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Superparasitism and Host Discrimination by *Neodohrniphora elongata* (Diptera: Phoridae), a Parasitoid of the Leaf-Cutting Ant *Atta sexdens rubropilosa* (Hymenoptera: Formicidae)

by

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ABSTRACT

The leaf-cutting ant *Atta sexdens rubropilosa* Forel is parasitized by several species of Phoridae flies, one of which is *Neodohrniphora elongata* Brown. The female of this fly oviposits inside the head of larger workers of the *Atta* colony, emerging only one fly from each cephalic capsule. The objective of this study was to investigate the occurrence of superparasitism by *N. elongata* on workers of *A. sexdens rubropilosa* in the laboratory and to describe if the female of this parasitoid is able to discriminate between non-parasitized and previously parasitized workers by itself and between non-parasitized and previously parasitized workers by another female of the same species. The tests were conducted in a glass observation chamber where 15 parasitized and 15 non-parasitized workers and one fly were released at a time. The flies laid eggs preferentially in non-parasitized workers rather than workers parasitized by themselves (n=11) or parasitized by other females (n=18). In both cases females of *N. elongata* were able to discriminate the parasitized host, rejecting most of them, but did not avoid superparasitism. Based on the high indices of superparasitism found (29.4% of self superparasitism and 49.5% of conspecific superparasitism), the process of host discrimination by *N. elongata* seems to be of little efficiency at least under experimental conditions.

KEY WORDS: Phoridae, self superparasitism, conspecific superparasitism, recurrent attack

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INTRODUCTION

The leaf-cutting ant *Atta sexdens rubropilosa* Forel is host to several species of parasitoid flies of the Phoridae family, belonging to the genera *Apocephalus* Coquilett, *Myrmosicarius* Borgmeier and *Neodohrniphora* Malloch (Feener & Moss 1990, Brown 2001). The attack of phorids against workers of that ant subspecies usually results in low levels of parasitism (from 2 to 4% of the foragers). However, overflight and attacks by a single fly against the ants in a trail usually lead to a negative impact on the foraging behavior, thus reducing the number and size of foragers in the trails and increasing the loss of leaf fragments (Tonhasca 1996, Bragança *et al.* 1998, Tonhasca *et al.* 2001).

Neodohrniphora elongata Brown is the most common species of that genus in trails of *A. sexdens rubropilosa* in Southeast Brazil (Silva *et al.* 2008); its biological and behavioral characteristics have been recently described (Bragança *et al.* 2009). Observations in the field and lab tests show that females of this phorid chase the workers along the foraging trails touching several times any part of the worker's body if the head capsule width is greater than 1.6 mm, but they seem to prefer larger individuals. In a similar way to what occurs with other phorids of the same genus (Feener & Brown 1993, Tonhasca 1996, Bragança *et al.* 1998), during the attack, which lasts approximately 1 second, *N. elongata* inserts the ovipositor in the postero-dorsal extremity of the head and lays a single egg inside the head capsule where a larva feeds and develops before pupating between the mandibles of the ant (Bragança *et al.* 2008).

Studies conducted in the field and in the laboratory have shown that some species of Hymenopteran parasitoids and flies of the Tachinidae family may distinguish between parasitized and non-parasitized hosts by using visual, olfactory or tactile cues (Visser 1993, López *et al.* 1995, Gauthier *et al.* 1996). However, the ability for intraspecific discrimination between hosts parasitized or not does not always result in the absence of superparasitism, this being of advantage, for example, if the number of hosts is low for the parasitoid that lays eggs on hosts already parasitized by itself (self superparasitism) or by a conspecific female (conspecific parasitism) (Gauthier *et al.* 1996, Sirot & Krivan 1997).

There is a paucity of information on the occurrence of superparasitism and host discrimination in the relationships between phorid parasitoids and ants.

The female of *Apocephalus paraponerae* Borgmeier may lay one or more eggs on each worker of *Paraponera clavata* (Fabricius). More than one female is normally attracted to the worker and lays on it and the presence of 20 or more larvae developing in a single host is common (Brown & Feener 1991, Feener *et al.* 1996). The phorid *Apocephalus attophilus* Borgmeier superparasitizes the leaf-cutters *A. sexdens rubropilosa* and *Atta laevigata* (F. Smith), with one to 13 flies emerging from a host worker (Erthal & Tonhasca 2000, Bragança & Medeiros 2006, M. Bragança, unpublished data). The phorid *Neodohrniphora curvinervis* (Malloch) is a parasitoid of *Atta cephalotes* (L.), with only one fly emerging per host, but in 19% of the cephalic capsules dissected more than an egg or an egg and a larva were found together or more than a larva was found in different phases of development. This suggests independent oviposition (Feener & Brown 1993). These authors considered the index of superparasitism high when compared with that of Hymenopteran parasitoids and that this may represent a lack of ability of the phorid females to discriminate between parasitized and non-parasitized hosts.

In this study it was tested if females of *N. elongata* collected in the field were able to superparasitize workers of *A. sexdens rubropilosa* in the laboratory. For this, we formulated two questions: 1) Does the female of the parasitoid discriminate workers parasitized by itself? 2) Is the female able to distinguish workers parasitized by another female?

MATERIALS AND METHODS

This study was conducted at the Insectarium of the Animal Biology Department, Federal University of Viçosa (UFV) in the city of Viçosa (20° 45'S, 42° 51'W), in the State of Minas Gerais, Brazil, between the months of January and March, 2005. Females of *N. elongata* were captured between 6:00 and 10:00 a.m. while they attacked workers of *A. sexdens rubropilosa* in foraging trails of several nests located on the University campus.

The parasitoids were taken to the laboratory and fed with a 10% honey solution up to 12 hours before being released individually in an observation glass chamber (50 cm long x 25 cm of width and 25 cm of height) with two frontal circular openings of 10 cm in diameter through which the fly and workers were introduced (Bragança *et al.* 2008). The ants belonged to two laboratory-reared 3 and a half year-old colonies of *A. sexdens rubropilosa*.

The foraging workers caught at random from these colonies for use in this experiment were among the largest individuals (> 1.8 mm head capsule width) (Tonhasca *et al.* 1996, Bragança *et al.* 2002). Temperature and moisture in the laboratory were kept at $23 \pm 1^\circ\text{C}$ and $80 \pm 5\%$ R.H.

Two bioassays were conducted to evaluate the occurrence of new attacks by the same female of *N. elongata* or by another conspecific female against workers of *A. sexdens rubropilosa*.

Bioassay 1. Recurrent attacks by the same female

In this bioassay 30 workers from one of the laboratory colonies were released in the observation chamber with one female parasitoid. We registered the number of attacks by the fly during 20 minutes. Each attacked ant was immediately taken from the chamber with the help of tweezers and marked on the gaster with a non-toxic, odorless ink (Painters^R).

After 20 minutes the fly was immobilized inside the chamber by a glass tube inverted on it while 15 of the marked attacked ants were replaced into the chamber along with 15 non-attacked ants. When the number of attacked ants was greater than 15, other ants were taken from the laboratory colony and utilized to add to the 15 non-marked ants inside the chamber. After this, the fly was released from the glass vial into the chamber for another 20 minutes, when the number of attacks of the fly against marked and non-marked ants was annotated. This procedure was repeated 11 times, with different females of *N. elongata* and workers from the two colonies.

Control Test

This test was conducted to evaluate possible effects of the ink on the gaster of the workers and the exhaustion of the phorid on the number of attacks in the final 20 minutes in relation to the initial 20 minutes.

One fly and 30 ants of each of the two laboratory colonies were introduced into the observation chamber, 15 of them previously marked on the gaster. The numbers of the attacks on marked and non-marked ants were registered during 20 minutes, the ants being taken from the chamber immediately after each attack. Following this, the fly was immobilized inside the chamber and the number of the 15 marked and unmarked workers inside the chamber was remade with ants of the same colony. During the following 20 minutes the number of attacks by the same fly on the two ant groups was registered; each

worker was removed after the attack. Twelve essays were conducted using different flies and ants.

Bioassay 2. Recurrence of attacks by another female

Thirty workers from one of the laboratory colonies were released in the observation chamber along with a parasitoid female. This female remained in the chamber until it attacked 15 ants, each of those removed from the chamber after the attack and marked with yellow ink. After the phorid was removed, the 15 non-attacked ants were also removed from the chamber and given an orange color. Following this, the 30 marked ants were replaced inside the chamber with another *N. elongata* female. The attacks of the second fly against workers previously marked yellow or orange were recorded for 20 minutes, the ants being taken from the chamber immediately after the attack. Eighteen replicates of this essay were conducted using 36 different flies and ants of the two colonies.

Rearing the adult parasitoids

At the end of each replicate of the two bioassays and of the control test, the ants attacked by flies were segregated into groups of up to 10 individuals kept in Petri dishes (12 cm Ø) and taken to a climatic chamber kept at 26.5 ± 0.5 °C; $85 \pm 5\%$ R.H. and in absence of light for the development of the parasitoids. The ants were daily fed with a 10% honey solution until their death. Following this, the dead ants with a sign of parasitism, that is, with a pupa between its mandibles (Bragança *et al.* 2008) were individually taken to glass tubes (8.2 cm height x 2.3 cm Ø) that were closed with cotton and kept in a B.O.D. chamber for up to 30 days to evaluate the emergence of the flies. This period was considered sufficient for the emergence of *N. elongata* found in workers of *A. sexdens rubropilosa* (Bragança *et al.* 2009).

Several flies of those obtained in the laboratory were mailed to Dr. Brian V. Brown (Natural History Museum of Los Angeles County) for species name confirmation. The flies collected in the field and others obtained in the laboratory were deposited in the Entomological Collection of the Biological Science Course, Federal University of Tocantins.

Data Analysis

The difference in the number of ants attacked between the 20 initial minutes and the 20 final minutes and between the number of marked and non-marked ants in the first bioassay were analyzed by the t-test.

In the same manner, in the second bioassay the number of attacked ants and those with recurrent attacks were also analyzed by the t-test. The data of the control test were submitted to a factorial ANOVA to evaluate the effects of time, ink and the interaction between these factors on the number of attacks. Data on the number of attacks were submitted to log transformation ($\log x+1$) to reduce the effect of heterogeneity of variances.

RESULTS

In bioassay 1 (Recurrence of attack by the same female), the average number of attacked ants in the 20 initial minutes was significantly higher than in the 20 final minutes (Table 1). Of the total number of 88 attacked workers in these final minutes, 20 (29.4%) had already been attacked by the same females (self superparasitism). On the average, the flies attacked significantly more non-marked ants rather than marked ones; that is, they attacked preferentially ants that had not been parasitized during the 20 initial minutes (Table 1).

Table 1. Comparison between the number of workers (mean \pm standard deviation) of *A. sexdens rubropilosa* attacked by a same female of the parasitoid *N. elongata* during the first 20 minutes and the final 20 minutes and also between those ink-marked (attacked during the first 20 minutes) and not marked (not previously attacked) during the last 20 minutes.

	Number of workers	n	t	P
Attacked (first 20 min)	19.3 \pm 3.0	11	6.49	< 0.0001
Attacked (final 20 min)	8.0 \pm 3.2	11		
Ink-marked (attacked)	1.8 \pm 1.4	11	4.41	0.0003
Not marked (non attacked)	6.2 \pm 2.7	11		

The results of the control test show that at least part of the diminished attack capacity of the flies may be attributed to fly exhaustion; the ink applied to the gaster did not show an effect on this decrease (Table 2). Besides this, the lack of interaction between the factors time and ink (Table 2, Fig. 1) show that the lower number of attacks in marked ants in the first bioassay is an effect of the attacks made against these ants in the first 20 minutes, that is, the flies rejected and did not attack a great part of marked ants because they had already been parasitized.

In the second bioassay the females of *N. elongata* released in the 20 initial minutes attacked a total of 95 workers; 47 (49.5%) of these had already been attacked by other females of same phorid species (conspecific superparasitism).

The average number of ants that suffered recurrent attacks by females that were different from those that made the first attacks was significantly lower than the number of ants that were attacked only once (first attack) (Fig. 2). As in the first bioassay, the females liberated in the chamber in the final 20 minutes made a lower number of attacks on workers previously attacked by other females because they could discriminate such ants as already parasitized and rejected most of them.

Table 2. Factorial ANOVA to evaluate the effects of time (first 20 minutes or final 20 minutes), ink mark (yes or no) on the ant's body and the interaction between those two factors on the number of the workers of *A. sexdens rubropilosa* attacked by females of the phorid *N. elongata*.

Factor	df	Mean square	F	P
Time	1	0.21570	6.87	0.012
Ink mark	1	0.01192	0.38	0.541
Time x Ink marc	1	0.00399	0.13	0.723
Error	44	0.03141		

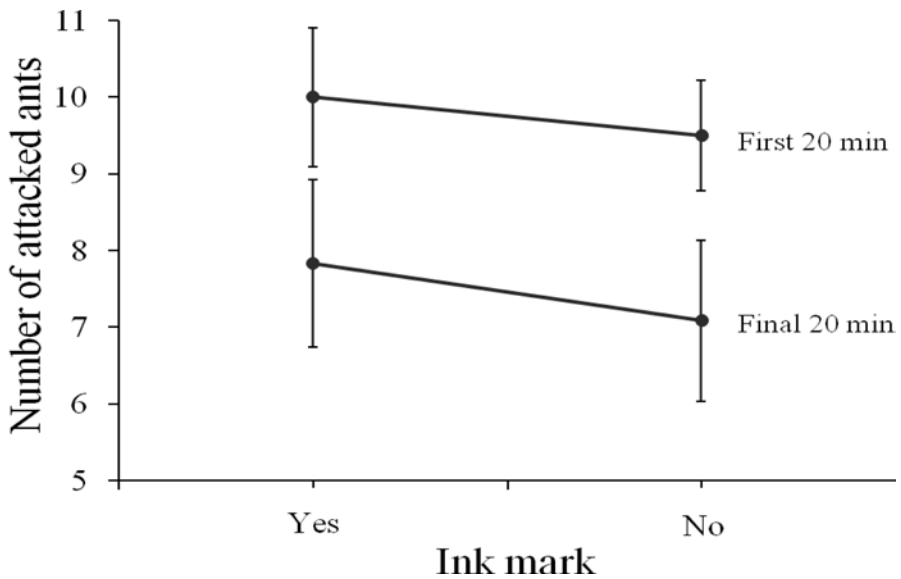


Fig. 1. Means \pm standard deviations of the number of workers of *A. sexdens rubropilosa* with or without ink marks attacked by the same females ($n=12$) of *N. elongata* in two consecutive 20 minute sessions.

DISCUSSION

Laboratory experiments have shown that visual stimulation is an essential component for the behavioral responses of *N. elongata* for locating and recognition of the host (Bragança *et al.* 2008). During the chase to ants, the females of *N. elongata* make more than five attacks, simply touching the host body for each attack with oviposition (Bragança *et al.* 2009). The absence of oviposition penetration during touches may indicate to the flies the adequacy of a potential host (Silva *et al.* 2008). Besides these, touches during the sorties also allow *N. elongata* to recognize and attack workers of *A. laevigata* and *Acromyrmex crassispinus* Forel under laboratory conditions although it is not known if such attacks are successful (Silva *et al.* 2008).

Our results have shown that even if the female of *N. elongata* is exhausted during the last 20 minutes, it is still able to discriminate workers that had or not been previously parasitized by itself or by other females of the same species, but did not avoid superparasitism. The females of *N. elongata* make

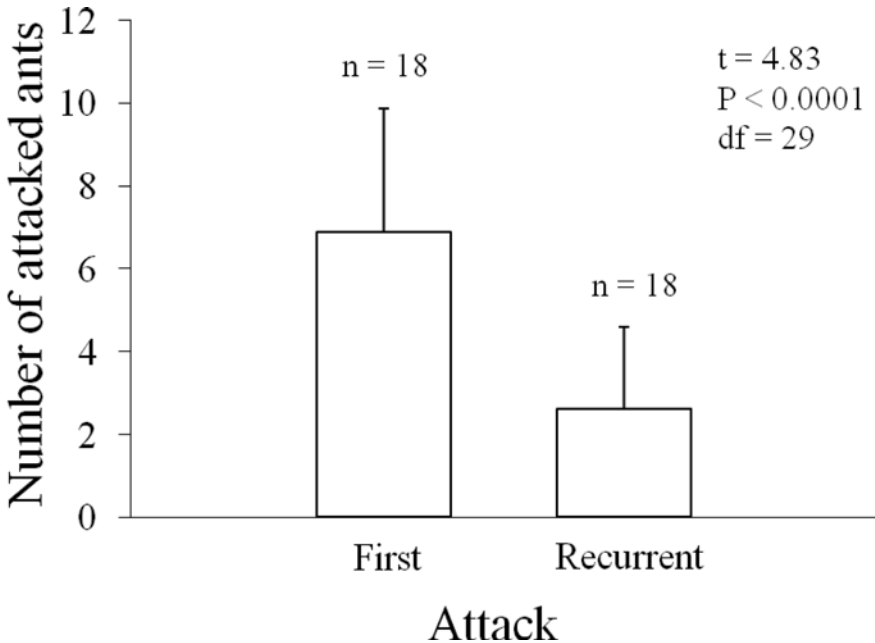


Fig. 2. Comparison between the number of workers (mean \pm standard deviation) of *A. sexdens rubropilosa* attacked by one female (first attack) or two females (recurrent attack) of the parasitoid *N. elongata*.

direct contact with the host during oviposition and this is the best opportunity to test the host quality (Feener & Brown 1997). This probably involves chemio- and mecanorreceptors on the tarsi of the flies (Lopez *et al.* 1995). It has already been shown that after the attack by *N. elongata* there occurs a gathering of the nestmates around the recently attacked worker (Bragança *et al.* 2009), which supposedly represents a grooming behavior due to the injury inflicted by the introduction of the fly ovipositor or because it deposits an unknown odor over the cuticle of the host (Wheeler *et al.* 2002).

The discriminating ability is not always implicated in avoiding superparasitism (Feener & Brown 1997). Superparasitism is common in the order Diptera, specially in Tachinidae (López *et al.* 1995, Feener & Brown 1997). In phorids of the genus *Neodohrniphora*, superparasitism is known from the interaction between *N. curvinervis* and the leaf-cutting ant *A. cephalotes* (Feener & Brown 1997).

Our results have shown that *N. elongata* is a parasitoid with an “imperfect ability” to discriminate between parasitized and non-parasitized hosts, as Rosenhein & Mangel (1994) and Yamata & Ikawa (2005) reported, because the flies did recognize attacked workers but still laid their eggs in some of them. However, the high indices of superparasitism here revealed (29.4% of self superparasitism and 49.5% of conspecific superparasitism) are possibly related to experimental conditions. This is because the discrimination rate, for instance, may depend on the density of parasitized and non-parasitized hosts available for the phorid females (Gauthier *et al.* 1996) or, in the case of interaction between *A. sexdens rubropilosa* and *Neodohrniphora* spp., on the density of workers of different sizes also available (Silva *et al.* 2007). In the absence of appropriate non-parasitized hosts, the female may accept parasitized hosts and superparasitize them (Feener & Brown 1997).

The manipulation of the number and size of non-parasitized ants in relation to those parasitized would produce superparasitism indices different from those found in this study. This is to be expected because females of *Neodohrniphora* spp. generally prefer the largest workers in the trails (Tonhasca 1996, Bragança *et al.* 2002) and because a larger number of non-parasitized ants in the glass observation chamber could avoid superparasitism. Both in the field and in the laboratory the workers of *A. sexdens rubropilosa* react aggressively to the attacks of *Neodohrniphora* spp., including *N. elongata*;

it is not uncommon that, when laying, the fly is killed by the mandibles of another worker (Tonhasca 1996, Bragança *et al.* 2002, Bragança *et al.* 2009). The oviposition on quick and aggressively reacting hosts such as ants must be fast and precise to avoid damage; this leaves not much time for the flies to accurately evaluate the host condition which results in high superparasitism (Feener & Brown 1997).

In this study, females of *N. elongata* were capable of discriminating between workers of *A. sexdens rubropilosa* parasitized or not by themselves or by other females, but superparasitism was not avoided. In the two bioassays the recurrence of attacks was tested immediately after the first attack. It is suggested that the occurrence of superparasitism be evaluated at different time intervals between the first and second attack to detect possible variation in host discrimination by *N. elongata*, and also to study the effect of varying parasitized and non-parasitized host proportions on superparasitism.

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