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Size-dependent precopulatory behavior as mate-securing tactic in the Japanese stag beetle, *Prosopocoilus inclinatus* (Coleoptera; Lucanidae)

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Abstract Males of the Japanese stag beetle *Prosopocoilus inclinatus* show dimorphism in body size. Since females frequently resist male courtship behavior, males often fail to mate after encounters with females. The males of two morphs showed different precopulatory behavior. During encounters with females, small males acted more persistently against females' resistance than large males by grasping the female more solidly and as a result, succeeded in copulation more frequently. This persistent precopulatory behavior could be regarded as an alternative mating tactic of small males that are inferior to large males in direct physical competition.

Key words Alternative reproductive behavior · Male dimorphism · Mating behavior · Stag beetles · Lucanidae

Introduction

In many horned beetle and stag beetle species, there is size dimorphism in male body size and ornamentation (Inukai 1924; Eberhard and Gutierrez 1991; Moczek and Emlen 1999; Kawano 1995, 2000). Size dimorphism in males suggests the existence of different mating strategies between the two types of males. In general, small males are inferior to large males in direct competition (Andersson 1994). Several studies have shown behavioral differences between the two morphs in getting access to females. For example, small males of the horned beetle *Podischnus agenor* emerge earlier than large males; this might result in the small males avoiding direct competition with large males (Eberhard 1982). Hornless males of *Onthophagus acuminatus* are known to sneak into tunnels guarded by other males in order to mate with females (Emlen 1997).

The importance of males getting access to females has been argued in several previous studies (Eberhard 1982; Andersson 1994; Emlen 1997; Moczek and Emlen 2000), but after getting access to females, there are often some hurdles that had to be cleared before successful mating can occur. For example, in dung flies and water striders, males have difficulty copulating after encounters with a mating partner because of behavioral resistance by females (Parker 1979; Arnqvist 1997). Males have to overcome female resistance to attain mating success. There is a similar struggle in the stag beetle *Prosopocoilus inclinatus*. Because of female resistance, reproductive success is influenced by the result of male–female interaction, which is a struggle between forcing male and resisting female.

Although many previous studies have mentioned various strategies for dimorphic insects to obtain mating partners (Alcock 1979; Hamilton 1979; Eberhard 1982; Emlen 1997; Moczek and Emlen 2000), there are few studies on behavior after the encounter with mating partners (but see Cook 1990). Thus, information about precopulatory behavior is important to understanding male size dimorphism and/or variation.

In this paper, we focused on the mating behavior of *P. inclinatus* after initial sexual encounters. To examine the ability of each morph to successfully mate, we observed how large and small males of *P. inclinatus* behave in mating sequences.

Materials and methods

We used a common Japanese stag beetle, *P. inclinatus*, that shows male body-size dimorphism (Inukai 1924). Sampling was carried out in Hobetsu-cho, Hokkaido, Japan from 7:00 to 11:00 p.m. from late June to early August 2002. Adult beetles were collected under streetlights. Males were kept individually in plastic containers (16 × 11 × 5 cm). Females were kept in large plastic containers (18 × 22 × 7 cm) in groups of ten. They were fed with fresh apples ad libitum. New apples were supplied every 2–3 days.

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For males, length was measured from the front of the head (excluding mandible) to the tip of the elytra as the index of body size. For females, total length including mandible was used as body size. Male size of *P. inclinatus* shows a dimorphism with approximately 35 mm being the border between the two morphs (Inukai 1924). To examine behavioral differences between morphs, we removed intermediate-sized individuals from the experiments (Fig. 1). We treated the individuals smaller than 34.4 mm as small males and ones larger than 36.8 mm as large males. Observations were carried out from 1:00 to 4:00 p.m. during July and August 2002, in a glass tank. The bottom of the tank was covered with a cork sheet so that beetles would not slip.

First, we introduced a male and a female into the tank but kept them separated using a clear board for 5 min. The board was then removed and we waited for 30 min for an encounter to occur. If an encounter did not occur within 30 min, the trial was abandoned. After the first encounter of the pair, behavior was observed for 30 min. Females that copulated in an experiment were removed from further

experiments, in order to minimize the effect of copulation on behavior. The virginity of the females used in this experiment was unknown.

Results

Male body-size distribution

Male body-size distribution is shown in Fig. 1. When divided according to body size with the boundary at 35 mm (Inukai 1924), large and small morphs differed in regression coefficient of mandible length to body size (Fig. 1 ANCOVA $F = 56.620$, $P < 0.0001$). The difference in regression coefficient was still significant when data were log-transformed (ANCOVA $F = 95.749$, $P < 0.0001$). Thus, size dimorphism was observed.

The sequence of mating behavior

Pairs of *P. inclinatus* showed a behavioral sequence during courtship (from encounter to genital insertion). Following the initial physical contact of the pair, the male touched the female's elytra with his antenna. This behavior ("antennation") is regarded as the beginning of mating behavior. We classified the sequence of male-female interactions into the following three stages (Fig. 2). In stage 1 (mounting/ignoring), the male climbed onto the female's back and held her with his limbs. When the male kept the female under his body, it was regarded as mounting. In some cases, males did not begin mounting within 30 min after antennation. This was judged as ignoring. In stage 2 (female resistance/acceptance), the female often kicked the male's limbs and/or genitalia, and/or tried to move away from him. When the female kicked the male and/or tried to move away from him, the female's behavior was recorded as female resistance. If female resistance was not observed after mounting, the female accepted a genital insertion (acceptance). In stage 3 (forcible copulation/forcible copulation failure), when a male that mounted a resisting female attained genital insertion, it was regarded as forcible copulation. In this study, insemination could not be confirmed. Genital insertion was regarded as mating success. Those pairs that separated before genital insertion were judged as forcible copulation failures.

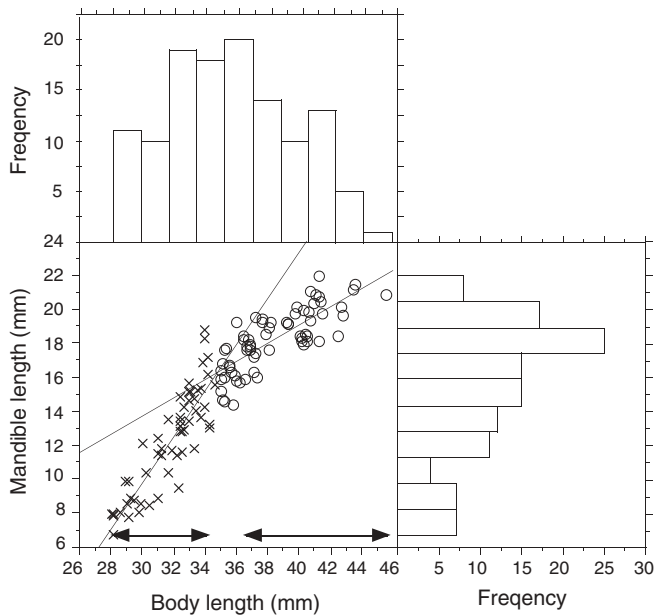
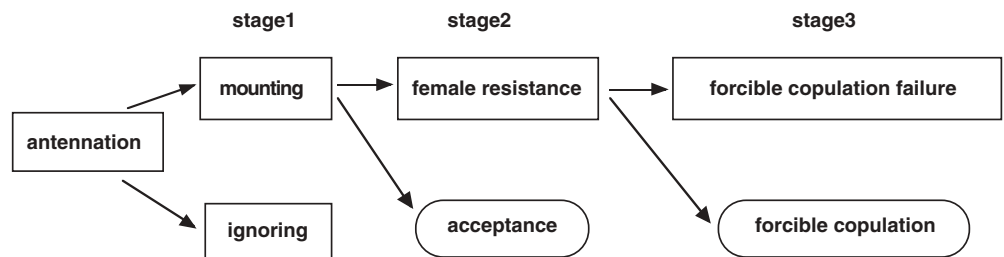


Fig. 1. Body-size distribution of male *P. inclinatus*. Open circles Large morph, $y = 0.538x - 2.429$, $r^2 = 0.622$. X Small morph, $y = 1.35x - 30.75$, $r^2 = 0.768$. Arrows indicate ranges of small and large morphs used in our experiment

Fig. 2. Mating sequence of *P. inclinatus*



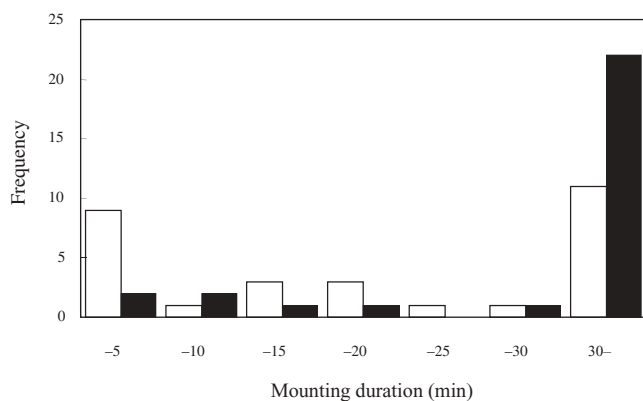


Fig. 3. Mounting duration of the males that showed mounting and subsequent mating sequence from 29 observations for each morph. *Open columns* Large males, *black columns* small males

Differences in mating behavior

During the above mating stages, the behavior of large and small males differed in two respects. First, at stage 1, a significantly greater proportion of the small males showed mounting behavior (29/30 = 97%) compared with the large males (29/37 = 78%; Fisher's exact probability test, $P = 0.035$). Second, at stage 3, a significantly greater proportion of small males attained forcible copulation (23/24 = 96%) compared with the large males (15/23 = 65%; Fisher's exact probability test, $P = 0.0102$). The frequency of female resistance at stage 2 did not differ significantly between large and small males (large 23/29 = 79%, small 24/29 = 83%; Fisher's exact probability test $P = 0.748$). In addition, the mounting duration of small males was significantly longer than that of large males (Fig. 3: $P = 0.0054$, $U_{29,29} = 241.5$; Mann-Whitney's U -test).

The number of males that did not encounter, i.e., did not "antennate" females within 30 min, was 8 out of 38 in small males and 6 out of 43 in large males, and there was no difference between the morphs ($P = 0.56$; Fisher's exact probability test). Thus, we concluded that the two morphs did not differ in sexual activity.

Females were allocated randomly towards large and small males. There was no significant difference in size between females that were allocated to large and small males (mean size of females allocated to small males 32.9 ± 2.55 ; to large males 32.37 ± 2.54 , $t = 0.900$, $P = 0.371$, t -test). For both large and small males, females that showed acceptance and resistance were not significantly different in size [for small males: acceptance ($n = 5$) = 33.88 ± 2.65 , resistance ($n = 24$) = 32.78 ± 2.59 ; $t = 0.861$, $P = 0.397$; for large males: acceptance ($n = 6$) = 32.47 ± 2.50 , resistance ($n = 23$) = 32.36 ± 2.74 ; $t = 0.085$, $P = 0.933$, t -test). In addition, all the "ignored" females (those females that were "antennated" but not mounted) were not significantly different in size from those mounted (ignored 32.18 ± 2.02 , mounted 32.62 ± 2.54 ; $t = -494$, $P = 0.623$, t -test). On the whole, the size of females did not affect their mating behavior irrespective of male morphs. Thus, all the females were lumped together in the analysis of male morph effect.

Discussion

Body-size distribution of males

Although male body-size distribution was not bimodal as previously reported in Inukai (1924), observed differences in allometric relationships between large and small males indicated dimorphism.

Differences in precopulatory behavior in males

In *P. inclinatus*, precopulatory struggle was observed and small males were more persistent against female resistance in precopulatory behavior. After antennation, small males began courtship behavior (mounting) at higher rates. They also had a tendency to grasp females securely (forcible copulation and longer mounting duration), whereas large males often let females escape (forcible copulation failed and shorter mounting duration). These results suggest that small males improve mating success through mating behavior.

In some other dimorphic beetles, behavioral differences of small males have been reported (Eberhard 1982; Siva-Jothy 1987; Emlen 1997). These studies have suggested that males of different morphs act differently towards other males. In our study, it was suggested that the different morphs act differently towards females. Alternative reproductive behavior might not only involve a behavioral change towards males, but also a different action towards females. The differentiation in the male mating behavior towards females implies a new aspect of alternative mating strategies.

If the mating chances vary between two morphs, it pays for small males to invest much in one mating event. In *Prosopocoilus dissimilis*, large males paired with females more frequently than small males in field observation (Siokawa and Iwahashi 2000). To make sure that persistence in precopulatory behavior works as a mate-securing tactic for small males, it is necessary to assess the mating chances of the two morphs, fertilization success and sperm competition through forcible copulation.

Female resistance and acceptability

Female resistance was not related to the body size of the paired male at stage 2. Although our experimental design did not allow females a choice of multiple males, females showed no preference for large males. The receptivity of females possibly depends on their mating experience and/or physical preparedness for oviposition. Considering females' condition and copulation experience would be a next step for understanding the basis of female resistance. After the acceptance of females, there are still possibilities of cryptic female choice and sperm competition before fertilization success (Eberhard 1996; Birkhead and Pizzari 2002). Further studies should confirm these possibilities to clarify mating success of both morphs.

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