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Multiple matings of Chrysolina aurichalcea (Mannerheim) (Coleoptera : Chrysomelidae)

By NOBUYUKI SHIMIZU and SHIZUO FUJIYAMA*

Department of Biology, Faculty of Science,
Shinshu University, Matsumoto, 390 Japan
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Abstract

The existence of multiple matings in both males and females of a chrysomelid beetle *Chrysolina aurichalcea* was examined by measuring the total duration of copulation in one multiple-mating bout and by observing the caged population every two hours for six days. The mean total duration of copulation in one multiple-mating bout was 3.2 ± 1.7 hrs under a plastic container. Observation for six days showed that all five females conducted an average of seven matings with three males. In case of all five males, an average of nine matings with four females were observed except two males which had performed no copulations. The significance of multiple matings of the species was discussed in relation to the effects of selection pressure on genetic variations of discreet populations.

Introduction

The adults of a chrysomelid beetle *Chrysolina aurichalcea* (Mannerheim) has two colour forms, the cupreous-form and the cyaneus-form. And the result of crossing experiment revealed that these colour forms are inherited on Mendel's law and that the cyaneus-form was dominant over the cupreous-form (FUJIYAMA, 1986). In the field, *C. aurichalcea* spends almost all life on the host plants, *Artemisia princeps*. These plant communities are patchily distributed and are often isolated each other. As a movement of the beetle is mainly dependent on walking (cf. SUZUKI, 1978, 1981), exchange of the members among different patches might occur scarcely. The fact that the frequency of two colour forms much differs between very close places (SUZUKI *et al*, 1976 ; BABA and KATO, 1978 ; FUJIYAMA, 1979) supports this possibility. When the population is isolated to small number of individuals and importation of new individuals from other populations scarcely happen, random genetic drift is liable to lose their genetic variation in a long period. But FUJIYAMA (1986) reported that the frequency of two colour forms in several sampling plots around Matsumoto City had been relatively

* Please address reprints to Shizuo Fujiyama.

stable through eight years. This indicates that the frequency of two colour forms might be fixed by some factor (*e.g.* natural selection). And genetic variability maintains for some way or other. In this article, the authors intend to ascertain the existence of multiple matings of this species and to suggest that the existence of multiple matings contributes to the preservation of the genetic variability of the population.

I. Measurement of the duration of one multiple-mating bout

Materials and Methods

Thirty-nine pairs of the adults of *C. aurichalcea* were used in this experiment. One group consisted of fifteen males and sixteen females. They were reared in 1981 from the eggs which were oviposited by the females collected at Utsukushigahara Heights (Lat. 36°N, Alt. 2000m) in September, 1980. Another group consisted of twenty-four males and twenty-three females that were collected at Utsukushigahara Heights on August 19, 1981. After eclosion or capture, in order to let the males and the females become sexually mature, all individuals of the two qroups were reared at L:D=11:13, 15°C. On the day before the experiment they were separated individually.

A laboratory experiment was carried out on August 8 and 15, 1981, at a temperature of c.a. 25°C under well-lit condition. At the beginning of the experiment, one male and one female were placed in a 10 cm diameter plastic container. After the initiation of copulation, the duration of copulation and that of mounting (pause of copulation on the back of the female) were measured. Each individual was used once, and a male and a female belonging to the same qroup were paired except one combination. In this experiment, sequential behaviour from the beginning of copulation to the descent by a male from a mating female was regarded as one mating bout.

Results

In the plastic container, the following sequence of activity occurred after a male detected a female. The male approached the female, landed on the dorsal part of the female's abdomen and inserted his aedeagus into the female. After the male conducted one copulation, either he left the female or stayed on the back of the female and sometimes tried to copulate with her more times (Fig.1).

Fig.2 indicates the frequency distributions of the number of copulations in one multiple-mating bout. The number of them ranges from once to seven times, with the average of 2.1 ± 1.4 times. Nearly half of them (48.7%, 19/39) are one copulation in one multiple-mating bout. The increase in number of copulations in one multiple-mating bout is accompanied with the decrease of frequency.

Fig.3 shows the frequency distributions of the total duration of copulation in one

multiple-mating bout. The duration ranges from 53 minutes to 6 hours 47 minutes, with the average of 3 hours 13 minutes. As the total duration of copulation in one multiple-mating bout increases, the rate of more than one copulation increases. A significant correlation exsists between the number of copulations in one multiple-mating bout and the total duration of copulation (Spearman $rs=0.37, P<0.05$).

Fig.4 shows the frequency distributions of the total duration of one multiple-mating bout. The duration ranges from 1 hour 15 minutes to 12 hours, with the average of 5 hours 17 minutes. Two peaks can be recognized, one peak between two and three hours and another one between six and seven hours. The former peak consists of one copulation in one multiple-mating bout and the small latter peak consists of more than one copulation in one multiple-mating bout. This indicates that the increase of the times of copulation in one multiple-mating bout increases the duration of one multiple-mating bout (Spearman $rs=0.88$, $P<0.01$).

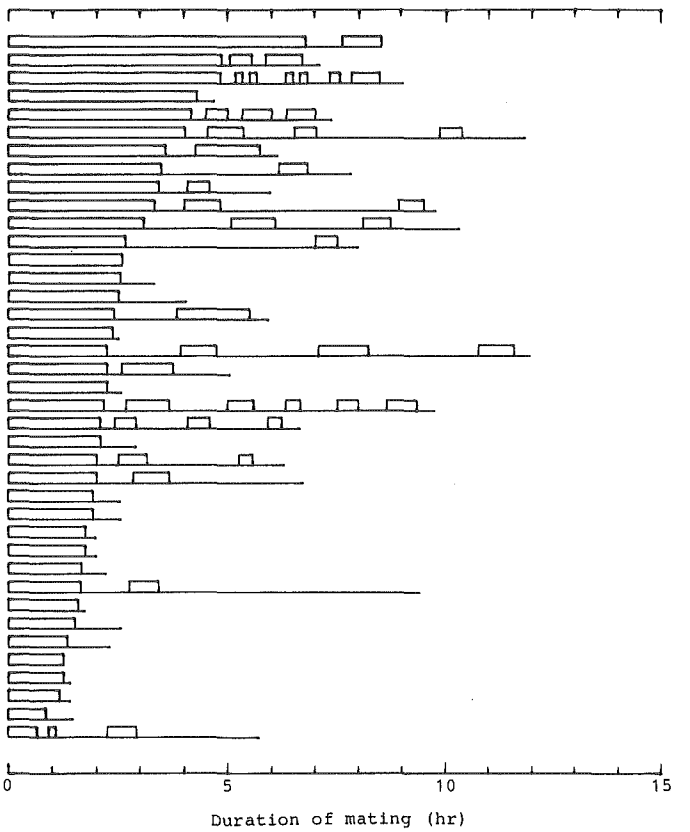

Fig. 1. A mode of copulation in one multiple-mating bout. Each column and each line indicate copulation and mounting, respectively. The order from top to bottom is arranged by the duration of the first copulation. (N=38)

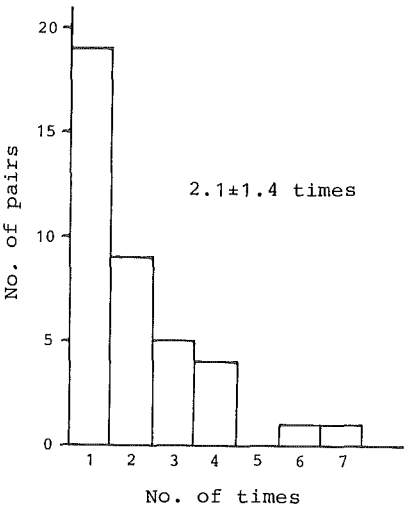

Fig. 2. Frequency distributions of the number of times of copulation in one multiple-mating bout. Numerals in the figure indicate mean times ± S. D.

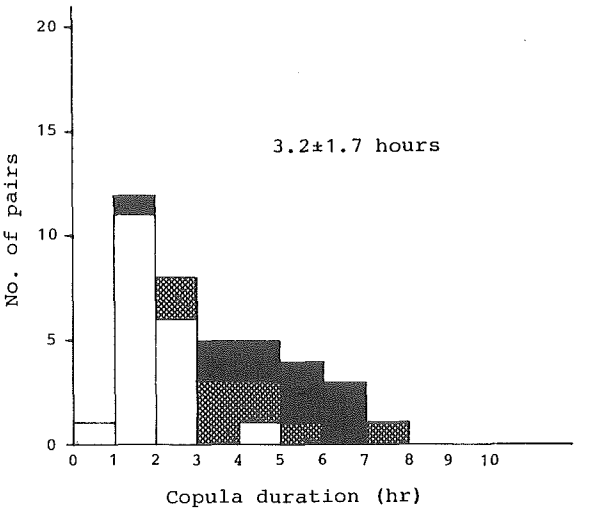

Fig. 3. Frequency distributions of the total duration of copulation in one multiple-mating bout. Numerals in the figure indicate mean hours ± S. D.

□-One copulation in one mating bout

▩-Two copulations in one mating bout

■-More than two copulations in one mating bout

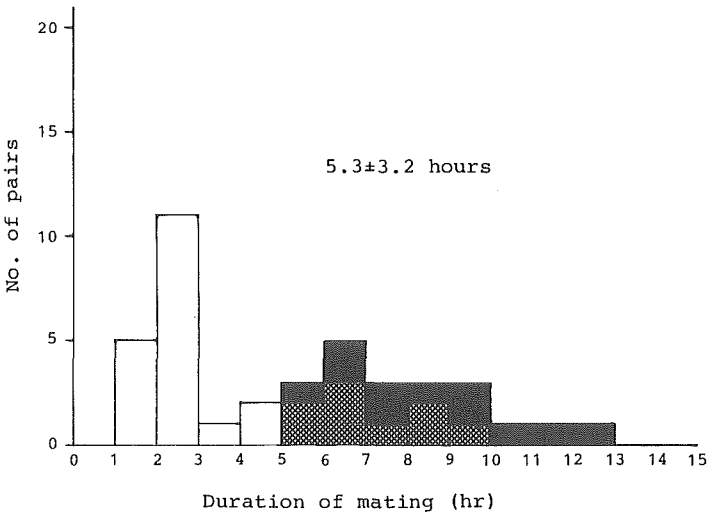

Fig. 4. Frequency distributions of the total duration of one multiple-mating bout. Numerals in the figure indicate mean hours ± S. D.
□-One copulation in one mating bout
▩-Two copulations in one mating bout
■-More than two copulations in one mating bout

II. Analysis of multiple matings of caged population

Materials and Methods

Five pairs of *C. aurichalcea* were used in this experiment. They were reared in 1981 from the eggs which were oviposited by the females collected around the campus of Shinshu University at Matsumoto (Lat. 36°N, Alt. 600 m) in October, 1980. After eclosion, in order to let the adults become sexually mature, they were kept at L : D= 11 : 13, 15°C. Before the experiment, these five pairs were individually marked with

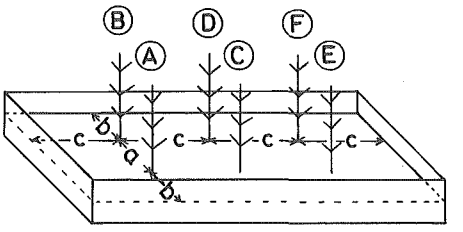

Fig. 5. Equipment used for analysis of the mating bout by caged population. Heights of plants A to F are 19 cm, 21 cm, 19 cm, 16 cm, 23 cm, and 18 cm, respectively. Small letters represent the distance, namely, a: 10 cm b: 12 cm c: 14 cm.

lacquer paint.

Six plants, *Artemisia princeps* were planted in two rows equidistantly (Fig. 5) in a square plastic pot (34×56cm). These plants were nearly 20cm in height. In order to prevent escape of the adults of *C. aurichalcea,* the wall of the plastic pot was spread with margarine. Experiments were conducted for six days in the middle of July, 1981, at L : D=11 : 13, 15°C. Five pairs of this species were released on the host plants. Observation was carried out once every two hours, considering that the average duration of copulation is 3 hours 13 minutes. The occurrence or non-occurrence of mating bout, *i.e.* copulation or mounting, and the combination between the members of both sexes were recorded. Insect pairs were considered as being copulation or mounting for two hours if they were observed together on one occassion. In this experiment, sequential behaviour of a male on the back of a female, which must include copulation, is regarded as one mating bout. After fifty-four hours had passed from the initiation of the experiment, the male No. 5 died. Soon after that, the male No. 6 was introduced and the experiment was continued. This male had been reared under the same condition as five pairs.

Results

Fig. 6 indicates four cases of mating bout of two males and two females. The male No. 1 mated with three females nine times, and the male No. 2 mated with five females fourteen times. While the female No. 1 mated with four males eight times and the

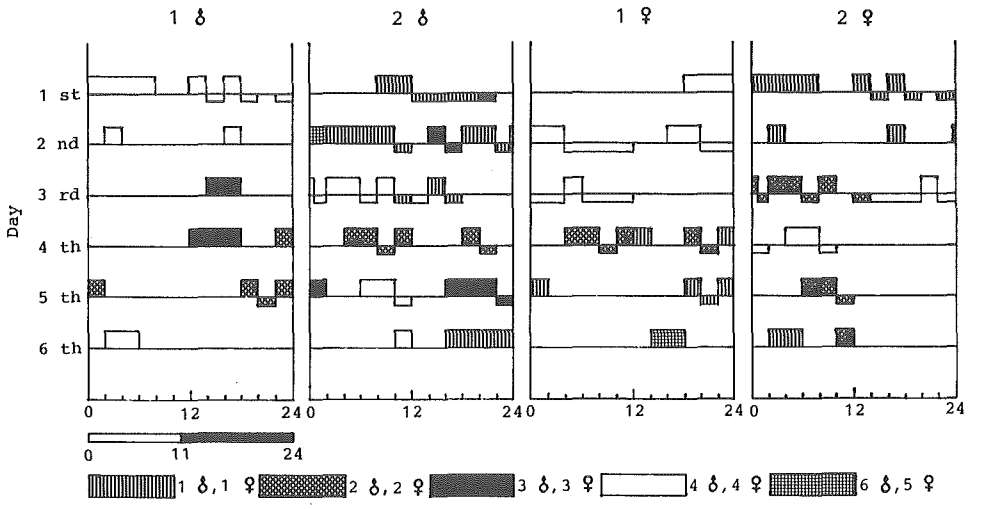

Fig. 6. Examples of multiple matings by both sexes in relation to the members of another sex. The number with a male and a female mark shows the individual number. Each upper column and each lower column in the figure indicate copulation and mounting, respectively.

Table 1. The number of times of copulation through the experiment in relation to the member of another sex.

No. of males \ No. of females	1♀	2♀	3♀	4♀	5♀	♀total
1♂	0	2	2	5	0	9
2♂	5	2	3	3	1	14
4♂	1	2	1	2	1	7
6♂	2	2	0	0	2	6
♂ total	8	8	6	10	4	36

female No. 2 mated with three males ten times.

Through the experiment, all the female conducted an average of seven matings with three males. In case of the males, an average of nine matings with four females were observed except No. 3 and No. 5 males which had performed no copulations.

Table 1 shows the mate selection by each individual in both sexes. It is noticeable that the male No. 1 mated five times with the female No. 4 and the male No. 2 did five times with the female No. 1. However, the mate selection by males is not significant in compliance with the member of the females (F-test, $P>0.05$). These results show that both males and females mated another sex multiple times without special preference.

Discussion

In a caged population, it was found that the adults of *C. aurichalcea* conduct multiple matings in both sexes with several members. As these results were given by the observation of two hours' interval, the frequency of multiple matings in both sexes might be underestimated. Each adult has about two months' reproductive period under rearing condition and the mating is frequently observed in whole period of breeding season, *e.g.* from late September to early December in Matsumoto (FUJIYAMA, 1986). So, even if the experimental condition are artificially made, these show that both males and females of this species have high possibility of doing multiple matings under natural conditions. For this species, subsequent matings by two males are reflected in their offspring (FUJIYAMA and WATANABE, unpublished). So, it is considered that multiple matings by both sexes of this species result in preserving genetic variability of locally isolated populations by maintaining a higher effective size of population as suggested in *Cepaea nemoralis* by MURRAY (1964). This might coincide with the following facts shown by FUJIYAMA (1886). At mountain areas around Matsumoto City, the populations tend to be isolated ones for the patchy distribution of host plants, *A. princeps*. Although the estimated gene frequency of cupreous-form in most populations examined were 80-89% (34 pop./40 pop.), there were no populations occupied by

cupreous-form. The frequency of two colour forms tends to change with the change of environment, *e.g.* frequency of wood coverage even between neighboring populations.

In view of mating systems, it could be imagined that an unpaired male encounters a mating pair more often in case of multiple matings in both sexes. The aggressive behaviour by an unpaired male against a mating male of *C. aurichalcea* (SHIMIZU and FUJIYAMA, 1986) might be ascribable to this style of mating.

Acknowledgements

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