NOTES AND COMMENTS

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Host defense in *Nicrophorus quadripunctatus* against brood parasitism by *Ptomascopus morio* (Coleoptera: Silphidae: Nicrophorinae)

Abstract Few studies have been conducted on the host defenses of insects against brood parasitism. We investigated whether the silphid beetle *Ptomascopus morio*, a brood parasite of related silphid species *Nicrophorus concolor*, can also parasitize another silphid species *Nicrophorus quadripunctatus* and the manner in which *N. quadripunctatus* defends itself against parasitism. Successful brood parasitism under natural conditions was not observed at the time of year when *P. morio* and *N. quadripunctatus* are both reproductively active. Follow-up experiments revealed that *P. morio* attempts to oviposit near *N. quadripunctatus* nests, but is rarely successful if adult hosts are present. When *P. morio* larvae were experimentally introduced to *N. quadripunctatus* broods, some *P. morio* larvae survived when the host and parasite larvae were at the same stage. We concluded that *N. quadripunctatus* defends itself against brood parasitism in two ways: (1) potential brood parasites are repelled, thus limiting their access to the resource; and (2) the young of the parasitic species are killed.

Keywords Host rejection · Facultative brood parasitism

Introduction

The relationship between host and parasite has been extensively studied in birds (Rothstein 1990) and eusocial hymenopterans (Wilson 1971; Sledge et al. 2001;

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Cervo et al. 2004). For example, parasitic cuckoos lay their eggs in the nest of the host species that incubate and rear the parasitic offspring; thereby, the hosts suffer severe reductions in reproductive success (Payne 1998; Soler et al. 2001). Parasitism favors the evolution of host defenses such as egg rejection (Soler and Soler 2000). Such host defense mechanisms may be specific to changes in parasitic behavior and may lead to an evolutionary arms race (Rothstein 1990). In contrast to the studies of birds and eusocial hymenopterans (Davies and de Brooke 1998; Soler et al. 2001), little research has been conducted on brood parasitism in noneusocial insects (Rasa and Heg 2004), and there is even less research on host defenses.

All species of the genus *Nicrophorus* (Silphidae, Coleoptera) care for their young, such as providing them with food (small vertebrate carrion), guarding against intruders exclusively, and regurgitating food for their young (Scott 1998). The genus *Ptomascopus* is believed to be a sister group of *Nicrophorus* (Dobler and Müller 2000; Peck and Anderson 1985; Szalanski et al. 2000). *Ptomascopus morio* shows simple parental care (Suzuki and Nagano, in press). Their larvae are able to grow without parents in laboratory conditions (Peck 1982; Trumbo et al. 2001), but it is difficult for them to grow without parents in field conditions because of predation and competition (Suzuki and Nagano, in press). At a study site near Kyoto, Japan, *P. morio* Kraatz was found to be a brood parasite of *Nicrophorus concolor* Kraatz (Trumbo et al. 2001). Larvae of *P. morio* live in the broods of *N. concolor* and grow under the care of host parents though the range of care is undetermined (Trumbo et al. 2001; S. Suzuki, personal observation). *P. morio* readily parasitized *N. concolor* in the field, and parasitism by *P. morio* reduced the brood mass of *N. concolor* in the laboratory (Trumbo et al. 2001). Since *Nicrophorus* beetles eliminate intruders exclusively for their brood, such as the fly, rove beetle, and other *Nicrophorus* spp. (Müller and Eggert 1990; Scott 1990; Suzuki 2000), the *P. morio* larvae will have ways to deceive *N. concolor* parents in order to stay in the latter's brood.

In contrast, *P. morio* seldom parasitized another host species, *Nicrophorus quadripunctatus* Kraatz, in the same locality (Trumbo et al. 2001). Since the reproductive seasons of *N. quadripunctatus* and *P. morio* differ slightly in Honshu, Japan (Nagano and Suzuki 2003), whether the low frequency of the parasitism of *N. quadripunctatus* by *P. morio* was caused by host defenses or by seasonal segregation has not been established. However, the phenology of *P. morio* in Otaru, Hokkaido is similar to that of *N. quadripunctatus* (Ohara 1995). Therefore, the aim of our study was to investigate the host defenses of *N. quadripunctatus* in Otaru where seasonal segregation between these two species can be negligible.

The aims of this paper are as follows: first to confirm the presence or absence of *N. quadripunctatus* parasitization by *P. morio* in the conditions in which *P. morio* is expected to parasitize *N. concolor*, and second to investigate the mechanism by which *N. quadripunctatus* defends itself against parasitization by *P. morio*.

Materials and methods

All experiments were conducted in the forest of the Naebo Forest Park in Otaru, near Sapporo, southwestern Hokkaido, North Japan.

Phenology of *N. quadripunctatus* and *P. morio*

Burying beetles were collected using hanging polyethylene traps (97 mm diameter, 166 mm depth), with pieces of chicken as bait. Three traps were installed at the site; they were arranged linearly at intervals of at least 5 m and were suspended from trees at a height of 1.5–2.0 m above the ground to prevent their removal by vertebrates. The sampling site was a natural mixed forest (*Fraxinus mandshurica* and *Ulmus davidiana* were common species). All traps were kept open for 5 days, and the beetles were collected on the last day. *Nicrophorus* and *Ptomascopus* were identified from among the collected beetles and were counted. Sampling was performed once or twice a month from May to September 2001.

Presence of brood parasitism

To assess brood parasitism under natural conditions, 36 pieces of chicken (15 g) were placed in soil-filled polyethylene bottles (90 mm diameter, 117 mm depth) that were suspended from trees at a height of 1.5–2.0 m. All bottles were open at the top to allow the beetles to freely enter and then colonize the resource. The containers were checked 10 days after baiting. Larvae and adult nicrophorines were morphologically identified, and adults with a brood were regarded as parents. The presence of *P. morio* larvae in the *Nicrophorus* brood can be regarded as evidence of brood parasitism.

A second experiment was conducted to determine the potential of *P. morio* to parasitize *N. quadripunctatus*. One pair each of *N. quadripunctatus* and *P. morio* and 15 g of chicken were placed in a polyethylene container (15×15×9 cm^3) that was half-filled with soil; the container was covered to prevent further colonization by insects ($n = 21$). All the containers were checked at 10 days after the introduction of *N. quadripunctatus* and *P. morio*, and the larvae were checked. The larvae were identified, and all adult beetles were checked for injuries to their legs and antennae. These containers were placed in scavenger-proof cages on the forest floor until the experiments were terminated.

All experiments were conducted between early to mid July 2002.

Parasitism of undefended carcasses

The rarity of parasitism by *P. morio* could be either due to few attempts at parasitism or due to the successful host defenses of *N. quadripunctatus*. In order to distinguish between these two possibilities, a pair of *N. quadripunctatus* and a piece of chicken (15 g, $n = 10$) were introduced into a soil-filled container (15×15×9 cm^3). After ensuring that the chicken was buried in the soil, the *N. quadripunctatus* pair was confined to a small, clear plastic cylinder (16 mm diameter, 39 mm depth) with many small holes to allow the passage of odor and sound; this cylinder was placed near the chicken carcass. A *P. morio* female was introduced into the container which held the undefended carcass. The container was checked for *P. morio* larvae 5–7 days later.

Host defense by *N. quadripunctatus*

To determine whether the presence of *N. quadripunctatus* adults prevented *P. morio* from oviposition near the carcasses, a pair each of *N. quadripunctatus* and *P. morio* and 15 g of chicken were introduced into a polyethylene container (15×15×9 cm^3) that was half-filled with soil ($n = 22$). After confirming oviposition by either species, all the adult beetles were removed. The larvae were identified after hatching.

To investigate whether *N. quadripunctatus* is capable of distinguishing and excluding parasitic larvae, a pair of *N. quadripunctatus* and 15 g of chicken were introduced into a soil-filled polyethylene container. When the hatched larvae appeared on the carcass, each brood was subjected to one of three treatments:

1. The same-stage treatment ($n = 14$): five of the *P. morio* first instars were introduced into the container with the *N. quadripunctatus* brood when these larvae reached the carcass.
2. The different-stage treatment ($n = 12$): five of the *P. morio* first instars were introduced into the con-

tainer with the *N. quadripunctatus* brood when these larvae had reached the second instar.

3. The parental-removal treatment (*n* = 13): the pair of *N. quadripunctatus* was removed when the *N. quadripunctatus* larvae appeared on the carcass and five first instars of *P. morio* were introduced into the container.

Broods in all treatments were checked 4 days after the introduction of *P. morio* into the containers, and the number of surviving *P. morio* larvae were counted. In addition, ten *N. quadripunctatus* broods were maintained for 4 days after the larvae appeared on the carcass (no-parasite treatment), and the number of larvae was counted in order to compare the size of the parasitized and unparasitized *N. quadripunctatus* broods.

Results

Phenology of *N. quadripunctatus* and *P. morio*

Two species of *Nicrophorus* (*N. maculifrons* and *N. quadripunctatus*) and one species of *Ptomascopus* (*P. morio*) were trapped. Their phenology is shown in Fig. 1. A significant correlation (*P* < 0.001, *r* = 0.98) was observed between the numbers of *N. quadripunctatus* and *P. morio*; this indicates a large overlap in their respective seasonal activities.

Presence of brood parasitism

In the field experiment in which carcasses were exposed to allow natural colonization, all carcasses were used by nicrophorine beetles (*n* = 36). *N. quadripunctatus* was the most successful species (colonized 25 carcasses). Eight of the remaining carcasses were colonized by *N. maculifrons*, two by an unknown *Nicrophorus* sp. (with *Nicrophorus* larvae but no adults) and one by *P. morio*. No *P. morio* demonstrated parasitism.

Brood parasitism was rare when *P. morio* was experimentally introduced with *N. quadripunctatus* into containers that contained carcasses in the field (*n* = 21). Two broods were parasitized by *P. morio*, and only a few parasitic larvae were observed (one and three, respectively). Behavior of the parasitic larvae in *N. quadripunctatus* broods appeared not to be different from that in *N. concolor* broods. Although injury in *N. quadripunctatus* was rare (one male), injuries were common in *P. morio* (three males and ten females). The injury rates of the two species differed significantly (*P* < 0.001, Fisher's exact test).

Parasitism of undefended carcasses

P. morio readily parasitized the broods of *N. quadripunctatus* on undefended carcasses. *P. morio* larvae were present on eight of ten carcasses that were previously used for oviposition by *N. quadripunctatus*.

Host defense by *N. quadripunctatus*

When *N. quadripunctatus* and *P. morio* were both allowed to oviposit for 2 days (adults were subsequently removed), the *P. morio* larvae hatched and emerged onto eight of 22 carcasses. On an average, 3.5 ± 1.3 (mean $\pm$ SE) *P. morio* larvae were found on these eight carcasses. This means that some *P. morio* females succeeded in ovipositing in the presence of *N. quadripunctatus*.

When *P. morio* larvae were introduced into *N. quadripunctatus* broods, it was clear that *N. quadripunctatus* had the ability to eliminate the *P. morio* young (Fig. 2). Most of the introduced *P. morio* larvae survived in the parent-removal treatment group. However, when the *N. quadripunctatus* pair was present, only a small number of *P. morio* larvae survived in both the same- and different-stage treatments. The number of surviving larvae in the two treatment conditions differed significantly (*P* < 0.01; Steel–Dwass test). The *N. quadripunctatus* brood size in the same-stage treatment was 11.7 ± 1.3 and smaller than that in the no-parasite treatment, i.e. 15.2 ± 0.9 (*P* = 0.03, *t* = 2.26; *t*-test).
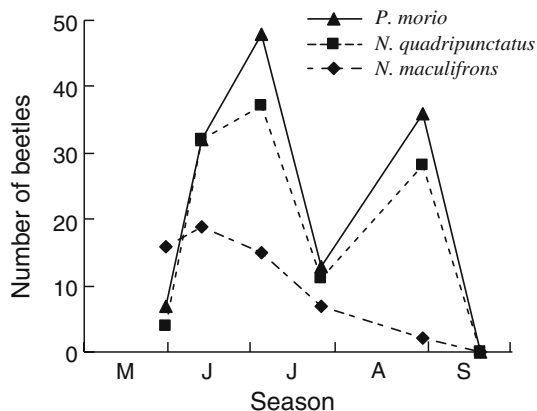

Fig. 1 Phenology of *Nicrophorus maculifrons*, *N. quadripunctatus* and *Ptomascopus morio* in Otaru. *M J J A S* May June July August September

Discussion

Trumbo et al. (2001) reported that *P. morio* from Kyoto (Honshu) successfully parasitized *N. concolor* broods in the field, and that *P. morio* derived from an Otaru population could parasitize *N. concolor* in the laboratory. However, the *P. morio* from Otaru could seldom parasitize the *N. quadripunctatus* broods. These results suggest that while *N. concolor* is susceptible to parasit-
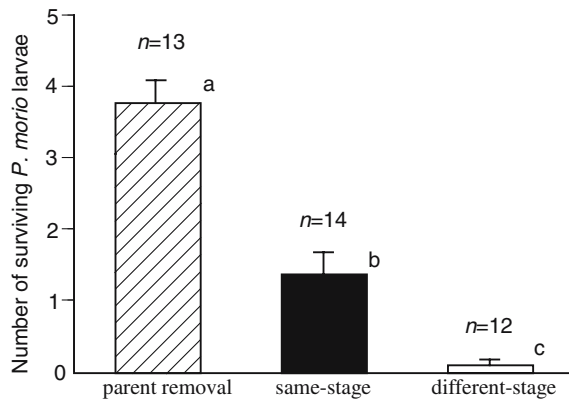
Fig. 2 Mean (+SE) number of surviving *P. morio* larvae in the parent-removal treatment (*hatched bar*), same-stage treatment (*solid bar*), and different-stage treatment (*open bar*). Bars with *different letters* are significantly different (*P* < 0.01; Steel–Dwass test)

ism, *N. quadripunctatus* has developed defense mechanisms against brood parasitism by *P. morio*.

Trumbo et al. (2001) suggested that *P. morio* is an obligate brood parasite since few *P. morio* reproduce on their own in the field. However, in Hokkaido, the susceptible populations of *N. concolor* are quite small. Further, the ecologically dominant *N. quadripunctatus* (Katakura et al. 1986; Ohkawara et al. 1998) can successfully prevent brood parasitism. It appears to be difficult for *P. morio* to reproduce by brood parasitism alone in Hokkaido. Therefore, the interaction between adult *P. morio* and *N. quadripunctatus* may be competition rather than host–parasite interaction. However, females of *P. morio* try to deposit eggs near the broods of *N. quadripunctatus*, larvae of *P. morio* try to parasitize, and parasitism by *P. morio* larvae is deleterious for the reproduction of *N. quadripunctatus*. Thus, it may be advantageous for *N. quadripunctatus* parents to defend against brood parasitism irrespective of its frequency.

Intra- and inter-specific brood parasitism have been reported for the genus *Nicrophorus* (Müller et al. 1990; Trumbo 1994). The host species of *Nicrophorus* have two defense mechanisms against a brood parasite—they either repel it, thus limiting access to the resource, or kill the parasitic young (Trumbo 1994). Adults of *N. quadripunctatus* appear to use both these mechanisms. The low oviposition success of *P. morio* was likely due to direct attacks by *N. quadripunctatus*; this was indicated by the high number of injuries observed in *P. morio* that were sustained while attempting to parasitize *N. quadripunctatus*. *N. quadripunctatus* also kill parasitic larvae. *Nicrophorus* does not have an absolute mechanism that discriminates against congeneric larvae; however, it indirectly discriminates by killing the young that do not come to the carcass at the expected time (Müller and Eggert 1990). Similarly, in our experiments, the parasitic young at a different stage of larval development than the host young were killed at a higher rate than the young at the same stage (Fig. 2). In our experiments, many *P. morio* larvae were killed by *N. quadripunctatus* even if they were at the same developmental stage as the host larvae. *N. quadripunctatus* appears to have an additional mechanism that discriminates against *P. morio* larvae. *P. morio* larvae usually remain under the carcass (Peck 1982), whereas *Nicrophorus* larvae congregate at the top of the carcass (Eggert and Müller 1997). It is possible that this behavioral difference contributes to the ability of *N. quadripunctatus* to distinguish between the host and parasitic young.

If it is difficult for *P. morio* to reproduce by brood parasitism, how can a *P. morio* population persist? The spatio-temporal distributions of *N. concolor* and *P. morio* largely overlap (Nagano and Suzuki 2003), but those of *N. quadripunctatus* and *P. morio* can either be similar (Ohara 1995), as they were in the present study, or very different (Katakura et al. 1986; Nagano and Suzuki 2003). Most facultative brood parasitism in birds occurs among independent breeders that are occasionally parasitic (Payne 1998). We hypothesize that brood parasitism by *P. morio* is facultative and that when rejected by a host, *P. morio* will reproduce in a place inhabited by few competing *Nicrophorus*. Previous studies have shown the niche differentiation between a *Nicrophorus* species and *P. morio* in habitat (Katakura and Ueno 1985) and in season (Nagano and Suzuki 2003; Trumbo et al. 2001). However, we did not observe any niche differentiation between *N. quadripunctatus* and *P. morio* in Otaru. Sometimes different *Nicrophorus* species coexist without any apparent difference in spatio-temporal distribution (Ohkawara et al. 1998). This observation warrants further investigation; heretofore unknown niche differentiation between *N. quadripunctatus* and *P. morio* may exist.

Why do *N. quadripunctatus* and *N. concolor* differ so markedly in their respective abilities to defend themselves against brood parasitism by *P. morio*? Since parasitism is typically deleterious to the host's reproductive success (Trumbo et al. 2001; this paper), we expected the hosts to develop counter-adaptations. Winfree (1999) noted that there is often a bimodal distribution of rejection frequencies among host species in birds, with each species rejecting close to either 0 or 100%. There are several hypotheses for such bimodality, including evolutionary lags and cost-benefit equilibria, but all models suggest that intermediate rejection rates are unstable (Winfree 1999). Trumbo et al. (2001) noted that *N. tomentosus* with a North American distribution was parasitized despite being completely allopatric to *P. morio*. We conclude that *P. morio* will attempt to parasitize any brood of a *Nicrophorus* species and that the degree of success of the host's defense will vary among the species and/or populations of *Nicrophorus*.

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