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SHORT COMMUNICATION

Mating behavior and genital damage during copulation in the leaf beetle *Lema coronata* (Chrysomelidae, Criocerinae)

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<H1>**Abstract**

The criocerine leaf beetle *Lema coronata* Baly has extremely long genitalia that reach more than twice the body length in both sexes. We observed mating behavior of this species in the laboratory and inspected the male genital morphology using a scanning electron microscope. The males did not perform pre-copulatory courtship and post-copulatory guarding of the mates, and copulation lasted only for about 30 min. The surface of male genitalia is smooth without any special structures at the tip. A fragment of broken male genitalia was detected in the spermathecal duct of one female. We discuss the adaptive significance of male genital damage and the selective factor of elongated genitalia.

The species of the genus *Lema* (Chrysomelidae, Criocerinae) have conspicuously elongated genitalia in both sexes (male median ejaculatory guide and female spermathecal duct, Fig. 1). There is a large variation in the length of the genitalia among the species (Matsumura & Suzuki 2008). In *Lema coronata* Baly, which has the longest genitalia, the male and female genitalia are more than twice as long as the body (male genitalia ~ 10 mm, female genitalia ~ 14 mm, body length ~ 6 mm). Comparative studies using many insect orders (Eberhard 1985; Arnqvist 1998) have provided evidence for the important role of female choice and sexual conflicts in driving genital divergence. In order to clarify the function and selective advantages of the elongated genitalia in both sexes, we observed the mating behavior of *L. coronata* and inspected the external structure of the male genitalia using a scanning electron microscope.

To obtain virgin males and females for observation of mating behavior, we collected adults from the field, made the females oviposit in the laboratory, and reared the offspring to adulthood. Male and female adults that were under hibernation in the soil were collected (18♂, 22♀) in Toyama Prefecture, Japan on 5 May 2007, and the adults were brought live to the laboratory at Hokkaido University, Sapporo. During transportation, they were maintained in plastic bags with their host plant. Immediately after arrival at the laboratory, they were put in a plastic cage (27 cm × 17 cm × 18 cm) with a whole shoot of the host plant *Commelina communis* (Commelinaceae), and the cage was maintained at room temperature. Mating was observed during the day and night under natural light conditions. Females laid eggs on the leaves, which the larvae fed on. After oviposition, these field-collected females were preserved in 80% ethanol

until the observation of the genital morphology. Last instars were individually isolated in a plastic cage (36 mm × 36 mm × 14 mm), where they pupated. After emergence, the males and females were separately maintained in plastic bags with cut shoots of the host plant until observation.

Twenty-nine pairs were prepared for observation. One male and one female were randomly selected, and each pair was transferred into a plastic centrifuge tube (118 mm long, 28 mm diameter) capped with wiping paper. Mating behavior was observed at room temperature from 28 July to 6 August. We observed the pairs continuously from 12:00 to 15:00 hours daily. Females two weeks after emergence were used for this experiment. Male genital morphology for adults collected from the field and for their offspring was observed using a scanning electron microscope (JSM-6301F, Hitachi) and a binocular microscope (SZ60, Olympus).

Mating was established in 20 of 29 pairs (70%) within three hours of the start of the experiment. In the remaining pairs, mating was not observed because some males were less vigorous and others were absolutely refused by the mate. The time until mounting started varied from 0 to 159 min. In all the pairs for which mating was observed, the males always mounted the female's back from behind. No pre-copulatory courtship behavior was observed. Copulation lasted for 22–46 min (33 min ± 5.4 (SD), $n = 18$). In 2 of the 20 pairs, copulation lasted for less than 10 min, and the females laid unfertilized eggs. After the termination of copulation, no males conducted post-copulatory guarding.

In 14 of the 20 pairs (70%) in which mating was established, the females accepted the male without reluctance; the females walked slowly or rested during copulation. In the remaining six pairs, the females did not accept copulation immediately after mounting. In one of the six pairs where females were reluctant to mate, the female

refused the male by lifting the tip of her abdomen against the ventral surface of her elytra so as to prevent the male from inserting his aedeagus at the beginning of the mounting. The refused male moved about actively on the female's back while tapping her back with his antennae. When the female was tapped, she finally allowed copulation. In five of the six pairs in which the females exhibited reluctance to mate, the females intensively shook themselves from side to side before or during copulation. The males kept holding the mate during this behavior, and then these pairs copulated successfully. Immediately after the start of copulation, in three of these five pairs, the males moved their aedeagus back and forth in the genitalia of the females.

We dissected 22 field-collected non-virgin females preserved in 80% ethanol. Of them, one female had a fragment of a broken male median ejaculatory guide in her spermathecal duct (Fig. 1b).

Observations of male genitalia using a scanning electron microscope (Fig. 1c) and a light microscope (Fig. 1d) showed that the surfaces of the median ejaculatory guide and aedeagus are smooth without special apparatus.

The present observations indicate that males did not perform either pre-copulatory courtship or post-copulatory guarding. Virgin females were passive and sometimes unreceptive to their partners. In the Chrysomelidae, this simple mode of mating is common, although a mating duration of about 30 min is relatively short (reviewed in Dickinson 1997; Jolivet 1999). The presence of a fragmentary median ejaculatory guide of a male in a spermathecal duct provides two perspectives. First, the male median ejaculatory guide is actually inserted into the long spermathecal duct of females. Secondly, having a long median ejaculatory guide entails fitness costs to males because it breaks during copulation. This observation poses a question as to why elongated genitalia have been selectively favored in *L. coronata* males despite the cost of genital

damage. Kamimura and Matsuo (2001) reported that field-collected females of *Anisolabis maritima* (Dermaptera) had a piece of broken male genitalia in their spermatheca. Kamimura (2003) suggests that a piece of broken male genitalia that was artificially induced acted as a mating plug against other males in *Euborellia plebeja* (Dermaptera). Elongated genitalia of *L. coronata* males may have a similar function during copulation.

The males of *Euborellia plebeja* (Dermaptera) can remove rival sperm from the spermatheca of their mates using the brim-like structure of their elongated genitalia (Kamimura 2000). In contrast, the lack of special apparatus at the tip of the median ejaculatory guide and aedeagus in *Lema coronata* (Fig 1a,d) suggests that the males can not remove rival sperm physically. Therefore, in future studies, it is necessary to test whether or not females assess the quality of the mate by using the male's genital length as a cue, as in *Cherymorpha alternans* (Coleoptera) (Rodríguez, 1995; Rodríguez *et al*. 2004) and *Lygaeus simulans* (Heteroptera) (Tadler 1999).

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Figure 1 A mating pair and male and female reproductive organs in *Lema coronata*. (a) Schematic drawings of a mating pair of *L. coronata* with male and female reproductive organs. Male genitalia: Aed, aedeagus; Ed, ejaculatory duct; MEG, median ejaculatory guide (the elongation of armature on the tip of IS); IS, internal sac (enveloped in BC); Female genitalia: BC, bursa copulatrix; Ov, oviduct; R, rectum; SptD, spermathecal duct with MEG; S9, ninth sternite; T9, ninth tergite. (b) Opening of the spermathecal duct (red-stained with acetofucsin) into BC (upper) with a fragment (brown tube) of the male median ejaculatory guide in a female of *L. coronata*. (c) Tip of the male median ejaculatory guide. (d) Apex of the aedeagus in the dorsal view.

1

Ed

T8

R

BC

IS

Aed

MEG

Ov

S8

Female genitalia

SptD

2

0.10 mm

3

1 μm

4

0.25mm

MEG

SptD with MEG