Sexual Selection and Leg Morphology in *Nicrophorus orbicollis* and *Ptomascopus morio*

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Abstract.   Contest competition between same-sex individuals is known to be an important component of sexual selection in burying beetles. In this study we examine morphological sex differences which we suggest are related to mating behavior. In two nicrophorine species, *Nicrophorus orbicollis* Say and *Ptomascopus morio* Kraatz, males possess longer leg segments, in relation to pronotal width, than do females. These sex differences are greatest for the tarsi, and of the tarsi, are greatest for the foreleg. The foretarsi of male nicrophorines also have more expanded pulvilli. Mating experiments using *P. morio* demonstrated that a male which is smaller than a female has difficulty completing a copulation attempt. We suggest that differences in leg morphology have been maintained by selection to increase mating ability of males. Females can attempt to reject copulations by holding their abdomen down, by a quick upward flip of the abdomen, or by running away from a male. Males employ their pretarsi as hooks to grasp the edge of the female's elytra when initiating copulation. This may present a difficult challenge when small males attempt to mate with large females.

Key words: Burying beetle, *Nicrophorus*, *Ptomascopus*, sexual selection, mating behavior, morphology.

Introduction

Sexual selection operates in many ways. Sexual selection may enhance morphological and behavioral characters which enable individuals to contest effectively for reproductive opportunities or it may enhance characters which allow individuals to locate, court and copulate with mates (Darwin, 1871). Contest competition is known to be important in the burying beetles (Silphidae: *Nicrophorus* spp.). Both males and females compete intensely with consexuals for small vertebrate carcasses which are used as a breeding resource (Pukowski, 1933). These sometimes fatal contests are more often won by the larger individual (Otronen, 1988; Otronen, 1990). The winning male then pairs with the winning female (Pukowski, 1933). Recent experimental work suggests that *Ptomascopus morio* has a much different life history (Trumbo *et al.*, in press). *Ptomascopus morio* does not contest for carcasses and does not provide parental care. At least some populations are brood parasites of *Nicrophorus* on small carcasses.

The clear importance of contest competition and the passive pairing of contest winners in *Nicrophorus* has obscured more subtle male-female interactions. A male that does not locate a carcass attempts to attract a mate by the alternative strategy of releasing sex pheromone. Females sometimes reject male attempts to copulate (Eggert & Müller, 1989). Once on a carcass a male will mate numerous times with the resident female. Repeated copulations ensure that the resident male sires over 90% of the offspring (Bartlett, 1988; Müller & Eggert, 1989). In the present study we examine sex differences of nine leg segments of *Nicrophorus orbicollis* and *Ptomascopus morio* and investigate the ability of *P. morio* males of different size to mate. We suggest that sex differences in leg morphology, traits likely plesiomorphic within the family Silphidae, may be maintained in nicrophorines by continuing sexual selection.

Methods

Morphology

Nicrophorus orbicollis (Silphidae: Nicrophorinae) was collected in pitfall traps baited with carrion in Naugatuck State Forest (New Haven Co., Connecticut, USA) and preserved for analysis by freezing. Eighteen long segments of the leg (femur, tibia and tarsus for each of 6 legs) were measured for 63 males and 68 females. Males and females possessed statistically similar mean and variance for pronotal width

(Mann–Whitney U test, $P > 0.80$). Pronotal width was measured to the nearest 0.01 mm using calipers, leg segments to the nearest 0.05 mm using a stage micrometer set on an Olympus SZ30 microscope. The *left and right leg segments did not differ in size (all nine comparisons, $P > 0.20$, ANOVA). The left and right segment for each individual was therefore* summed and divided by 2, and this value divided by the individual's pronotal width. Experimenters performing these measurements were not informed of the hypotheses being tested.

A laboratory population of *Ptomascopus morio* (Silphidae: Nicrophorinae) was established from individuals collected in baited pitfall traps from the Yase forest, Kyoto, Japan during July, 1998 (15L : 9L). In the second laboratory generation, 22 males and 22 females were used to examine sex differences in leg segments as outlined above. Means and variances for pronotal width did not differ between the sexes (Mann–Whitney U test, $P > 0.80$).

Ratios of leg segment to pronotal width were distributed normally about the mean and were analyzed using ANOVA (Wilkinson, 1989).

Illustrations of the foretarsi of *Nicrophorus orbicollis* and *Ptomascopus morio* were done by D. S. Sikes using a Leica WILD M3C microscope and a drawing tube.

Mating Experiment

To examine the effect of body size on mating ability, 24 male and 24 female *P. morio* were isolated in containers without soil for 4 days on a standard laboratory diet of chicken liver. Trials were run under standard laboratory lighting at 22–24°C. A single male and female were placed in a covered petri dish,

the bottom of which was lined by a thin la compacted clay (preventing digging and e ior). For 8 pairs the male was larger in difference in pronotal width), for 8 pa *and female were of similar size (< 5% differe pronotal width) and for 8 pairs the male was smaller* (> 20% difference in pronotal width). To control fr. possible mating differences by males of different absolute size, the mean pronotal width of males was similar in all 3 treatments ($P > 0.20$, ANOVA). A copulation attempt was recorded when the male mounted the female and turned so that he was in the norma copulatory orientation. A successful copulation was scored when the male inserted his aedeagus and maintained copulation for greater than 10 sec (see Eggert & Müller, 1989). The trial was terminated after the first successful copulation or after a male made 5 unsuccessful attempts.

Results

Morphology

The leg segment/pronotal width values for 9 long leg segments for *N. orbicollis* and *P. morio* are shown in Table 1. For each comparison, males possessed a longer leg segment than females, although not all comparisons were statistically significant. In both species the male–female difference was more pronounced for the tarsi than for the femur and tibia. Of the three tarsal differences, the foretarsus showed the greatest difference (*N. orbicollis*–10%, *P. morio*– 11%).

The foretarsi of *N. orbicollis* and *P. morio* were not only longer in males but possessed expanded pulvill

Table 1.   Ratios of segment length to pronotal width for nine leg segments of male and female *Nicrophorus orbicollis* and *Ptomascopus morio*.

Segment	Segment length/pronotal width (mean±se) *Nicrophorus orbicollis*		Difference	P[b]	Segment length/pronotal width (mean±se) *Ptomascopus morio*		Difference	P[b]
	Female (N=68)	Male (N=63)			Female (N=22)	Male (N=22)		
FL[a]-femur	.564 (.004)	.571 (.005)	1.2%	>0.20	.609 (.007)	.618 (.008)	1.5%	>0.20
FL-tibia	.448 (.003)	.450 (.005)	0.4%	>0.20	.440 (.004)	.450 (.006)	2.3%	>0.10
FL-tarsus	.479 (.008)	.527 (.009)	10.0%	<0.001	.473 (.007)	.525 (.007)	11.0%	<0.001
ML[a]-femur	.630 (.005)	.640 (.006)	1.6%	>0.20	.694 (.007)	.717 (.007)	3.3%	=0.02
ML-tibia	.613 (.004)	.624 (.005)	1.8%	=0.08	.641 (.008)	.661 (.008)	3.1%	=0.05
ML-tarsus	.763 (.009)	.790 (.009)	3.5%	=0.05	.789 (.009)	.835 (.013)	5.8%	=0.005
HL[a]-femur	.761 (.007)	.781 (.007)	2.6%	=0.06	.802 (.006)	.823 (.008)	2.6%	=0.04
HL-tibia	.707 (.007)	.730 (.007)	3.3%	=0.02	.717 (.008)	.747 (.009)	4.7%	=0.02
HL-tarsus	.732 (.010)	.756 (.009)	3.3%	=0.08	.740 (.008)	.785 (.012)	6.1%	=0.003

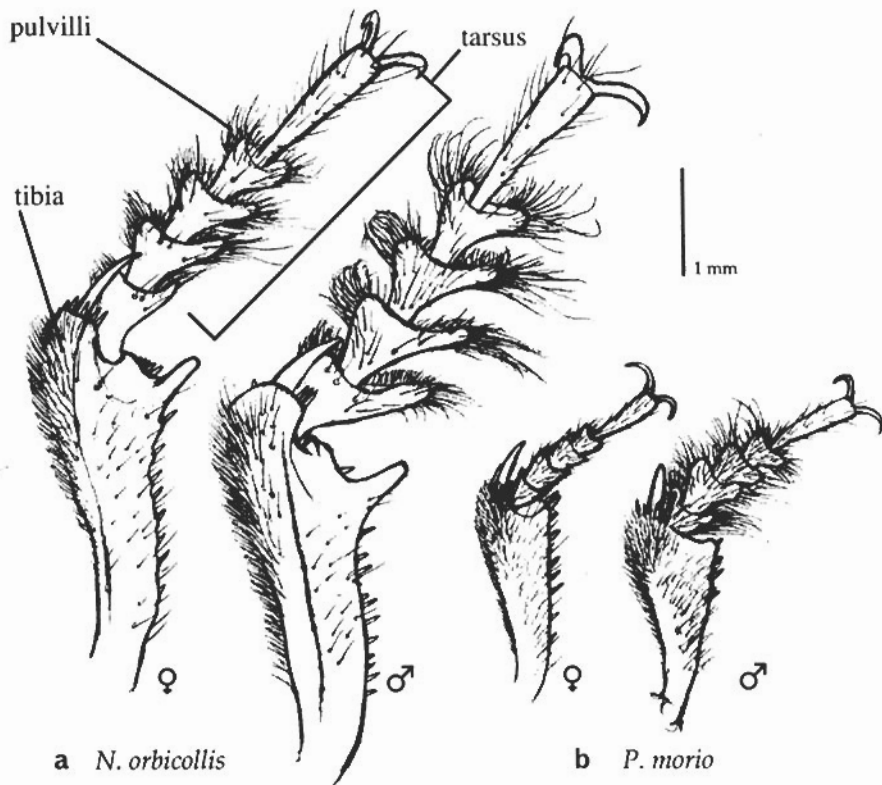[a] FL = foreleg, ML = midleg, HL = hindleg
[b] ANOVA

Fig. 1. (a) *Nicrophorus orbicollis* fore tibia and tarsus. Female and male from USA: Indiana, Porter Co. Indiana Dunes N. L. inland marsh 16 viii–15 ix 1995, R. Grundel leg. (b) *Ptomascopus morio* fore tibia and tarsus, male and female from Japan: Honshu, Chiba Pref. Funabashi, Kowakama, Hachiohji Shr. 2 vii 1995, S. Nomura leg.
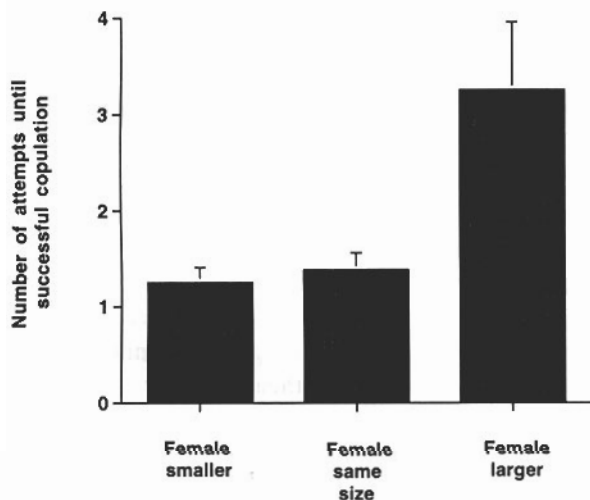

Fig. 2. The mean (±se) number of copulation attempts required for a successful copulation by male *Ptomascopus morio* when paired with a smaller female, a similarly sized female, or a larger female.

compared to females (Fig. 1).

Mating Experiment

When the male and female *P. morio* were of similar size, or the male was larger than the female, the male was always successful in mating on his first or second copulation attempt ($N=8$ per treatment). Males used their foretarsi and midtarsi to grasp the edge of the female's elytra when initiating copulation. Only 3 of 8 small males were successful in one of their first two attempts, and 2 small males were not successful in 5 attempts. Measures of copulatory success, scored as the first attempt for which copulation was successful, were significantly different among treatments (Fig. 2; Kruskal–Wallis statistic $=7.59$, $P=0.023$). A *post hoc* test, combining the results from the male larger and male similar-size treatments, gives a highly significant result (Mann–Whitney U test, $U=23.5$, $P=0.006$). Females that were reluctant to mate exhibited three behaviors. Two rejection behaviors, holding the abdomen down and moving away from the male, were also recorded for *Nicrophorus vespilloides* Herbst by Eggert and Müller (1989). *Ptomascopus morio* females exhibited a third rejection behavior, previously undescribed. Females gave a quick upward flip of the abdomen as a male attempted to mount. This behavior was very successful in dislodging a male approaching posteriorly. A male which mounted a female attempted to grasp the edge of the female's elytra with his fore- and midtarsi. Once copulation was initiated, males relaxed their grasp, holding their legs out to the

side. After copulation was completed, the male and female quickly separated. *Ptomascopus morio* males did not guard their mates as occurs following copulations near a carcass (Trumbo *et al.*, in press).

Discussion

Sexual selection takes many forms in *Nicrophorus*. The most obvious is intrasexual contest competition (Pukowski, 1933; Otronen, 1988). Sex differences in leg morphology are not likely to be explained by contest competition because both sexes engage in fights to win carcasses, and both sexes aggressively defend larvae in the nest. We suggest that the sex difference in tarsal length may reflect the use of these structures as prehensile structures during copulation.

Although some aspects of mating behavior in *Nicrophorus* may be considered passive (the winning male on a carcass pairs with the winning female), there are means by which leg morphology might enhance mating success. (1) Males which have not located a carcass will advertise for females by releasing sex pheromone. Attracted females typically mate only once and have some ability to accept or reject potential mates (Eggert & Müller, 1989). (2) More than one male often discovers a carcass and the smaller defeated male often stays near the nest and attempts to mate with the female (Bartlett, 1988; Müller & Eggert, 1989). The smaller male may get but one or a few mating attempts and may be interrupted by the larger male. It is therefore important that the smaller male mates quickly and effectively. (3) The dominant male sires most of the brood by mating frequently with the female, averaging more than 29 copulations/day in *N. vespilloides* (Müller & Eggert, 1989). The dominant male also will mate with defeated females which have been expelled from the carcass (Robertson, 1994). Effective mating performance is likely to increase the dominant male's mating advantage on the carcass and to help inseminate defeated females which may reproduce at a later time. (4) Burying beetles sometimes breed in larger communal groups with less aggression on larger carcasses which cannot be defended by a single pair (Eggert & Müller, 1992; Trumbo, 1992; Scott & Williams, 1993; Trumbo & Wilson, 1993; Scott, 1997). Mating competition could be as important as contest competition in communal groups where multiple females mate with multiple males.

There are considerable size differences among same-sex individuals. The results of our mating experiments demonstrate that size affects the ability to mate. We suggest that the lack of success by smaller males can best be explained by their inability to maintain copulatory contact. It is also possible that larger-bodied females have more stringent mate-choice criteria than small or medium-size females. In either case, the result would be lower success for smaller males and likely selection for male characteristics to enhance mating performance. To distinguish between the hypotheses of varying male mating ability and varying female mate preferences, it would be necessary to incorporate experimental trials using males of different size attempting to mate with similar-size females.

We observed that small *P. morio* males had extreme difficulty grasping a moving female and were more likely to be unsuccessful in their mating attempts. We suggest that the longer foretarsi of males increases mating success of males attempting to grasp the elytral edge of potential mates. This may be most important in establishing copulatory contact. Once copulation has been initiated, the female remains still and the male relaxes his hold.

The foretarsi of *N. orbicollis* and *P. morio* males have expanded pulvilli compared to females, a character noted for several *Nicrophorus* spp. (Arnett, 1944; Bliss, 1949). The function of this sexual dimorphism is not understood. If these structures were found to be sensory then we would speculate that they assist the male in finding and maintaining an effective hold on the female's elytra.

Sex differences in tarsal length and shape appear to be plesiomorphic in *Nicrophorus*, occurring in *P. morio* and silphids outside the subfamily Nicrophorinae. Elaboration of the midtarsi is clearly present in *Diamesus* spp. (Silphidae: Silphinae). *Diamesus* and other silphines use large carcasses for reproduction for which they do not compete. The resource is not prepared in any way and no parental care is provided. Male reproductive success is therefore predominantly determined by mating with gravid females. Male silphines mate and guard their mates for long durations between bouts of oviposition (Ratcliffe, 1996); the ability to grasp the female effectively is likely to be crucial in this mating competition. *Ptomascopus morio* is somewhat intermediate in behavior between silphines and *Nicrophorus*. Males of *P. morio* will not stay mounted on their mates for hours at a time, as do many silphines, but they will follow their mate near the carcass subsequent to copulation. This behavior is very different than *Nicrophorus* in which both sexes return to preparation of the resource following copulation. The presence of leg sexual dimorphism in *Nicrophorus* suggests that this trait has been maintained despite the change to contest competition and resource

preparation in this group.

Additional sex differences are present in nicrophorines. For individuals of the same mass, in most *Nicrophorus* species males tend to possess a larger clypeus, larger postocular bulges, and expanded pulvilli on the foretarsi (Horn, 1880; Portevin, 1926; Arnett, 1944; Bliss, 1949). The ninth abdominal segment is incorporated into the genitalia of female nicrophorines whereas this segment is unmodified in males allowing extra flexibility to bend the aedeagus anteriorly during copulation. This dimorphism is useful for determining the sex of live *Nicrophorus* (Trumbo, 1996), but is not always reliable for museum specimens (Bliss, 1949). Some nicrophorines (e.g., *N. concolor*) show no dimorphism excepting the extra visible abdominal segment of males (although leg size has not been examined). Males also stridulate audibly prior to copulation. If the stridulatory structure is made inoperative, normal mating and pair formation is disrupted (Huerta & Halffter, 1992). Although contests for carcasses are of primary importance for the reproductive success of *Nicrophorus*, we suggest that sexual selection also operates on leg and abdominal morphology as well as on chemical and auditory communication of males.

Acknowledgments

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