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Effect of morph types, body size and prior residence on food-site holding by males of the male-dimorphic stag beetle *Prosopocoilus inclinatus* (Coleoptera: Lucanidae)

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Abstract Mate-securing tactics of small males in male-polymorphic species exhibiting male–male combat is an important issue in behavioral ecology. While most studies have focused on the outcomes of such combat encounters, the holding of a mating resource like a feeding site has a greater impact for obtaining reproductive success. We examined the effects of the prior residence at a feeding site on resource acquisition in the male-dimorphic stag beetle, *Prosopocoilus inclinatus*. More than 70 % of encounters did not result in combat. While larger males tended to occupy a food site after a combat, smaller males with prior residence tended to occupy food sites when no fighting occurred. Morph types or body size have no effect on the occurrence of combat, meaning that small males do not hesitate to fight with large males. These findings show that, under experimental conditions, the prior residence has a positive effect to hold food site in *P. inclinatus*.

Keywords Alternative strategy · Male dimorphism · Male-male combat · Prior residence

Introduction

Numerous animal species exhibit sexual dimorphism, with males frequently possessing weapons that are used to fight with other males in order to gain access to mates

(Darwin 1871; Andersson and Iwasa 1996). Several coleopteran species are known to exhibit male dimorphism in weapon size (Siva-Jothy 1987; Eberhard and Gutierrez 1991; Moczek and Emlen 1999; Kawano 2000; Okada and Miyatake 2007; Okada et al. 2008). In such male-dimorphic species, major males have large weapons and minor males have either small or no weapons. Since large males with large weapons are at an advantage in male–male combat, small males are regarded to be at a disadvantage in competitions for mating resources (Siva-Jothy 1987; Moczek and Emlen 2000; Hongo 2003; Okada and Miyatake 2004, 2007). Although, in many cases, small males compensate for their weakness in combat by various alternative strategies (Eberhard 1982; Siva-Jothy 1987; Moczek and Emlen 2000; Shiokawa and Iwahashi 2000; Hunt and Simmons 2001; Okada and Hasegawa 2005), it is unclear that whether they secure a similar degree of mating success comparing with large males.

In competition for resources, an individual that already holds the resource (=resident) is known to be at an advantage to keep the resource and in subsequent mating successes (Davies 1978). In the beetle, *Librodor japonicus*, males with prior residence of a food site tended to win in male–male combat even when they were smaller than the opponents (Okada and Miyatake 2004, 2007). In male dimorphic beetles, small males have been known to come earlier to food sites than large males (e.g., *Trypoxylus dichotomus*, Siva-Jothy 1987; *Prosopocoilus dissimilis*, Shiokawa and Iwahashi 2000). In these species, small males may secure mating success by the advantage of prior residence in the resource competition.

In a butterfly, individuals with prior residence always defend their territories irrespective of its prior status (Davies 1978), suggesting that an intruder hesitates to fight. No previous study, however, has focused on the positive

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effect of prior residence on resource-holding in species with male–male combat. Indeed, even Okada and Miyatake (2007) who have published extensively on this topic have only considered encounters in which combat occurred, and they did not provide data for the final ownership of the resource in the cases with no combat. However, since acquisition of the final ownership is crucial to secure mating success, we need to examine the effects of body size or prior residence on the final ownership including in the cases of encounters without combat.

In this study, we examined the effects of body size and prior residence on the final ownership of the food site using a male dimorphic stag beetle, *Prosopocolius inclinatus*. Females of this species showed no mating preference for the male morph (Okada and Hasegawa 2005), and, thus, the final ownership of the food site directly reflects the mating success of males. We conducted behavioral observations under a condition in which a male introduced into an experimental arena with a food site that has already been occupied by another male. We studied the following three points; (1) whether small males avoid combat when they encounter larger males; (2) whether large males tend to win when combat occurs; and (3) whether prior residence has a positive effect on the final ownership of the food site when combat did not occur. In addition, we compared the probability of keeping the food site in each male morph in order to estimate the fitness of each morph.

Materials and methods

Animal sampling and maintenance

We used both morphs of the male-dimorphic stag beetle, *P. inclinatus*. Adult males that have fled to streetlights were collected from 19:30 to 20:30 hours on four evenings between June 24 and July 9, 2009, at Hobetsu-Tomiuchi in Mukawa-cho, Hokkaido, Japan. Males were collected and placed individually in plastic cups and transported to the laboratory where they were maintained in plastic containers (195 × 298 × 210 mm). The containers were divided into two compartments using a plastic board, and one male was placed into each separated compartment. Pulverized decaying wood mulch (Kunugi-Konchu-MatTM, M-200; Marukan, Japan) was used to line the bottom of each container. As a food site, a cup of insect jelly (Konchu-No-MitsuTM) was placed in the recess of a circular wooden holder (diameter 6–8 cm, height 5–6 cm). Jellies were replaced every 3–6 days.

Classification of morphs

Elytral length of each male was measured to the nearest 0.05 mm with a vernier caliper (C-15; Kenis, Japan) and

was used as an index of body size (see Okada et al. 2008). Classification of morph type was based on the allometric relationship between the elytral length and mandible length (Okada et al. 2008). Males with values that exceeded this threshold (19.70 mm) were considered to be the major morph and males with values that were smaller than the threshold were considered to be the minor morph.

Behavioral observations

General methods

Males were used for observations after at least 2 days from the collections. We introduced a male (hereinafter referred to as an intruder) to a compartment in which another male (hereinafter referred to as a resident) had been maintained. After the intruder was placed on the food site, the behaviors of the two males were observed. This procedure was repeated 69 times with each male only being used once to avoid pseudo-replication. Since small males in several horned beetles tend to come earlier to food sites (Siva-Jothy 1987; Shiokawa and Iwahashi 2000), we tested for a positive effect of prior residence of small males. For this purpose, we made three categories of male pairing, i.e., minor-resident (MiR) versus minor-intruder (MiI) ($n = 18$), MiR versus major-intruder (MaI) ($n = 28$), and major resident (MaR) versus MaI ($n = 23$). The limited number of collected males prevented us from having a category of the MaR versus MiI combination, but this combination has no information for the effect of prior residence in small males.

Combat frequency

“Combat” was considered to have occurred when any of the following aggressive behaviors were observed in a pair: (1) opening of mandibles, (2) biting the opponent, (3) lifting up the opponents with mandibles, and (4) rushing to the opponent. In the absence of any aggressive behavior on the food site, the interaction was recorded as “no-combat”. In our observations, there were no encounters in which only one male exhibited aggressive behavior.

Competition for food sites

We observed interactions between two males during 30 min. When the combat encounters occurred, the observations were terminated when either of the males pushed the opponent off the food site. When either of the males walked away from the food site without any aggressive behavior, the observation was terminated and the event was recorded as “no combat”. A male on the food site at the end of the observation was considered to be

the “winner” and the other male was considered to be the “loser” in each case. When both the males remained on the food site for 30 min or simultaneously walked away from the food site, we considered the encounter to be no-combat without a winner.

Statistics

We used Fisher’s exact test to compare the frequencies of combat among combinations of the morphs. The frequencies of encounters won by resident or intruder were compared for the cases of “combat” or “no-combat”. A binomial test was used to compare the number of residents and intruders that won encounters. Welch’s *t* test was applied to compare the average body size of paired males or the difference in body size between paired males between “combat” and “no-combat”, and a paired *t* test was applied to compare the body size of winners and losers. To examine which effects, the average body size of paired males or the difference in body size between paired males, affected the incidence of combat, we applied a generalized linear model using the free statistic software R 2.13.0 with the incidence of combat as the dependant variable, “binomial” as family, “logit” as link function, and the average body size of paired males and the difference in body size between paired males as explanatory variables.

Results

Combat frequency

Table 1 shows the occurrence of the combat in each combination of the morphs. More than 70 % of trials resulted in no-combat in every combination. The occurrence rate of the combat did not differ among the combinations ($p = 0.941$, Fisher’s exact test). Figure 1a, b show the average size of pairs and the difference in the size of two individuals in a pair for combat and no-combat cases,

Table 1 Occurrence of combat in each combinations of morphs of the stag beetle, *Prosopocoilus inclinatus*

Morph combination	Combat	No-combat
MaR vs. MaI	6 (26.1 %)	17 (73.9 %)
MiR vs. MaI	8 (28.6 %)	20 (71.4 %)
MiR vs. MiI	4 (22.2 %)	14 (77.8 %)
Total	18 (26.1 %)	51 (73.9 %)

The occurrence rate of combat did not differ significantly among different morph combinations (Fisher’s exact test: $p > 0.05$)

MaR major residents, MaI major intruders, MiR minor residents, MiI minor intruders

respectively. Neither of the parameters were different between the combat and no-combat cases (for the average size: $t = 0.384$, $df = 30.572$, $p = 0.703$; for size difference: $t = 0.279$, $df = 29.106$, $p = 0.782$; Welch’s *t* test). The GLM also showed that neither the parameters have any effect on the occurrence of combat (for the average size: $p = 0.723$; for the size difference: $p = 0.873$). These results suggest that the small morph did not hesitate to have combat with the large morph and that the size difference between individuals in a pair did not affect the occurrence of combat.

Competition for food sites

Figure 2 shows the effect of prior residence in the cases with and without combat. In the combat cases, the intruder is tended to be winner ($p = 0.025$, binomial-test). In 51 no-combat cases, only 6 cases were the case in which there is no winner. In the other no-combat cases (=with winner), the resident tended to hold finally the food site ($p = 0.036$,

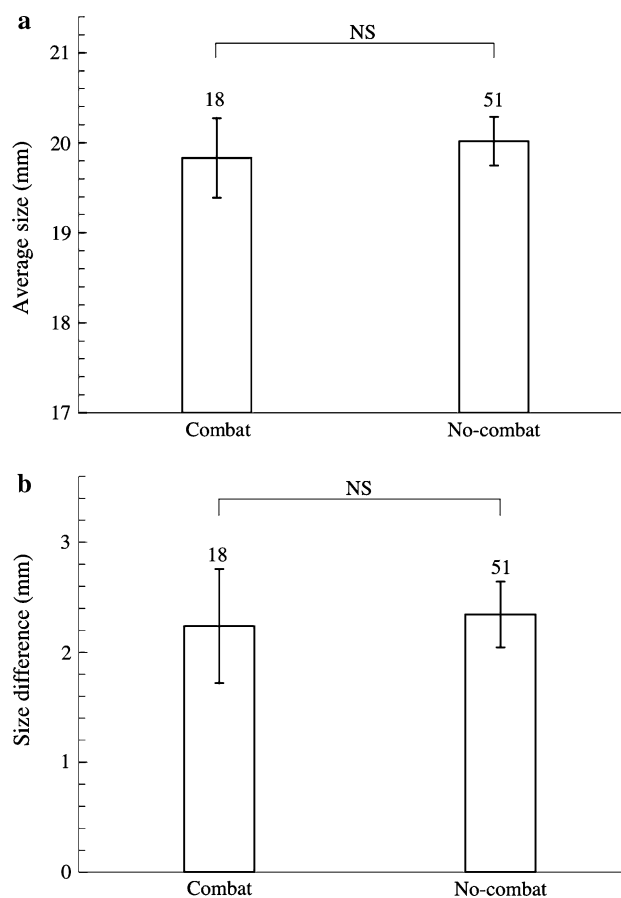


Fig. 1 a Average body size and b difference in body size between paired male stag beetles, *Prosopocoilus inclinatus*, in combat and no-combat encounters. Error bars standard error. NS not significantly different (Welch’s *t* test: $p > 0.05$). Numbers above bars indicate sample size

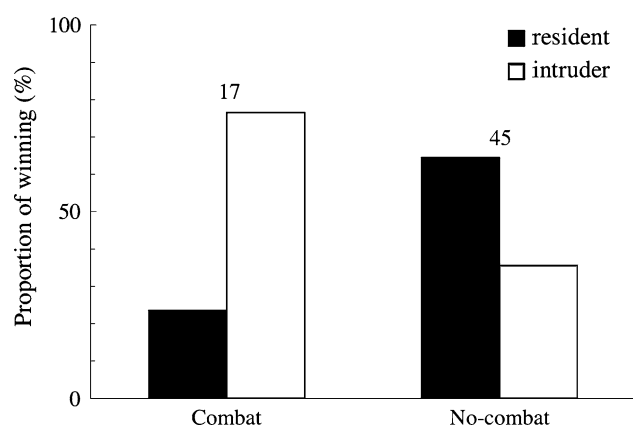


Fig. 2 Effect of resource ownership on winning with and without combat. Intruding males were significantly more successful than resident males at obtaining access to the food source when interactions involved combat, while resident males were significantly more successful than intruding males at retaining access to the food source when interactions did not involve combat (Fisher's exact test: $p > 0.05$). Numbers above bars indicate sample size

binomial-test). Proportion in which the resident win is higher in the no-combat cases than in the combat cases. Thus, a resident tended to hold the food site when a combat did not occur.

Figure 3a, b show the relationship in body size of the winner and loser in the combat and the no-combat cases, respectively. In the combat cases, large males tended to be the winner ($t = 1.987$, $df = 16$, $p = 0.032$; paired t test), but, in the no-combat cases, there is no difference in body size between the winners and the losers ($t = -1.041$, $df = 45$, $p = 0.303$; paired t test). Thus, when a combat occurred, larger males are stronger in combat.

Figure 4 shows the winning rate of the resident in each combination of the morphs. No significant difference was detected among the combinations ($p = 0.555$; Fisher's exact test). For any combination, the proportion in which the resident won was not different from the proportion in which the intruder won (MaR vs. MaI: $p = 0.192$; MiR vs. MaI: $p = 0.500$; MiR vs. MiI: $p = 0.402$; binomial test). Furthermore, this trend held when we pooled the data ($p = 0.615$; binomial test). These results suggest that the probability of winning did not change with combinations of the morphs. In other words, a resident male obtained a similar level of success in food-site holding irrespective of his morph.

Discussion

This study showed that the larger males of *Prosopocoilus inclinatus* tend to win when two individuals fight for a food site (Fig. 3a). This result is consistent with the previous reports that show the advantages possessed by males with

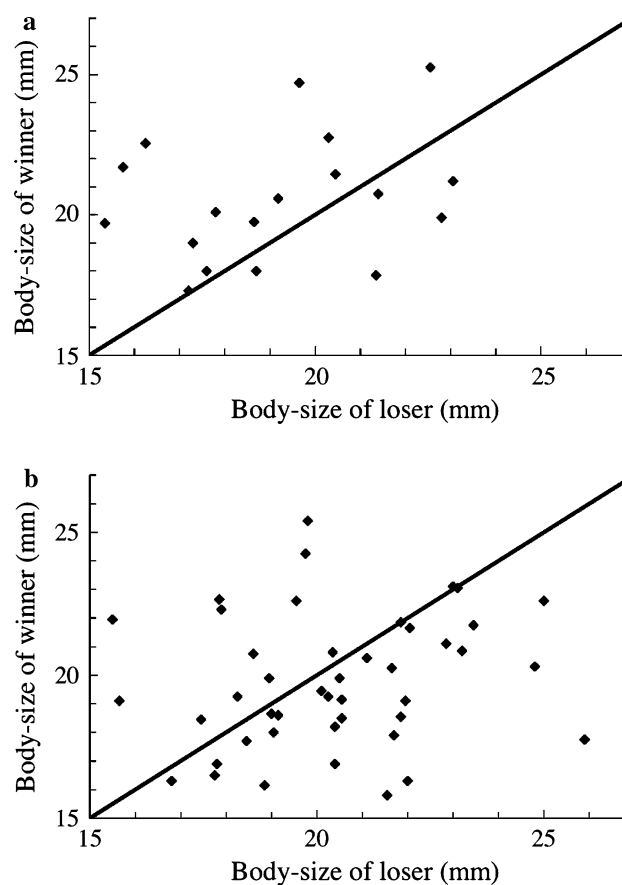


Fig. 3 Relationship between the body size of winners and losers in combat (a) and no-combat encounters (b). Points above the solid line show winners that are larger than losers. Larger males were significantly more likely to win interactions involving combat than smaller males ($p > 0.05$; paired t test). The difference in the body size of winners and losers was not significant in interactions with no combat ($p > 0.05$; paired t test)

larger bodies or weapons (Siva-Jothy 1987; Moczek and Emlen 2000; Okada and Miyatake 2004, 2007). However, the occurrence of combat was not affected by any of the combination of the morphs, the average body size of the pair, or the difference in the body size (Table 1; Fig. 1). These results suggest that body size or morph type was not the determinant of the decision making for combat. At this point, *P. inclinatus* differs from other species in which smaller individuals hesitate to fight with larger individuals (Whitehouse 1997; Hongo 2003). Moreover, when direct combat did not occur, the resident male tended to finally hold the food site even when he was the smaller individual (Figs. 2, 3b). In every combination of the morphs in the encounters, there was a high proportion (>70 %) of no-combat cases. When a combat did not occur, a resident tended to hold the food site (Fig. 2). The high probability of no-combat resulted in no difference in the final holding rate of the food site by the resident in our experiment (Fig. 4). Thus, there is a possibility that the advantage of prior

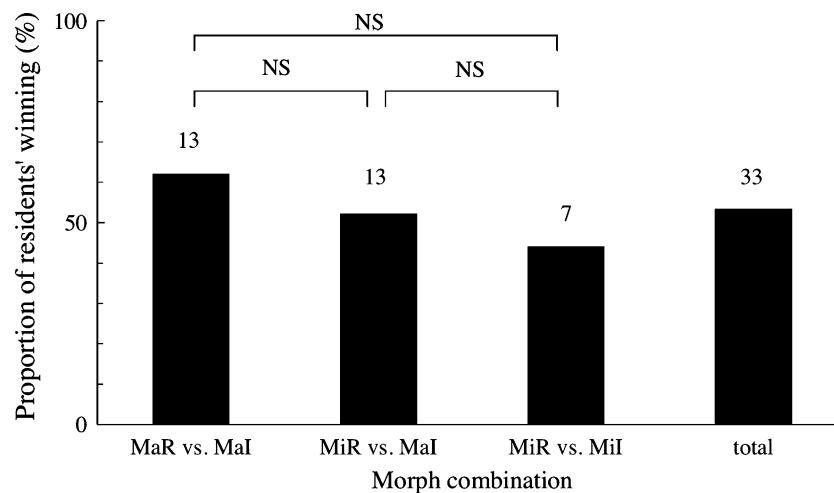


Fig. 4 Number of winners classified by ownership (residents vs. intruders) for each combination of morph types. The proportion of winning residents did not differ significantly among combinations of morph types ($p > 0.05$; Fisher's exact test). The proportion of males

retaining the food site did not differ significantly between residents and intruders for each combination of morph types and in total ($p > 0.05$; binomial test). *NS* not significantly different ($p > 0.05$; Fisher's exact test). *Numbers above bars* indicate sample size

residence and the high occurrence of no-combat encounters compensate the small males for the disadvantage in the field. In other species showing male dimorphism (*P. dissimilis* and *Trypoxylus dichotomus*), males of the small morph appear earlier at the food site than do males of the large morph (Siva-Jothy 1987; Shiokawa and Iwahashi 2000). These early appearances of small males may raise the probability of the final holding of the food site through the advantage of prior residence. In order to estimate fitness of small males in the field, it is important to know how many proportion of male encounters result in no-combat in the field.

For males of *P. inclinatus*, the holding of a food site will contribute to high reproductive success because males frequently copulated with females at the food sites under both field and rearing conditions (A. Inoue, personal observation). In addition, females of *P. inclinatus* did not prefer a special morph as mates (Okada and Hasegawa 2005). Therefore, for males, increasing the probability of food-site holding is directly connected to the chance of mating. Further field studies are needed to elucidate the role of prior residence for the final holding of the food site.

As large males are at an advantage when a combat occurs, the probability of the final holding of a food site by a male (especially for a small male) is seriously affected by the probability of the occurrence of the combat in encounters. If combats occur at high probabilities, then large size is advantageous, but if at low probabilities, then prior residence is advantageous. In our experimental condition, more than 70 % of encounters were not accompanied by direct combat. As a result, there is no difference in the probability of the final holding of the food site between

the morphs under the experimental condition (Fig. 4), meaning that both morphs may have a similar level of reproductive success in the field. However, we need data for the probability of combat in encounters in order to assess the fitness of males of *P. inclinatus* in the field. In addition, other factors (e.g., the result of combat just before starvation or the degree of starvation) are known to affect the occurrence and degree of combat (see Whitehouse 1997; Kemp 2000; Nosil 2002). Further studies including such data will bring us many new insights into the reproductive strategies of males in male-polymorphic species.

There are behavioral differences between morphs in male-dimorphic insects (Siva-Jothy 1987; Moczek and Emlen 2000; Okada and Hasegawa 2005; Okada and Miyatake 2007). For example, small males of a beetle, *Podischus agenor*, appear at earlier seasons than do large males, and the dispersal ability is higher in the small males (Eberhard 1982). In *T. dichotomus* and *L. japonicus*, small males avoid direct encounters with other males and mate with females at sites hidden from large males, so-called sneaking or satellite mating (Siva-Jothy 1987; Okada and Miyatake 2007). In *P. inclinatus*, since small males are more attached to mates than large males, they succeed in mating with a female, overcoming the female's resistance (Okada and Hasegawa 2005). However, there are few studies in which the effects of such differences in fitness have been measured (but see Tsubaki et al. 1997; Hunt and Simmons 2001). In order to understand the evolution and maintenance of male dimorphism, we must measure and compare the reproductive success of both the morphs in the field by considering differences in reproductive tactics between the morphs.

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