a chemical reaction between the substance in spermatheca and the material injected by the male. After 30 min of mating, the sperm-like mass was found in the spermatheca but the female produced no eggs. It seems that in A. womersleyi a wall-like structure was first formed around the injected fluids and then fertile sperm was injected into the vesicle. As sperm-like masses were observed in spermathecae of females that had mated with a sterile male, there are two possibilities: either there is no sperm in the spermathecae or the sperm is sterile. In T. rickeri, the females re-mated and began to deposit eggs again.

### Table 3. Changes in mate-receptive behavior in *Amblyseius womersleyi* under different mating conditions

<table>
<thead>
<tr>
<th>Modea</th>
<th>First mating</th>
<th>Second mating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of females examined</td>
<td>No. of females that accepted matingb</td>
</tr>
<tr>
<td>Full</td>
<td>48</td>
<td>43 a</td>
</tr>
<tr>
<td>60 min</td>
<td>14</td>
<td>14 a</td>
</tr>
<tr>
<td>Sterile</td>
<td>7</td>
<td>7 a</td>
</tr>
</tbody>
</table>

a Full, the first mating was completed; 60 min, the first mating was interrupted at 60 min from the start; Sterile, the first mating was performed with a sterile male.
b Values followed by different letters are significantly different at p<0.05 by Fisher’s exact test.

### Table 4. Reproductive capacity of *Amblyseius womersleyi* females under different mating conditions

<table>
<thead>
<tr>
<th>Mating duration</th>
<th>First</th>
<th>Second</th>
<th>No. of females</th>
<th>Total number of eggs laid per femalea</th>
<th>Oviposition period (days)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>—</td>
<td>6</td>
<td>6</td>
<td>37.8±8.4 a</td>
<td>10.0±1.4 a</td>
</tr>
<tr>
<td>60 min</td>
<td>Full on the 2nd day</td>
<td>6</td>
<td>6</td>
<td>40.2±7.9 a</td>
<td>10.8±1.2 a</td>
</tr>
<tr>
<td>Full</td>
<td>Full on the 10th day</td>
<td>6</td>
<td>6</td>
<td>49.8±5.3 b</td>
<td>13.0±1.3 b</td>
</tr>
</tbody>
</table>

a The results are shown as mean±SD. Means followed by different letters in a column are significantly different at p<0.05 by Mann-Whitney U-test.
after they initially had ceased producing eggs (Mc-
Murtry and Scriven, 1964). *A. womersleyi* females in this study lost interest in males after a single full mating, but mating receptivity resumed as the females ceased egg production. We hypothesize that the termination of egg production will be the key stimulus for re-mating. Consequently, the females that had copulated for only 1 h had a short oviposition period (up to five days), but their mate-recep-
tive behavior decreased only slightly on the fol-
lowing day. These results suggest that acquisition of sperm or seminal fluids is a critical factor responsi-
ble for the loss of mating interest in *A. womersleyi* females.

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