Mating aggregation and copulatory success by males of the stink bug, *Megacopta punctatissima* (Heteroptera: Plataspidae)

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(Received 27 May 1999; Accepted 17 September 1999)

Abstract
In the stink bug, *Megacopta punctatissima*, which forms mating aggregations, male mating behaviors and the copulatory success of males were examined in experimental populations with 4 different sex ratios. In the male-biased sex ratio, more males tended to stay in "copulatory aggregations" including at least a mating pair rather than in "non-copulatory aggregations" including no mating pair, despite a scarcity of non-copulating females in such aggregations. However, when copulating females were included, there were more females in the copulatory aggregations than those in the non-copulatory aggregations. Therefore, the results appear to suggest that males also regarded copulating females as potential mates. Males which had immigrated to an aggregation showed a higher copulatory success than resident males in the aggregation at a male-biased sex ratio. Moreover, males showed a large variation in mating success, and those which had a higher mating success tended to stay in aggregations of female-biased sex ratios. These results suggest that females may choose their mates in mating aggregations, and that the choice may influence male mating success more acutely in a male-biased sex ratio.

Key words: *Megacopta punctatissima*, mating aggregation, copulatory success, female choice, sex ratio

INTRODUCTION

Many insect species form aggregations and mate in them. Insect aggregations can be divided into two major categories (Alexander, 1975). One is that in which females aggregate at a particular place (i.e., oviposition site, feeding site) where males then visit (e.g. Fujisaki, 1980, 1981; Mitchell, 1980; McLain, 1984, 1992; Miyatake, 1995), and the other is that in which males aggregate and appear to attract females; these are often called leks (see review by Shelly and Whittier, 1997). It is considered that in leks, aggressions between males and/or courtship by males allow females to choose mates among the aggregated males. It isn’t difficult to explain the significance of the choice when males offer resources to a female such as food and territories, especially when paternal care occurs. However, females may often receive no tangible benefits from their choice, and so the lek system is one of the most enduring problems in evolutionary biology (Reynolds and Gross, 1990; Kirkpatrick and Ryan, 1991; but see Pomiankowski and Möller, 1995).

Adults of the stink bug, *Megacopta punctatissima*, form mating aggregations (Hibino and Itô, 1983), and Hibino (1985) indicated that males play an active role in the process of formation and maintenance of the aggregations. Hibino (1986) showed that females of the bug accept courtships with a higher probability when males court in the aggregations compared to when they are solitary, and he suggested that female choice may be a selective force for the gregarious behavior of males. Moreover, increasing numbers of males in an aggregation resulted in a shorter residence time of a given male in the aggregation (Hibino, 1985), implying that males may compete for mates within the aggregation. However, how the gregarious habit of males influences their mating success has not been precisely examined as yet.

In this study, we investigated male mating behaviors and the copulatory success of males in experimental populations with 4 different sex ratios to elucidate the mating strategies of males in aggregations and the effects of sex ratios on the copulatory success of males.
MATERIALS AND METHODS

Adults of *M. punctatissima* were collected from the kudzu-vine, *Pueraria lobata*, at Kobe City on September 13, 1997. They were brought to a laboratory, and released on a soybean plant grown in a 1/5,000 a pot and enclosed in an acrylic cylinder of about 32 cm in diameter and 50 cm in height with a nylon net at the top. Bugs were reared at 20–25°C under constant light. The adults became sexually mature 30–40 days after the commencement of rearing. Thus, we regarded bugs collected from the kudzu-vine as newly emerged adults, because Tayutivutikul and Yano (1990) showed that the duration of the pre-reproductive period of *M. punctatissima* was about 30 days.

Bugs were individually marked with paint markers (Mitsubishi Paint Marker PX-21®). In order to synchronize physiological states in terms of reproductive activity, males and females were separately reared with some soybean pods in petri dishes (90 mm in diameter, 20 mm in depth) for more than 12 h before the commencement of each experiment.

Twenty adults were released at the base of a soybean plant grown in a pot with an enclosure as described above in four different sex ratios (male : female = 5 : 15, 7 : 13, 13 : 7, 15 : 5). To refer to these ratios, the term "population sex ratio" (= σ / (σ + 9)) was used. The experiments were carried out in a laboratory at 20–25°C under constant light. The same soybean plant was used repeatedly in the experiments for the four sex ratios. Therefore, the spatial structure of the soybean plant on which the bugs wandered was almost constant except for slight growth of the plant. The positions of each individual on the plant and copulating behavior were observed every hour for 48 h. Bugs wandering on the enclosure at the time of observation were immediately captured and released at the base of the soybean plant. Each bug was classified into a solitary (nongregarious) individual and a gregarious at each observation. Hibino and Itô (1983) regarded an aggregation as two or more individuals with a distance from the nearest neighbor of less than 3 cm, but in this study, considering the size of the plant, we used a distance of less than 1 cm. Ordinary size and composition of aggregations were examined from the data of all aggregations for 48 observations in each population sex ratio.

RESULTS

Two types of aggregations

There were two types of aggregations, one was the copulatory aggregation which consisted of at least one copulating pair and several or no individuals, and the other was the non-copulatory aggregation which consisted of only non-copulating male(s) and female(s).

Aggregation size

The size of copulatory aggregations was not significantly different among population sex ratios 5 : 15, 7 : 13 and 13 : 7 (Fisher's PLSD, *p* > 0.05), but that at 15 : 5 was larger than that in the other sex ratios (Fisher's PLSD, *p* < 0.05) (Table 1). The size of non-copulatory aggregations was not significantly different among all population sex ratios (Fisher's PLSD, *p* > 0.05). The size of copulatory aggregations was significantly larger than that of non-copulatory aggregations at all four sex ratio (Mann-Whitney U-test, *p* < 0.05) (Table 1).

<table>
<thead>
<tr>
<th>Population sex ratio (♂ : ♀)</th>
<th>Copulatory aggregationa mean ± SE</th>
<th>Non-copulatory aggregationa mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 : 15</td>
<td>3.988 ± 0.114 a (n=172)</td>
<td>2.237 ± 0.122 a (n=38)</td>
</tr>
<tr>
<td>7 : 13</td>
<td>3.718 ± 0.159 a (n=195)</td>
<td>2.286 ± 0.101 a (n=28)</td>
</tr>
<tr>
<td>13 : 7</td>
<td>3.671 ± 0.168 a (n=155)</td>
<td>2.493 ± 0.107 a (n=69)</td>
</tr>
<tr>
<td>15 : 5</td>
<td>5.837 ± 0.212 b (n=98)</td>
<td>2.517 ± 0.097 a (n=60)</td>
</tr>
</tbody>
</table>

aWithin copulatory and non-copulatory aggregations, significant differences are indicated by different letters (Fisher's PLSD, *p* < 0.05), and within each population sex ratio, there are significant differences between copulatory and non-copulatory aggregations (Mann-Whitney U-test, *p* < 0.05).
Non-copulating males in aggregations
The average number of non-copulating males staying in copulatory aggregations was significantly larger than that in non-copulatory aggregations at a population sex ratio of 15 : 5 (Mann-Whitney U-test, \( p < 0.05 \)), but there were no differences at the other population sex ratios (Mann-Whitney U-test, \( p > 0.05 \)) (Fig. 1).

The ratio of non-copulating females to non-copulating males (n.f./n.m.) in copulatory aggregations was not significantly different from that in non-copulatory aggregations at population sex ratios of 5 : 15 and 7 : 13 (Fisher’s PLSD, \( p > 0.05 \)) (Table 2), but the ratios in both copulatory and non-copulatory aggregation were more than one. At sex ratios of 13 : 7 and 15 : 5, n.f./n.m. ratios in copulatory aggregations were nearly equal 0, and significantly lower than that in non-copulatory aggregations (Fisher’s PLSD, \( p < 0.05 \); Table 2). The ratio of all females (both copulating and non-copulating females) to non-copulating males (f./n.m.) was higher in copulatory aggregations than in non-copulatory aggregations at sex ratios of 5 : 15, 7 : 13 and 13 : 7 (Fisher’s PLSD, \( p < 0.05 \)), but no difference was seen at a population sex ratio of 15 : 5 (Fisher’s PLSD, \( p > 0.05 \)) (Table 2).

Copulatory success of males
We defined a “resident male” as a male that remained at the same aggregation between successive observations, and an “immigrated male” as a male that immigrated into the aggregation during the interval of observations. We regarded the sum of immigrated and resident males when a new copulation occurred in the aggregation as the total number of potentially courting males (i.e., total copulatory attempts).

Then, we calculated the copulatory success rate of immigrant (I) and resident males (R) as the total number of copulations divided by the sum of immigrated and resident males, respectively.

Potentially courting males were more abundant in resident males than in immigrated males in all population sex ratios (Table 3). However, the copulatory success rate by immigrated males was significantly higher than that by resident males in a male-biased population sex ratio (Fisher’s exact probability test; combined 5 : 15 and 7 : 13, \( p = 0.508 \); combined 13 : 7 and 15 : 5, \( p = 0.0003 \)). The ratio of copulatory success by resident males to that by immigrated males (R/I) decreased with increasing population sex ratio (Table 3). The proportion of copulations by resident males to all copulations was over

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**Table 2.** Ratios of non-copulating females to non-copulating males (n.f./n.m.) and those of all females to non-copulating males (f./n.m.) in copulatory and non-copulatory aggregations

| Population sex ratio (♂ : ♀) | In a copulatory aggregation* | | In a non-copulatory aggregation* |
|-----------------------------|-----------------------------|-----------------------------|
| n                          | n.f./n.m. mean ± SE         | f./n.m. mean ± SE           | n                          | n.f./n.m. (= f./n.m.) mean ± SE |
| 5 : 15                     | 14                          | 1.643 ± 0.269 a             | 2.964 ± 0.248 b             | 13                          | 1.538 ± 0.312 a |
| 7 : 13                     | 16                          | 1.594 ± 0.286 a             | 3.000 ± 0.387 b             | 16                          | 1.052 ± 0.105 a |
| 13 : 7                     | 52                          | 0.128 ± 0.041 a             | 1.404 ± 0.116 b             | 39                          | 0.846 ± 0.060 c |
| 15 : 5                     | 88                          | 0.101 ± 0.025 a             | 0.734 ± 0.061 b             | 38                          | 0.711 ± 0.055 b |

*Within each population sex ratio, significant differences are indicated by different letters (Fisher’s PLSD, \( p < 0.05 \)).
50\% in low population sex ratios (5 : 15 and 7 : 13), but under 50\% in the high population sex ratios (13 : 7 and 15 : 5), indicating that resident males could mate more frequently than immigrated males in low sex ratios, and vice versa in high population sex ratios (Table 3).

**Variation in copulatory success of males**

There was a large variation in the number of copulations among males (Fig. 2). The average number of copulations decreased with increasing population sex ratio (mean = 4.60, 5.00, 2.23 and 0.60 in population sex ratios of 5 : 15, 7 : 13, 13 : 7 and 15 : 5, respectively), and the variance in terms of the coefficient of variation was larger in higher population sex ratios (CV = 52.4, 49.0, 71.2 and 122.8, in population sex ratios of 5 : 15, 7 : 13, 13 : 7 and 15 : 5, respectively).

When all data for the 4 population sex ratios were combined, it was found that males that had copulated more frequently tended to remain in aggregations with lower sex ratios (Spearman’s rank correlation coefficient, $r_s = -0.356$, $p < 0.0001$, $n = 1,106$), and also to remain for a shorter time in the aggregations in which they could not copulate (Spearman’s rank correlation coefficient, $r_s = -0.277$, $p < 0.0001$, $n = 277$).

**DISCUSSION**

In some Hemipteran species, male-male competition in which males fight with their hind legs occurs in mating aggregations (Mitchell, 1980; Fujisaki, 1981; Miyatake, 1993, 1995, 1997; McLain et al., 1993; Eberhard, 1998), and males also disturb copulations of other pairs physically (McLain, 1980; Carroll and Loye, 1990; Carroll, 1991; Tsukamoto et al., 1994). In *M. punctatissima*, we occasionally observed both in laboratory experiments and in the field that non-copulating males disturbed copulatory males by thrusting their bodies, not by using their legs.

In this study, it may be suspected from n.f./n.m. values (Table 2) that the male-male competition for mates was not apparent in the low population sex ratios (5 : 15 and 7 : 13), while it appeared to be more severe in the high population sex ratios (13 : 7 and 15 : 5). Nevertheless, the number of males in copulatory aggregations was nearly equal to that in non-copulatory aggregations at the 13 : 7 sex ratio, and was significantly larger at 15 : 5 (Fig. 1). In the population sex ratio of 13 : 7, the ratio of all females to non-copulating males (f./n.m.) in a copulatory aggregation was significantly larger than that of non-copulating females to non-copulating males (n.f./n.m.) in a non-copulatory aggregation, and at 15 : 5, the two ratios were nearly equal (Table 2). Therefore, it could be supposed that non-copulating males in copulatory aggregations may regard even copulating females as candidates for mates in the high population sex ratios (13 : 7 and 15 : 5).

Females of *M. punctatissima* usually reject courtships of males (Hibino, 1986), as seen in some other Hemipteran species, which may be

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**Table 3. Copulatory success rate by resident males in an aggregation and by those which immigrated to an aggregation**

<table>
<thead>
<tr>
<th>Resident males</th>
<th>5 : 15</th>
<th>7 : 13</th>
<th>13 : 7</th>
<th>15 : 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of potentially courting males</td>
<td>9</td>
<td>7</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Number of copulations</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Copulatory success rate (R)</td>
<td>89%</td>
<td>86%</td>
<td>20%</td>
<td>7%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Immigrated males</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of potentially courting males</td>
<td>4</td>
<td>6</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Number of copulations</td>
<td>4</td>
<td>6</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td>Copulatory success rate (I)</td>
<td>100%</td>
<td>100%</td>
<td>70%</td>
<td>36%</td>
</tr>
<tr>
<td>$R/I$</td>
<td>0.89</td>
<td>0.86</td>
<td>0.29</td>
<td>0.18</td>
</tr>
<tr>
<td>% Copulation by resident males</td>
<td>67%</td>
<td>50%</td>
<td>26%</td>
<td>20%</td>
</tr>
</tbody>
</table>
residences in aggregations in which they could not copulate. However, the present study did not investigate the difference of quality between immigrated and resident males. We need to make sure it in future researches.

In spite of advantages of immigrated males, many males tended to remain in an aggregation and to court as resident males (Table 3), which may imply that movements between aggregations may involve any cost. The cost-benefit balance between movement to another aggregation and remaining resident in the aggregation may determine the mating strategy of males.

Shelly and Whittier (1997) suggested some reasons why females prefer males that aggregate. One of them is that females prefer aggregated males for facilitation of female choice. Hibino (1986) found that *M. punctatissima* females prefer males in aggregations rather than solitary males. Thus, there is a possibility that females of *M. punctatissima* choose better mates among potential mates in aggregations. Furthermore, our results showed a large variation in the intensity of possible female choice. In the high population sex ratios, the number of copulations by resident males was smaller than that by immigrated males, though resident males copulated more frequently than immigrated males in the low population sex ratios (Table 3). These results indicate that the proportion of courtship acceptances by females may decrease with increasing population sex ratio, coinciding the prediction that female choice becomes more apparent when the number of males increase (Andersson, 1994; but see Arnyqvist, 1997). This seems to have brought about the result that the males which copulated more frequently tended to remain in the aggregations with lower sex ratio. The decrease of courtship acceptance by females may be caused by the following mating strategies of females. In the low population sex ratio, females should not be meticulous when choosing a mate. Rather the acquisition of any mate should take precedence because of the shortage of available mates. On the other hand, in the high population sex ratio, she should choose a mate carefully by evaluating the relative quality of males. These strategies by females might result in large variation in the copulatory success of males (Fig. 2) and an in-

Fig. 2. Number of copulations by males in different population sex ratios.

an evidence that intersexual selection had occurred (McLain, 1992; Tsukamoto et al., 1994). Females may be able to choose better mates by a relative evaluation through many courtships (Jang and Greenfield, 1998).

In this study, immigrated males could copulate more successfully than resident males in male-biased population sex ratio (Table 3). This result suggests the possibility that female choice occurred in the following manner. If females select their mates carefully or have a particular preference for mates, they may copulate with new immigrating males rather than with resident males that the females might have rejected once. Therefore, as our results showed, males may have higher mating success by shortening
crease in the variance with increased population sex ratio (Bateman, 1948; Thornhill and Alcock, 1983; but see Sutherland, 1985).

In summary, our results suggest that females of *M. punctatissima* choose their mates in mating aggregations and the choice influences male mating success more severely in higher sex ratios. Furthermore, male-male competition, perhaps more weakly than female choice, also seems to influence the mating success of males. However, though the bugs may have a polygamous mating system, we did not investigate whether or not the males more successful in copulation can gain higher fitness, i.e., whether sperm competition and cryptic female choice (Eberhard, 1996) occur. This problem, as well as clarification as to which males are superior in male-male competition and are chosen by females, should be investigated in future researches.

ACKNOWLEDGEMENTS

We thank Dr. L. Filippi, Faculty of General Education, Saga Medical School, for her valuable English corrections. We also are grateful to Dr. K. Takasu, Faculty of Agriculture, Kobe University, for providing the methods for rearing the bugs.

REFERENCES


