Host choice decisions in the polyembryonic wasp *Copidosoma koehleri* (Hymenoptera: Encyrtidae)

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Abstract. Female parasitoids often reject hosts of poor quality, where the survival and fitness of their offspring are expected to be low. In polyembryonic parasitoid wasps, a clone of genetically identical embryos develops from one egg in a host. In the wasp *Copidosoma koehleri*, each female clone produces one soldier larva that attacks competing clones inside the host. Aggression by soldiers is directed usually towards unrelated clones. Accordingly, it may be predicted that females will prefer nonparasitized over parasitized hosts, especially if the latter have been parasitized previously by a mated unrelated female, as a result of the reduced chances of survival for their offspring inside these hosts. In accordance with these predictions, females prefer nonparasitized hosts over self-parasitized hosts when they are presented simultaneously. By contrast to the predictions, females prefer hosts parasitized by an unrelated conspecific over nonparasitized hosts when presented simultaneously. Females do not distinguish hosts parasitized by conspecifics from self-parasitized hosts when presented simultaneously. They reject self-parasitized hosts significantly more often than hosts parasitized by conspecifics when each host type is presented alone. Females faced with two previously parasitized hosts are not affected in their choice by the mating status (i.e. virgin or mated) of the previous parasitizing females. The combined results suggest that females are limited in their ability to assess the risk that their offspring will be attacked by a soldier, or that this risk is balanced by the relative advantages of ovipositing in a host parasitized by conspecifics. A possible advantage may be increased out-breeding opportunities for the emerging offspring.

Key words. *Copidosoma koehleri*, host choice, host quality, polyembryonic parasitoid wasps, soldier larva, superparasitism.

Introduction

Habitat choice may have high importance for the survival, foraging and reproductive success of an individual. In parasitoids, the host body serves as a habitat for the developing offspring (Godfray, 1994). Host quality affects the survival of offspring, their developmental rate and their size at emergence (King, 1996; Godin & Boivin, 2000; Harvey, 2000; Chong & Oetting, 2006; King & Napoleon, 2006; Ozkan, 2006). Accordingly,

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female parasitoids often adaptively choose hosts according to their species, size and developmental stage (King, 1996; Sait *et al.*, 1997; Chow & Mackauer, 1999; Godin & Boivin, 2000; Mansfield & Mills, 2004; Daane *et al.*, 2005; Chong & Oetting, 2006).

Host evaluation may include several steps, such as antennation, probing and drumming the host surface (Drost & Carde, 1990; Higuchi & Suzuki, 1996; Conti *et al.*, 1997; Ardeh *et al.*, 2005), and may be based on physical features of the host, such as size, shape and texture. Additionally, host choice may be based on chemical cues from the host or from markings by previously parasitizing females (Godfray, 1994). Host marking is reported for many parasitoid species and may provide the

marking female, as well as other females, with the information that the host is parasitized (van Alphen & Visser, 1990; Nufio & Papaj, 2001). Accordingly, females often avoid ovipositing in previously parasitized hosts (King & Rafai, 1970; Conti *et al.*, 1997; Netting & Hunter, 2000). In addition, females more often avoid hosts that have been parasitized previously by themselves than those parasitized by conspecifics (Volkl & Mackauer, 1990; Holler *et al.*, 1991; van Dijken *et al.*, 1992; Cronin & Strong, 1993; van Baaren *et al.*, 1994; Marris *et al.*, 1996; Field & Keller, 1999), most likely because the avoidance of self-superparasitism decreases competition among siblings (Nufio & Papaj, 2001).

Host discrimination may have high importance in polyembryonic parasitoid wasps. In this group, each egg produces a clone of genetically identical embryos inside the host (Strand, 2003). Thus, competition for the host resources is expected to be intense. In some species of polyembryonic wasps, a proportion of embryos develop into sterile soldier larvae (Silvestri, 1937; Doutt, 1952; Cruz *et al.*, 1990) that attack inter- and intra-specific competitors inside the host (Cruz, 1981, 1986). Soldiers are mostly females, developing from clones produced by mated females (Grbic *et al.*, 1992; Segoli *et al.*, 2009a). Soldiers develop faster than the rest of the embryos and may eliminate competing clones at a relatively early stage of development (Giron *et al.*, 2007). Soldiers are reported to attack according to relatedness, with higher levels of aggression being directed toward unrelated individuals (Giron *et al.*, 2004; Giron & Strand, 2004). Thus, offspring survival may be especially low in hosts that have been previously parasitized by an unrelated mated female.

On the basis of the above, it is hypothesized that females of polyembryonic wasps perceive parasitized hosts as being of low quality because of the high direct (aggression) and indirect (resource) competition that their offspring are likely to face. Furthermore, it is hypothesized that, because of the high risk of soldier aggression, hosts parasitized by a mated unrelated female are perceived as having extremely low quality. To test these hypotheses, several sub-questions are addressed: (i) do females discriminate parasitized from nonparasitized hosts; (ii) do females discriminate hosts parasitized by a virgin female from hosts parasitized by a mated female; and (iii) do females discriminate self-parasitized hosts from hosts parasitized by conspecific unrelated females? It is predicted that females would prefer nonparasitized hosts over parasitized hosts, and hosts parasitized by virgins (i.e having sons with no soldiers) over hosts parasitized by mated females (i.e. that are more likely to contain a soldier). If females can assess the risk for a soldier attack, and if it exceeds the cost of inducing sibling competition, hosts parasitized by unrelated conspecifics may become less attractive than self-parasitized hosts. Otherwise, females would prefer conspecific parasitized hosts over self-parasitized hosts, thereby avoiding sibling competition, as in other parasitoids (Nufio & Papaj, 2001).

Materials and methods

Study species

The present study used the polyembryonic egg-larval parasitoid wasp *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae), a polyembryonic wasp that parasitizes the potato tuber moth (*Phthorimaea operculella* Zeller). A female releases normally one egg per oviposition, although superparasitism by the same or by a different female may occur (Doutt, 1947; Keasar *et al.*, 2006). The host larva hatches from the egg and continues to develop, whereas the wasp embryos proliferate inside its body to produce a clone of approximately 40 individuals. If more than one egg was laid, several clones would compete inside the host. Wasp larvae actively feed on the host tissues until they fully consume the host and pupate. Wasps emerge from the host cuticle as adults. Observations suggest that each female clone produces one soldier, whereas male clones never produce a soldier (Doutt, 1952; Keasar *et al.*, 2006; Segoli *et al.*, 2009a). Additionally, there is evidence that the soldier more often attacks unrelated clones than related ones (Segoli *et al.*, 2009b). Evidence from a field population indicates that superparasitism is common (Segoli *et al.*, 2009b). However, it is not known whether this results from indiscriminative behaviour by ovipositing females or from ecological constraints on the number of available hosts.

Rearing and using of wasps

A laboratory stock of *C. koehleri* was used in these experiments. The stock originated from field-collected individuals from South Africa (courtesy of Dr R. Kfir, Plant Protection Institute, Pretoria). Parasitoids were housed at 27°C, natural daylight, and fed with honey. A laboratory stock of potato tuber moth was housed at 27°C, natural daylight, and fed with honey and water. Potato tuber moth eggs were collected daily and were used within 24 h because the age of the eggs is known to influence the parasitoids' oviposition decisions (Ode & Strand, 1995). Adult wasps were used within 5 days after emergence. Females from mating treatments were exposed each to one male and observed until copulation occurred. Females that did not oviposit within 3 min from the first contact with the host were excluded from the experiment. Because females from the same brood are likely to be related, only one female per brood was used as the focal female for each experiment. Focal females in the two-choice experiments were either virgins ($n = 71$) or mated ($n = 67$) but, as the results did not differ between these groups, they were combined within each treatment. In the single-choice experiment, focal females ($n = 86$) were all mated.

Parasitized versus nonparasitized host

A two-choice experiment was conducted in which each female was exposed to two hosts: one nonparasitized and one that had previously been parasitized either by the same female

($n = 37$ tests) or by an unrelated conspecific female (i.e. from a different brood; $n = 50$ tests). This was carried out by placing two host eggs of a similar size and age in the centre of a Petri dish. Host eggs were not in direct contact but were sufficiently close to be touched by one female simultaneously. Soon after, a female was introduced to the Petri dish and directed to its centre. This was achieved by rotating the dish when holding it vertically, using the tendency of wasps to walk upwards on vertical surfaces. As soon as the female was in close proximity to the hosts, the Petri dish was placed under the dissecting microscope and was not moved further. The female was removed immediately after she inserted her ovipositor fully into one of the hosts. The location of the parasitized host was indicated by a small inconspicuous marking at the side of the Petri dish, aiming to avoid bias by the choosing wasp or by the experimenter. Approximately 2 h later, the same female or a different unrelated female was introduced to the Petri dish containing the host eggs. This time gap was introduced to induce developmental delay of the second clone, possibly increasing the risk of superparasitism. The first host in which this focal female oviposited was recorded. In this experimental design, focal females exposed to self-parasitized versus nonparasitized hosts have always had one previous host encounter. To control for possible effects of previous experience on host choice, some of the focal females presented with a nonparasitized and a conspecific parasitized host were experimentally naïve, whereas others had a previous experience of oviposition into a nonparasitized host. Because the results did not differ between females with previous experience ($n = 28$) and females without previous experience ($n = 22$), these groups were combined.

Hosts parasitized by a virgin versus mated female wasp

A two-choice experiment was conducted in which a female was exposed to two hosts; one parasitized by a virgin unrelated female and one parasitized by a mated unrelated female. As in the previous experiment, two host eggs were placed first on a Petri dish. Soon after, a virgin or a mated female was introduced and was allowed to oviposit in one host. Then, a female of a different mating status (i.e. mated or virgin, respectively) was introduced and was directed to the host that was not yet parasitized. The second female was not allowed to oviposit in the already parasitized host. Approximately 2 h later, a third female (i.e. the focal female) was introduced to the plate and allowed to choose between the two hosts. In this experiment, focal females had no previous experience. The sample size was 23.

Self-parasitized versus conspecific parasitized hosts

Two-choice experiment. Each female was exposed to two hosts: one that had been previously parasitized by the same female and one that had been previously parasitized by an unrelated female. First, a female was allowed to oviposit once in one of these hosts. Soon after, a second female was

directed to the yet nonparasitized host and observed until she oviposited in it. Finally, the first female (i.e. the focal female) was introduced again and allowed to choose between the two hosts. Thus, each focal female from this experiment had one previous experience of oviposition into a nonparasitized host. The sample size for this experiment was 28.

Single-choice experiment. Whether females tended to accept or reject hosts according to their relatedness to a previously parasitizing female was tested when only one host was presented to them. Each focal female was exposed to a single host that had been parasitized previously by the same female ($n = 38$) or by an unrelated female ($n = 48$). To control for previous experience, females presented with a host parasitized by an unrelated female had a previous experience with a single nonparasitized host. If a female did not oviposit within 3 min after the first contact with the host, rejection was scored.

Statistical analysis

Whether the distribution of choices between two options in each two-choice experiment deviated from the expected in a random choice was tested using the binomial test. Fisher's exact test was used in the single-choice experiment to test whether the rejection level differed between females presented with self-parasitized hosts and those with host parasitized by conspecifics.

Results

Parasitized versus nonparasitized host

When allowed to choose between a self-parasitized and a nonparasitized host, females more often selected the nonparasitized host. The frequency was different from that expected by random choice (binomial test, $P = 0.047$; Table 1). Unexpectedly, when offered a conspecific parasitized and a nonparasitized host, females more often chose the conspecific parasitized host. The frequency was different from that expected by random choice (binomial test, $P = 0.032$; Table 1).

Hosts parasitized by a virgin versus mated female

Females did not show a preference to hosts that were previously parasitized by a virgin over hosts that were previously parasitized by a mated female, or vice versa (binomial test $P = 0.57$; Table 1).

Self-parasitized versus conspecific parasitized hosts

Two-choice experiment. Females did not show a significant preference for self-parasitized hosts over hosts parasitized by conspecific, or vice versa (binomial test, $P = 0.68$; Table 1).

Table 1. Number of females that made a specific choice in the different experiments.

Experiment	Female's first choice		
1: Nonparasitized versus parasitized	Parasitized host	Nonparasitized host	Binomial test
Nonparasitized versus self-parasitized host	12	25	$P = 0.047$
Nonparasitized versus conspecific parasitized host	33	17	$P = 0.032$
2: Parasitized by virgin versus by mated	Host parasitized by virgin	Host parasitized by mated	Binomial test
Host parasitized by virgin versus by mated	10	13	$P = 0.68$
3: Self-parasitized versus conspecific parasitized	Self-parasitized host	Conspecific parasitized host	Binomial test
Self-parasitized versus conspecific parasitized host	12	16	$P = 0.57$
4: Self-parasitized or conspecific parasitized	Rejected host	Accepted host	Fisher's exact test
Self-parasitized	11	27	$P = 0.049$
Conspecific parasitized	5	43	

Experiment 1: Each female had the choice between one nonparasitized host, and one host that was previously parasitized by herself (self-parasitized host) or by an unrelated female (conspecific parasitized host), in a two-choice experiment. Experiment 2: Each female had the choice between one host that was parasitized previously by a mated female and one host that was parasitized previously by a virgin female, in a two-choice experiment. Experiment 3: Each female had the choice between one conspecific parasitized host and one self-parasitized host, in a two-choice experiment. Experiment 4: Each female was presented with a single host that was either self-parasitized or conspecific parasitized. The number of females that accepted or rejected were scored.

Very often, females displayed a distinct behaviour that was not observed in previous tests: they moved from one host to another examining them repeatedly with their antennae and often did not oviposit at all.

Single-choice experiment. When only a single host was presented (self-parasitized or parasitized by a conspecific), females more often rejected self-parasitized hosts than hosts parasitized by conspecifics (Fisher's exact test, $P = 0.049$; Table 1).

Discussion

The present study tests whether host choice by polyembryonic wasps is affected by the anticipated future survival prospects of their offspring. It was hypothesized that ovipositing females perceive parasitized hosts, especially those parasitized by an unrelated mated female, as comprising extremely low quality hosts, as a result of the reduced chances of survival of their offspring inside these hosts. In accordance with these predictions, females prefer nonparasitized hosts over self-parasitized hosts. By contrast to the predictions, females do not avoid, and sometimes even prefer, hosts parasitized by a conspecific over other host types. The results may be explained both at the proximate level and at the ultimate level.

Proximate level

The lack of avoidance of hosts parasitized by conspecifics may be explained by the limited ability of females to assess the potential risk of soldier aggression toward their offspring. The results support the possibility of limited host discrimination by ovipositing females. This is because hosts parasitized by a virgin female (i.e. that do not contain a soldier) are not preferred over those parasitized by a mated female (i.e. that may contain a soldier and hence a greater future risk to offspring).

Females show an active preference for conspecific parasitized hosts over nonparasitized hosts, rather than mere non-avoidance, which requires an additional explanation. The results could be biased if the first and second female from the same trial prefer to oviposit in the same host, regardless of its parasitism status, as a result of some other differential quality that cannot be detected by the observer. Although, in the present study, hosts are of a similar age and size, and are subjected to a similar treatment, this possibility cannot be ruled out. Perhaps the avoidance of self-superparasitism is strong enough to compensate for this bias in the choice between self-parasitized and nonparasitized hosts. Because variation in host quality is expected to be larger in the field, such a bias should be expressed also under natural conditions, resulting in the occurrence of conspecific superparasitism.

The lack of discrimination between conspecific and self-parasitized hosts in the two-choice experiment does not coincide with the results obtained in other experiments (i.e. experiments 1 and 4). In general, in this two-choice situation, females appear to be disoriented and often do not choose any host. Chemical external markings of the host by previously parasitizing females are perhaps mixed as a result of the proximity of host eggs at the time of oviposition. Although this could be controlled experimentally by allowing the first and second females to oviposit on separate substrates before placing the host eggs together, the present set-up mimics natural conditions more closely because host eggs are often

glued to the substrate and are occasionally in small clusters. Thus, a mixing of chemical markings is probably common and may result in reduced discrimination ability.

Ultimate level

In addition to the mechanistic hypotheses offered above, the adaptive implications of superparasitism in the present study should be considered. The results may suggest that the risk of conspecific superparasitism is balanced through the relative advantages of ovipositing in a conspecific parasitized host. One factor that may affect this risk is the time gap between the first and second oviposition. As the time gap increases, the risk for the younger offspring may increase. In accordance, it is reported that parasitoid females of several species avoid superparasitism only when sufficient time has elapsed subsequent to the first parasitism (King & Rafai, 1970; Godfray, 1994). Giron *et al.* (2007) show that, in the polyembryonic wasp *Copidosoma floridanum*, a time gap of 12 h is sufficient to provide the first clone with a total advantage in competition. On the basis of the above, it may be that the time between the first and second oviposition in our experiments is not large enough to impose a sufficient risk for the younger clone. Additional experiments of varying time gaps are necessary to test this hypothesis.

A preference for conspecific parasitism is known from the parasitoid *Ooencyrtus nezarae* where females use a previously drilled hole to deposit their eggs, thereby reducing handling time (Takasu & Hirose, 1991). *Copidosoma koehleri* females are never observed using previously drilled holes but do reduce the duration of insertion when ovipositing in a parasitized host (Segoli *et al.*, 2009c). This may confer some advantage for conspecific parasitism, although it is not likely to compensate for the increased resource competition and the risk for offspring inside the host.

Another possible explanation for the females' preference for conspecific parasitism may be to enhance out-breeding opportunities for their offspring upon emergence. Indeed, male and female *C. koehleri* that develop within the same host emerge during a short time window and often mate soon after emergence, at least in the laboratory (M. Segoli, personal observations). Thus, offspring may compensate for lower survival in hosts parasitized by conspecifics through increased out-breeding opportunities. This hypothesis, however, should be treated with caution: local mating under crowded conditions, such as imposed in the laboratory, does not necessarily indicate local mating under natural conditions (Ode *et al.*, 1995; Godfray & Cook, 1997), and information on the mating system of this wasp under natural conditions is lacking.

Overall, the results of the present study suggest that host choice may be subjected to several constraints as well as to different and sometimes opposing selective forces. Additional data on host availability, mating opportunities and offspring survival under natural conditions are required to increase our understanding of the evolutionary forces shaping host choice decisions in polyembryonic parasitoids

Acknowledgements

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