

## Mating competition and parentage assessment in *Ptomascopus morio* (Coleoptera: Silphidae): A case for resource defense polygyny

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**Abstract.** *Ptomascopus morio* of both sexes are attracted to vertebrate carcasses, a necessary resource for reproduction. The stage during reproduction that resource defense was most intense and the hypothesis that large beetles were better competitors and sired a larger share of the offspring were supported and tested. Male-male aggression (pushing, biting and mounting) was commonly observed before and during oviposition, but rarely after the larvae hatched. Few female-female aggressive interactions were observed at any time. Parentage analysis of the offspring of six groups of two males and two females each reproducing on a separate carcass revealed that the large males sired more of the offspring than small males. Paternity analysis, using AFLP markers, revealed that larger males had higher paternity than smaller males, but the number of eggs produced by each female did not differ between large and small females. This suggests that competition among males is intense until the end of oviposition and that resident (large) males can acquire more mates and sire more offspring than smaller males; competition among females was not evident at any time.

### INTRODUCTION

Mating systems in insects are diverse (Thornhill & Alcock, 1983). While males compete to fertilize the greatest number of eggs, the form that competition takes is determined by many factors. The spatial and temporal distributions of females and resources will determine whether males defend resources or females (Shuster & Wade, 2003). Thus, competition for females should be most intense at those resources with the highest female density. Resource defense polygyny, with males defending territories or resources against conspecific males, has independently evolved in a wide variety of insects (Thornhill & Alcock, 1983). In these insects, large males often have a greater mating success than smaller males (e.g. Alcock et al., 1977; Emlen, 1997; Forsyth & Alcock, 1990; Serveringhaus et al., 1981). In contrast, variance in reproductive success is smaller among females than among males, and usually competition for mates is less intense in females (Davies, 1991).

Carrion beetles are good models for studying mating systems, parental care and sexual selection. For instance, *Nicrophorus* spp. use small vertebrate carrion, which they bury and prepare as food for their larvae, and are well known for their extended parental care (reviewed by Eggert & Müller, 1997; Scott, 1998). There is intraspecific competition for unburied carcasses and the larger individuals usually win (Bartlett & Ashworth, 1988; Otronen, 1988; Pukowski, 1933). Dominant males spend more time on a carcass attracting females than smaller males (Eggert, 1992) and thus achieve higher paternity (Eggert, 1992; Müller & Eggert, 1989; Scott & Williams,

1993; Trumbo & Fiore, 1991). Both males and females defend their carcass and brood even after larvae hatch by attacking intruders cooperatively (Robertson, 1993; Scott, 1990; Trumbo, 1990a; 1994).

The genus *Ptomascopus*, which has three extant species, is one of three genera in the subfamily Nicrophorinae, and is closely related to the genus *Nicrophorus* (Dobler & Müller, 2000; Peck & Anderson, 1985; Szalanski et al., 2000). *Ptomascopus morio* Kraatz displays many of the characteristics of *Nicrophorus*. They use vertebrate carrion as food for their larvae (Peck, 1982) but may also use large carcasses, although this has not been documented. Males emit pheromones to attract females (Trumbo et al., 2001) and try to monopolize carcasses, but the females do not (Suzuki et al., 2005). Females often refuse to copulate with small males (Trumbo & Sikes, 2000). The presence of parents reduces the negative effects of competition with fly larvae (Trumbo et al., 2001). However, unlike *Nicrophorus* they do not control carcass decomposition, regurgitate food for their larvae, or adjust clutch size (Peck, 1982; Trumbo et al., 2001). Nevertheless *P. morio* parents may stay with a carcass and their broods for more than 10 days. (Trumbo et al., 2001).

The mating system of *P. morio* has been suggested to be resource defense polygyny (Suzuki et al., 2005) because of the male-male aggression and lack of female-female aggression. Since many *P. morio* males are attracted to a carcass (Nagano & Suzuki, 2003) there is intense competition. Subordinate males often try to access a carcass repeatedly (Suzuki et al. 2005), which the resi-

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dent males guard continuously. In contrast, aggressive interactions between females are rare, even though several females may lay eggs on the same carcass (Suzuki et al., 2005). Thus, it is hypothesized that paternity is biased towards the resident male but maternity is shared more equitably.

In this study the duration and the effect of resource guarding by *P. morio* was examined. Especially, male-male interactions before, during and after oviposition, the effect of guarding (repelling competitors, preventing infanticide, etc) and whether females guard resources against other females at any time were investigated. In addition, parentage analysis using AFLPs (Amplified Fragment Length Polymorphism: Vos et al., 1995) was conducted to assess the effect of resource guarding on reproductive success. Finally, the reproductive strategy and behavior of *P. morio* and *Nicrophorus* were compared and discussed.

## MATERIAL AND METHODS

### Behavioural observations

All beetles were caught in the field using hanging traps baited with rotten meat in Naebo Forest Park in Otaru, near Sapporo, situated in southwestern Hokkaido, Japan. To facilitate identification, beetles were individually marked on the elytra with lacquer paint. For the experiments, *P. morio* were sorted into large (elytral length > 6.5 mm) or small (elytral length < 6.0 mm) size classes. Beetles of intermediate size were not used. Large male and large female (residents) were placed together with a small piece of chicken meat (approximately 15 g) in a plastic arena (50 × 250 × 50 mm) with a soil substrate 1 cm deep. Arenas were kept under standard laboratory conditions of lighting and ambient temperature. They were checked daily. Beetles were assigned to treatments randomly and then placed into a new arena with little soil to facilitate observation. The treatments were:

Treatment 1 (pre-oviposition: N = 22): resident pairs were moved to a new arena several hours after being released. None of the females had begun to oviposit.

Treatment 2 (post-oviposition: N = 18): resident pairs were moved to a new arena after confirming that oviposition had begun.

Treatment 3 (post-hatching: N = 22): resident pairs were moved to a new arena along with their neonate larvae.

Small male and small female (intruders) were then placed in each arena, and the following behavioural interactions between the resident and intruder pair recorded for one hour: aggression – pushing or biting with mandibles; male-male mounting – one male climbing on to the elytra of the other male; copulatory attempts – a male mounting a female (Suzuki et al., 2005). When an aggressive interaction occurred, the beetle that moved away was regarded as the loser and the beetle that stayed as the winner.

All observations were made under standard laboratory conditions of lighting and ambient temperature. Behaviour was recorded continuously for one hour, between 16:00 and 19:00.

### Parentage analysis

Laboratory-reared virgin males and females were used in this analysis. There were eight replicates. Two males and two females were allowed to reproduce using one piece of 30 g chicken meat in a plastic box (20 × 13 × 15 cm) half-filled with soil. All beetles in a replicate were selected from non-relatives. Elytral length of conspecific beetles differed by more than 0.5

mm. All replicates were monitored until larvae crawled away from the carcass to pupate. All parents and larvae were later killed and stored in 99% ethanol.

Total DNA was extracted from flight muscle (parents) or abdomen (larvae), following Boom's method (Boom et al., 1990). DNA was suspended in 40 µl TE buffer and stored at -80°C.

Amplified fragment length polymorphism (AFLP) fingerprinting can be used to determine parentage (Mueller & Wolfenbarger, 1999; Vos et al., 1995) and has recently been applied to some insect species (Garcia-Gonzalez et al., 2003, 2005; Simmons et al., 2004). AFLPs were resolved according to the AFLP™ Plant Mapping Kit protocol (Perkin Elmer). Enzymatic digestion with *Mse* I and *Eco* RI and ligation of the adaptors at 25°C for 14 h in a final volume of 11 µl were performed at the same time. The pre-selective and selective PCR was conducted under protocol conditions. The primer for the selective amplification PCR was *Mse* I-CTA and fluorescent *Eco*RI-TC. Two microlitres of the PCR product were then added to 20 µL of deionized formamide, 0.5 µL of Genescan-500 LIZ-labeled size standard and subsequently concentrated under vacuum until the final volume was 2.5 µL. The loading mixture was denatured and loaded on an ABI PRISM™ 3100 Avant DNA sequencer.

DNA samples of parents and 20 randomly selected larvae from each brood were used for AFLP analysis. All peaks were scored for presence/absence in each individual using the Genescan™ analysis software in the 50–500 bp range. All peaks with a height above 150 fluorescent units we considered for small fragments (Questiau et al., 1999), since two diagnostic peaks seem to be sufficient for detecting parentage in most cases (Garcia-Gonzalez et al., 2003, 2005; Questiau et al., 1999; Simmons et al., 2004). All the fragments present in the offspring and the two potential fathers and mothers were scored. For paternity assignment, fragments present in larvae, but absent in two potential mothers were assumed to be derived from father. When one male and a larva had two or more common diagnostic peaks absent in the other male, the former male was identified as the father of the larva. The same procedure was used for the maternity analysis and parentage of all larvae confirmed.

## RESULTS

### Behavioural observations

Aggression and mounting were common in males in treatment 1 (pre-oviposition) and treatment 2 (post-oviposition), but uncommon in treatment 3 (post-hatching) (Figs 1 and 2). The numbers of male aggressive acts and mounting were significantly different among treatments (aggression:  $F_{2,59} = 16.92$ ,  $P < 0.001$ , mounting:  $F_{2,59} = 6.42$ ,  $P = 0.003$ , one-way ANOVA). Few aggressive acts between females were observed and there was no difference among treatments ( $F_{2,59} = 0.91$ ,  $P = 0.41$ , one-way ANOVA, Fig. 1). No aggression was directed toward opposite sex. Intruders showed no aggression towards larvae and residents did not guard their larvae from intruders. A total of 138 male-male aggressive acts (pre-oviposition: 60, post-oviposition: 63; post-hatching: 18) and 27 mountings (pre-oviposition: 18, post-oviposition: 9; post-hatching: 0), were observed and in all cases the winner was the large male. Both females and the large male usually stayed with the carcass, but the small male was often repelled from near the carcass.

The number of copulations performed by each male also differed among treatments (Fig. 2,  $F_{2,59} = 15.23$ ,  $P <$

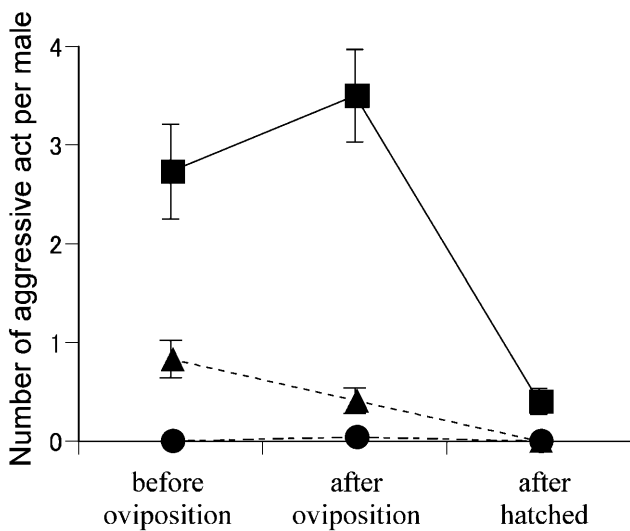


Fig. 1. Number of intrasexual aggressive acts in pre-oviposition, post-oviposition, and post-hatching periods (mean  $\pm$  SE). Squares show male-male aggression, triangles male-male mounting, circles female-female aggression.

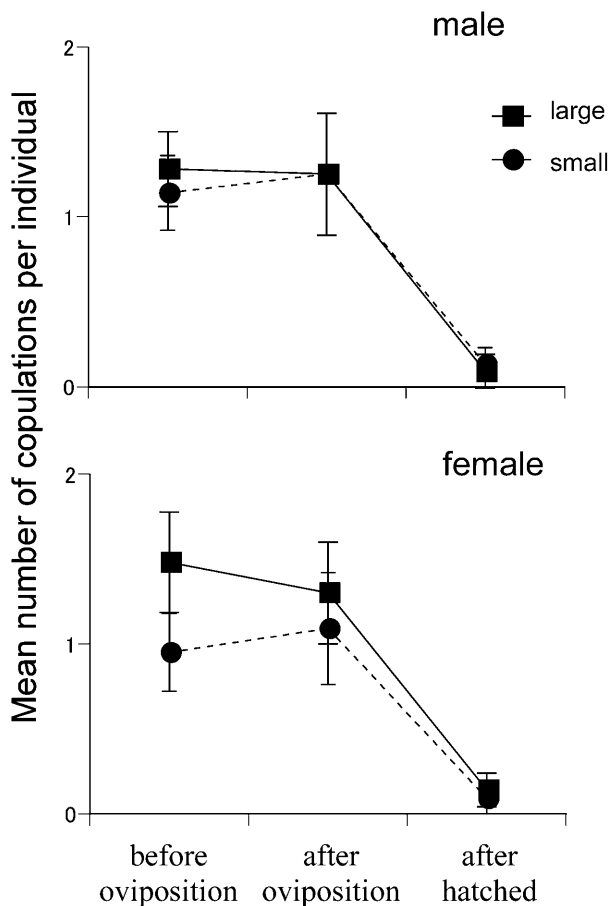


Fig. 2. Number of copulations performed by males or experienced by females in pre-oviposition, post-oviposition, and post-hatching periods (mean  $\pm$  SE). Squares show the number of copulations for large individual, circles those for small individuals.

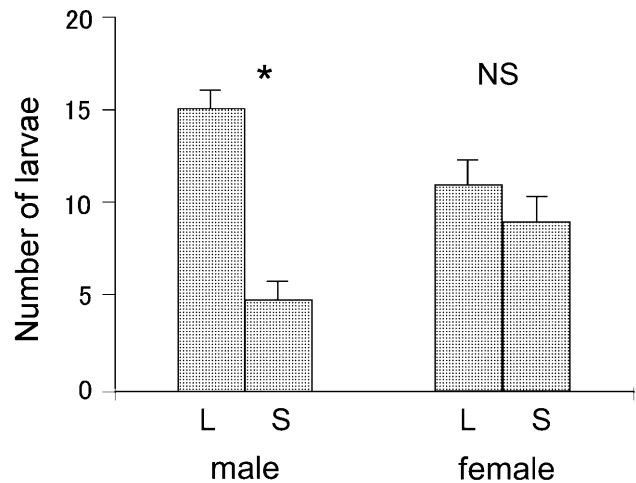


Fig. 3. Mean ( $\pm$  SE) number of larvae attributed to each parent using AFLP. L indicates a large and S indicates the small male or female. \*:  $P < 0.05$ , Wilcoxon rank test.

0.001). Many were observed during the pre- and post-oviposition periods, but fewer were observed in post-hatching period. There was no difference in the number of copulations performed by the large and small male (Fig. 2A,  $F_{1,2} = 0.05$ ,  $P = 0.82$ , two-way ANOVA). The number of times each female copulated also differed significantly among treatments (Fig. 2B,  $F_{2,59} = 13.78$ ,  $P < 0.001$ ) but was similar for large and small females ( $F_{1,2} = 1.04$ ,  $P = 0.31$ , two-way ANOVA).

#### Parentage analysis

Average size of the broods included in the parentage analysis was  $41.3 \pm 2.5$  (mean  $\pm$  SE). Two broods had too few diagnostic peaks. Six broods for paternity and maternity were examined. The number of fragments generated per individual was  $30.2 \pm 0.9$ , and the number of diagnostic peaks was  $2.6 \pm 0.2$ .

Paternity assignment to large and small males was biased ( $U = 2.2$ ,  $P = 0.03$ ) towards the large male, but maternity of females was not significantly biased ( $U = 6.0$ ,  $P = 0.43$ , Wilcoxon rank test, Fig. 3). Number of the larvae sired by large males was not significantly different for the large and small females (large females:  $6.6 \pm 1.8$ , small females:  $7.2 \pm 1.7$ ,  $U = 11.5$ ,  $P = 0.83$ ) and vice versa (large females:  $3.4 \pm 1.3$ , small females:  $1.0 \pm 0.7$ ,  $U = 20.0$ ,  $P = 0.11$ , Wilcoxon rank test).

#### DISCUSSION

Resident male *P. morio* actively guard the resource and are aggressive toward conspecifics until after oviposition and are no longer aggressive after the larvae hatch (Fig. 1). The frequency of copulation decreased after the larvae hatched (Fig. 2). Thus it can be concluded that most mating in *P. morio* occurs before the larvae hatch.

Trumbo et al. (2001) reported that *P. morio* males are present on or near the carcass more often than females before the larvae eclose, whereas females are more often on or near the carcass after the larvae eclose. This pattern of male presence coincides with frequency of aggression,

male-male mounting and copulation. In all *Nicrophorus* species studied, large males win contests (Bartlett & Ashworth, 1988; Otronen, 1988), as in *P. morio* males (Suzuki et al., 2005). Male *P. morio* copulate many times (Suzuki et al., 2005) and oviposition continues over 4 days (Trumbo et al., 2001). Thus, if another male usurps the carcass he can copulate with the female and sire some offspring. Therefore, males are aggressive on the first day after the onset of oviposition (when we observed them) possibly because usurping males can still father some of the brood.

Although the number of times resident males (large) and intruder males (small) copulated were similar (Fig. 2A), paternity was very biased (Fig. 3). Paternity of the large males was about 76%, which is similar to that of *N. tomentosus* (about 70% in double-mating trials) (Scott & Williams, 1993). This indicates that the very high paternity of large males is independent of copulation frequency. Small males more frequently copulate when the large male is absent (Suzuki et al., 2005). Since large males win contests and remain with the carcasses longer they are likely to be the last to copulate with the females. The bias in paternity suggests the presence of last sperm precedence in *P. morio*. Last male sperm precedence in *N. vespilloides* and *N. orbicollis* in similar double matings is over 90% (Müller & Eggert, 1989; Trumbo & Fiore, 1991). Resident males can be the last to mate with females because they remain with the carcass for the duration of oviposition. In addition, females prefer large males (Suzuki, unpubl. data; Trumbo & Sikes, 2000), both large and small females copulate and are fertilized by large males. Thus if there is last sperm precedence, guarding females near a carcass will effectively guarantee high paternity.

Why do not males guard carrion after the larvae hatch? The pattern of aggression coincided with that of copulation. Nicrophorine beetles do not guard mates but the resources (Suzuki et al., 2005) necessary for reproduction, which attract the mates. *P. morio* males do not guard their larvae (Suzuki et al., 2005; Trumbo et al., 2001). Thus carrion guarding reflects sexual selection and not parental investment. Copulation in *Nicrophorus* rarely occurs after larvae hatch (Müller & Eggert, 1989). Male *P. morio* shows little aggression in the absence of resources (Suzuki et al., 2005). Adult *P. morio* do not influence the decomposition of carcass (Trumbo et al., 2001). By the time the larvae hatch, the carcass is unsuitable for oviposition and it is no longer advantageous to guard it. Thus, *P. morio* males only guard resources suitable for reproduction. This is supported by the absence of copulation between small males and females, since these beetles are still in "pre-oviposition" condition.

In *Nicrophorus*, aggression is also common among females (Otronen, 1988; Suzuki et al., 2005); a carcass is usually monopolized by a single male and female (Müller et al., 1990; Trumbo, 1992) although they will reproduce communally on large carcasses (Scott, 1996, 1997). In contrast, and unlike males, few aggressive interactions and no significant maternity bias occurred among *P.*

*morio* females but the sample size was rather small. Absence of aggression between *P. morio* females means two or more females can reproduce on the same small carcass. Trumbo et al. (2001) found that female *P. morio* spend little time on or near carcasses at the beginning of reproduction, which may reduce aggressive interactions between females. In addition, females of *Nicrophorus* kill the young of rivals (Trumbo, 1990b) but those of *P. morio* do not (Trumbo et al., 2001). Usually prolonged larval guarding is shown by females of *Nicrophorus*, which results in semelparity. Females of *P. morio* stay near carcasses for less time than those of *Nicrophorus*, and are as a consequence iteroparous. It is possible that females of *P. morio* avoid the cost of fighting for the next reproduction. The strong male-male competition and little female-female competition indicates sexual selection on males and a mating system characteristic of resource defense polygyny.

When females and/or resources are aggregated, males are usually polygynous because they can defend the females or the resource. When males are abundant they may reduce the costs of defense by restricting their patrolling to the most likely oviposition sites rather than searching widely for females (Poethke & Kaiser, 1987). In such a situation, males often show resource defense polygyny (Reynolds, 1996). The oviposition site of *P. morio* is carrion (Peck, 1982) and its mating system resource defense polygyny (Suzuki et al., 2005). There is no intersexual size dimorphism in *P. morio* (Nagano & Suzuki, 2003) as body size is environmentally determined and usually large males repel small males (Suzuki et al., 2005). Since large males acquire females and there is little reproductive competition among females, territorial males can acquire more mates and sire more offspring when they guard a carcass until oviposition ceases.

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