

State-dependent host acceptance in the parasitoid *Copidosoma koehleri*: the effect of intervals between host encounters

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Abstract Choosing suitable hosts is an important component of parasitoid fitness. Many parasitoids reject already-parasitized hosts. In some species, females reject hosts in which they had recently oviposited, but accept these hosts minutes or hours later. Parasitoids were suggested to mark hosts with repellent pheromones at oviposition and to accept them again after the mark fades. We tested a complementary hypothesis, stating that parasitoids' host acceptance thresholds decrease with time since their previous oviposition because of a change in internal state, independent of the hosts' deterrent marking. We scored the acceptance of hosts (eggs of the moth *Phthorimaea operculella*, Gelechiidae) by *Copidosoma koehleri* (Encyrtidae) parasitoids in single-choice experiments. Acceptance of self-parasitized (low-quality) hosts increased with time since the wasps' previous host encounter. The wasps accepted two non-parasitized (high-quality) hosts within a 5-s presentation interval, indicating that they are physiologically able to oviposit twice in quick succession. Parasitized hosts, presented after varying time intervals to conspecifics with uniform host encounter experience, were accepted at similar frequencies. However, as predicted by the working hypothesis, increasing the time elapsed since the wasps' last oviposition significantly enhanced the acceptance rates of hosts that had been parasitized by conspecifics 2 min earlier. Learning of host-associated cues during oviposition may enable wasps to identify and reject parasitized hosts, while fading of this association from their short-term

memory during periods with no host encounters could trigger acceptance of parasitized hosts. State-dependent host acceptance may adaptively allow wasps to avoid self-superparasitism and to adjust conspecific superparasitism rates to foraging conditions.

Keywords Parasitoid · Host choice · Learning · Superparasitism · Acceptance threshold

Introduction

Parasitoid insects lay their eggs in or on the body of other arthropods, which later become hosts for the parasitoids' offspring larvae. Parasitoid females do not feed their young and generally do not guard them (Hardy and Blackburn 1991). Hence, host selection is one of the few components of maternal investment in these organisms. The quality of a host may depend on factors such as its species (Strand and Obrycki 1996), age (Colinet et al. 2005), size (Wang et al. 2008), bacterial symbionts (Feldhaar 2011), and diet (Lampert and Bowers 2010) and can seriously affect the fitness of the developing parasitoids. Host quality is also greatly affected by the parasitism status of the host because multiply parasitized hosts offer fewer resources to each developing parasitoid than singly parasitized ones (Godfray 1994). Moreover, in solitary parasitoids, only one of the competing larvae (often the first-comer) eventually survives and completes its development. Consequently, parasitoids often inspect prospective hosts with their antennae or ovipositor and reject hosts that are already parasitized (van Baaren et al. 1994).

Rejection of parasitized hosts is frequently based on volatiles, produced by the host or by the first-coming parasitoid (reviewed by Nufio and Papaj 2001; Ruther 2013). An

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example of the role of chemicals discharged by the parasitized hosts was described by Outreman et al. (2001): the cornicles of parasitized aphids exude a secretion that is detected by their parasitoid, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae), and that reduces the frequency of superparasitism (repeated oviposition in one host). Parasitoid-produced pheromones used in host marking include products of Dufour's gland, cuticular hydrocarbons, and juvenile hormones (Ruther 2013). The parasitoids deposit their deterring pheromone either on the parasitized hosts (e.g., Rosi et al. 2001) or on the hosts' food plants (e.g., Stelinski et al. 2007).

The time elapsed since the previous parasitism affects the oviposition behavior of parasitoids in a variety of ways. Some parasitoids accept recently parasitized hosts more often than hosts that contain more developed embryos from a previous oviposition (e.g., *Anisopteromalus calandrae*, Lebreton et al. 2010). Many others, on the other hand, increase acceptance rates of parasitized hosts as the time since the previous oviposition increases (Nufio and Papaj 2001).

The rise in acceptance of parasitized hosts over time was suggested to reflect the breakdown of repellent volatile marks. Recently parasitized hosts are assumed to carry a clear mark that strongly deters additional parasitism. Over the next minutes, hours, or days, the pheromone or kairomone gradually degrades, resulting in increased acceptance of the parasitized hosts. Thus, time-dependent changes in the state of the hosts are hypothesized to affect the parasitoids' oviposition choices (see Nufio and Papaj 2001 for a review). A second and complementary interpretation has been proposed in the context of self-superparasitism (repeated oviposition by one individual). It hypothesizes that changes in the state of the parasitoids themselves account for the increase in self-superparasitism over time. This interpretation was originally invoked by Roitberg and Prokopy (1983) to explain the host choices of the tephritid fly *Rhagoletis pomonella*. This fly lays its eggs in hawthorn fruit and marks them with a deterrent pheromone. Marked fruit were accepted by females that were removed from host fruit for a period of 5–80 min, and the frequency of acceptance increased with the duration of host deprivation. The increased exploitation of marked fruit was proposed to reflect a gradual decrease in the flies' host acceptance thresholds (Roitberg and Prokopy 1983). Ueno and Tanaka (1996) applied similar reasoning to parasitoid behavior, to explain how the wasp *Pimpla nipponica* (Hymenoptera: Ichneumonidae) discriminates between self-parasitized hosts and hosts parasitized by conspecifics. The parasitoids' acceptance of self-parasitized hosts increased with time since a previous oviposition. Experimental manipulations that interfere with short-term memory enhanced the frequency of self-parasitism as well, and no evidence for host marking was found. *P. nipponica* may achieve self-discrimination by remembering the individual odor or morphology of its most recent host, i.e., the parasitoid's information state may be

modified at oviposition. Fading of this short-term memory was proposed to account for its time-dependent decrease in discrimination between self- and conspecific-parasitized hosts (Ueno and Tanaka 1996).

It is difficult to tease apart the effects of host and parasitoid state on self-superparasitism rates, because experimental manipulation of host encounter rates affects both variables simultaneously. However, it is straightforward to study their relative contributions to host discrimination in conspecific parasitism, as the intervals between parasitism events can be varied independently for host and parasitoid. Here, we used this approach to explore the mechanism underlying host acceptance in the wasp *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae), a parasitoid of the potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae).

C. koehleri is a polyembryonic egg-larval endoparasitoid with arrhenotokous (haplo-diploid) reproduction. Adult females readily parasitize hosts both before and after having mated. A female normally releases one egg per oviposition (Keinan et al. 2012), which later proliferates into ca. 40 genetically identical, same-sex individuals. Superparasitism by the same or by a conspecific female occurs under lab conditions (Doutt 1947; Keinan et al. 2012). Superparasitism seems to be also common in the field, as 40 % of the parasitoids' broods in a field-collected sample were mixed-sex, hence must have been due to multiple parasitism (Segoli et al. 2009). Competition among developing embryos in superparasitized hosts leads to increased larval mortality and shifts in the brood sex ratio (Kesar et al. 2006; Bügler et al. 2013). In two-choice tests, *C. koehleri* females prefer healthy (non-parasitized) hosts to self-parasitized ones and do not show a clear preference between self- and conspecific-parasitized hosts. In single-choice assays, acceptance rates of conspecific-parasitized hosts are higher than those of self-parasitized hosts (Keinan et al. 2012). These findings indicate that *C. koehleri* discriminates between healthy and parasitized hosts and possibly also detects self-parasitism (Segoli et al. 2010).

Here, we first verified that *C. koehleri* female increases self-superparasitism with time between successive host presentations. We then manipulated the interval between the wasps' host encounters independently of the hosts' history of parasitism. This allowed us to test whether the time elapsed since a wasp's previous oviposition affects its host selectivity.

Methods

Rearing conditions A laboratory stock of *C. koehleri* was used. The stock originated from a field collection from South Africa in 2003. Parasitoids were housed at a constant temperature of 26 ± 1 °C and 14:10-h L:D schedule and fed with honey. A laboratory stock of potato tuber moth,

P. operculella, was housed at the same temperature and light conditions. Water was provided to the moths by placing water-soaked cotton in 1.5-ml Eppendorf tubes inside their oviposition cages. Potato tuber moth eggs (0–4 h old) were used as hosts for experiments.

Individuals used in the experiments Mummies from the laboratory stock were placed individually in 13 × 100 mm glass test tubes until the emergence of adult wasps. Virgin adult wasps from all-female broods were used within 24 h of emergence. Host acceptance varies with offspring sex in some parasitoids. For example, the parasitoid *Diglyphus isaea* (Hymenoptera: Eulophidae) produces a more female-biased sex ratio in higher-quality (larger) hosts than in low-quality (smaller) ones (Ode and Heinz 2002). To avoid this potential confounding effect, virgin females, capable of producing sons only, were tested. Host choices of one female from each brood were observed. Each individual was tested once.

Determination of host acceptance/rejection During behavioral observations, a single moth egg was placed in a petri dish of 50-mm diameter. One female from an all-female test tube was allowed to access the moth egg. This was done by placing the petri dish over the test tube until one wasp passed directly to the egg. The dish was then closed with a lid. In experiments that involved moving the wasp from one petri dish to another, the first dish was opened and tapped over the host in the second dish until the wasp dropped on the host.

The parasitoid usually immediately contacted the host with its antennae. The wasp's behavior was observed under a dissecting microscope. Ovipositor insertions into the host that were longer than 10 s were scored as host acceptance, based on previous findings (Keinan et al. 2012). This was confirmed by dissecting hosts and counting the parasitoid eggs in them. Wasps that did not form immediate antennal contact with the host were excluded from the experiment. Ovipositor insertions that were shorter than 10 s, or no ovipositor insertion within 2 min of antennal contact with the host, were interpreted as host rejection.

Experiment 1: acceptance of self-parasitized hosts at different intervals We first combined data from Segoli et al. (2010) with new observations to establish that the parasitoid's acceptance of self-parasitized hosts increases with time since the previous oviposition. A healthy host was presented to a naïve parasitoid. If the host was accepted, it was presented again to the same parasitoid, either 5 s ($n=16$, treatment A), 2 min ($n=20$, treatment B), or 2 h ($n=38$, data from Segoli et al. 2010, treatment C) later (Fig. 1). Host acceptance at the second presentation was recorded. Wasps that rejected the host at the first presentation were excluded from the experiment.

Experiment 2: acceptance of healthy hosts at short intervals between presentations The oviposition rate of the wasps may be physiologically constrained, limiting their ability to parasitize two hosts in quick succession, regardless of the hosts' state of parasitism. This possibility was investigated by presenting a healthy host to a naïve parasitoid. If the host was accepted, it was removed from the dish immediately after it was parasitized, and the wasp was presented with a second healthy host within 5 s. Acceptance of the second host was recorded (Fig. 1, treatment D, $n=20$). Wasps that rejected their first host were excluded from the experiment.

Experiment 3: acceptance of conspecific-parasitized hosts by naïve wasps Discrimination between parasitized and healthy hosts is known to require a previous oviposition experience in several species (Chow and Mackauer 1986; Santolamazza-Carbone et al. 2004; Ardeh et al. 2005). We therefore tested whether acceptance of conspecific-parasitized hosts varies between naïve and experienced individuals in *C. koehleri*, to decide which type of females to use in experiment 4. A naïve female was presented with a host that had been parasitized by a conspecific 2 min earlier, and host acceptance was recorded (Fig. 1, treatment E, $n=59$).

Experiments 4 and 5 aimed to separate the effects of host parasitism history and parasitoid oviposition history on host acceptance.

Experiment 4: acceptance of conspecific-parasitized hosts, host history manipulated Parasitoids with uniform previous experience were exposed to hosts that had been parasitized by conspecifics, either 2 min (this study) or 2 h (data from Segoli et al. 2010) earlier. If transient pheromonal marks are involved in host acceptance, then acceptance rates are expected to be lower for the more recently parasitized hosts.

A healthy host was presented to a naïve parasitoid and removed from the petri dish immediately after being parasitized. The focal wasp was left alone in the dish for 2 h and was then moved into a new dish with a different host, which had been parasitized by a conspecific either 2 h ($n=48$, data from Segoli et al. 2010, treatment F) or 2 min ($n=20$, treatment G) earlier. Acceptance of the second host was recorded (Fig. 1). Wasps that rejected their first host were excluded from the experiment.

Experiment 5: acceptance of conspecific-parasitized hosts, wasp history manipulated Here, parasitoid history was varied while keeping host history constant. Females were allowed to parasitize a single healthy host and subsequently kept in a host-free dish for either 5 s or 2 h. The wasps were then presented with a host that had been parasitized by a conspecific 2 min earlier, and their oviposition response was scored. As time since the previous parasitism was identical for hosts in

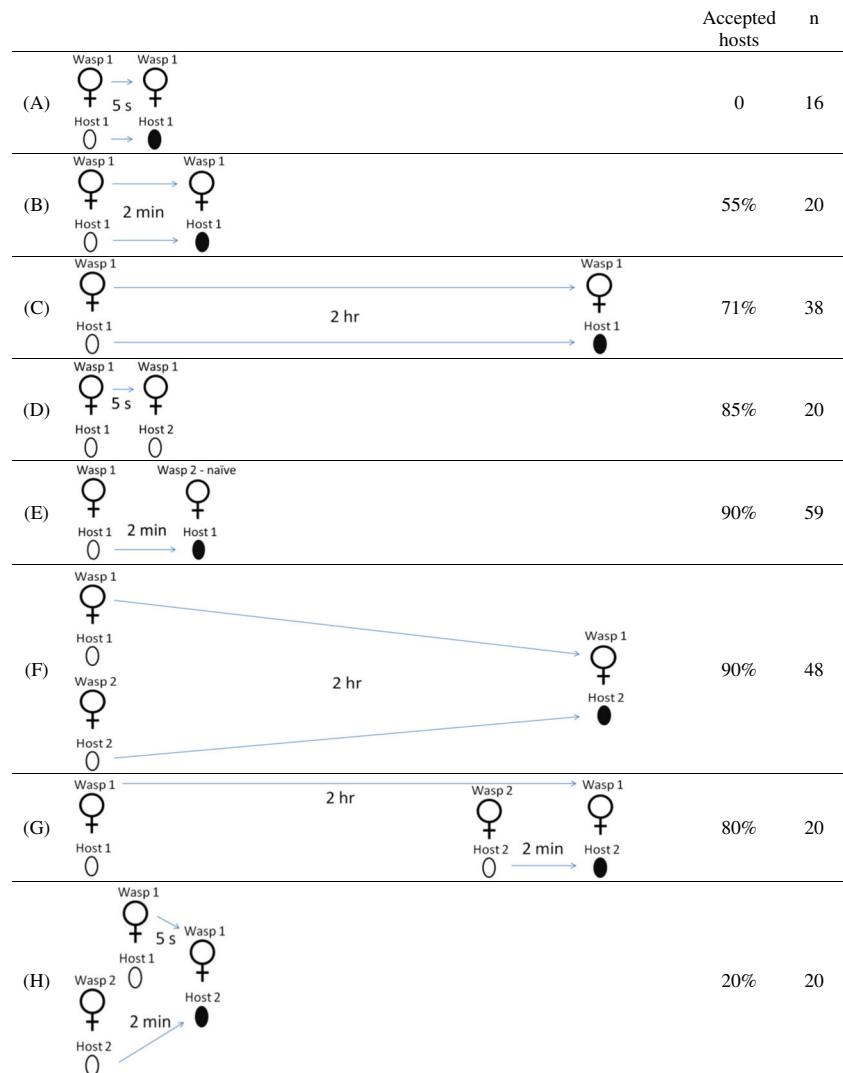
the two treatments, all should be marked by deterrent pheromones to the same degree. We therefore predicted that host acceptance rates will not vary between the treatments if they are guided by the strength of host marking. If, on the other hand, host acceptance rates are affected by the parasitoids' state, they are predicted to increase with the time elapsed since the wasps' last encounter with a host.

A healthy host was presented to a naïve parasitoid. If the host was accepted, it was removed from the petri dish immediately after the wasp withdrew her ovipositor. Five seconds later, the wasp was moved into a new dish that contained a different host, which had been parasitized by a conspecific female from a different brood 2 min earlier. Acceptance of the second host was recorded (Fig. 1, treatment H, $n=20$). Acceptance rates were compared with treatment G, where hosts had an identical history (parasitized by a conspecific 2 min earlier), but wasps waited for 2 h before receiving their

second host. Individuals that rejected their first host were excluded from the experiment.

Data analysis We used a generalized linear model with a binomial distribution of the dependent variable (host acceptance) to test for the effects of host type (self-parasitized, conspecific-parasitized, or healthy), parasitoid experience (none, 5 s, 2 min, or 2 h since previous oviposition), and host experience (none, 5 s, 2 min, or 2 h since previous parasitism event). Fisher's exact tests were used to compare proportions of accepted hosts between specific treatments, namely A-B-C (self-parasitized hosts at increasing presentation intervals), A-D (self-parasitized vs. healthy hosts), E-H (naïve vs. experienced wasps), F-G (2 min vs. 2 h since host was previously parasitized), and G-H (5 s vs. 2 h since the parasitoid oviposited previously). As data from treatments A, G, and H were used to test two hypotheses, the Bonferroni correction

Fig. 1 Scheme of experimental treatments A–H. Healthy host eggs are marked as *empty ovals*, and parasitized hosts are marked as *filled ovals*. Proportions of host acceptance were compared between specific treatments using Fisher's exact tests. Treatments A, B, and C: $P<0.0001$; treatments A and D: $P<0.001$; treatments E and H: $P<0.0001$; treatments F and G: $P=0.54$; treatments G and H: $P=0.0004$



was applied and $0.05/2=0.025$ considered as the cutoff value for rejecting the statistical hypotheses in Fisher's tests.

Results

Experiment 1: acceptance of self-parasitized hosts at different intervals None of the wasps accepted a self-parasitized host within 5 s of their first oviposition. Acceptance of self-parasitized hosts increased to 55 % when the interval between host presentations was increased to 2 min (treatments A–B). In a previous experiment (Segoli et al. 2010), conducted using the same protocol, 27 out of 38 self-parasitized hosts (71 %) were accepted after a 2-h interval (treatment C). Thus, self-acceptance of hosts by *C. koehleri* significantly increases with time since a previous oviposition (Fisher's extended exact test, $P<0.0001$).

Experiment 2: acceptance of healthy hosts at short intervals between presentations Eighty-five percent of the females that were presented with two healthy hosts within 5 s accepted the second host, while a self-parasitized host that was presented again within 5 s was never accepted (treatments A–D, Fisher's exact test, $P<0.001$).

Experiment 3: acceptance of conspecific-parasitized hosts by naïve wasps Hosts that were presented to naïve females 2 min after conspecific parasitism (treatment E) were accepted by 90 % of the wasps, while only 20 % of the experienced individuals accepted such hosts (treatment H). This difference was statistically significant (Fisher's exact test, $P<0.0001$), indicating that naïve wasps make different choices from experienced ones. Experienced females were therefore used in all further experiments.

Experiment 4: acceptance of conspecific-parasitized hosts, host history manipulated Parasitized hosts that were presented to a conspecific 2 min later were accepted in 80 % of the cases (treatment G). When parasitized hosts were presented to a conspecific after 2 h, they were accepted by 90 % of the parasitoids (treatment F). The proportion of acceptance did not differ significantly between the two treatments (Fisher's exact test, $P=0.43$).

Experiment 5: acceptance of conspecific-parasitized hosts, parasitoid state manipulated The acceptance rate of conspecific-parasitized hosts was 20 % in the group of wasps that had oviposited 5 s earlier (treatment H) and 80 % in the wasps that had oviposited 2 h earlier (treatment G). Acceptance frequencies differed significantly between the treatments (Fisher's exact test, $P=0.0004$).

Generalized linear model analysis: effects of host history, parasitoid history, and host type In agreement with the results from the individual experiments, the GLM analysis revealed a significant effect of parasitoid history (time elapsed since the parasitoid's previous oviposition) on host acceptance rates (Wald chi-square 26.515, $df=3$, $P<0.001$). Host history (time since a host was previously parasitized), on the other hand, did not significantly affect acceptance rates (Wald chi-square 1.094, $df=2$, $P=0.579$). In addition, host acceptance was significantly affected by the type of host (healthy, self-parasitized, or conspecific-parasitized) (Wald chi-square 4.476, $df=1$, $P=0.034$).

Discussion

This study tested for the mechanism that underlies time-dependent avoidance of superparasitism in a parasitoid wasp. Similarly to several other parasitoids, *C. koehleri* females strongly reject just-parasitized hosts, and their acceptance of self- and conspecific-parasitized hosts increases with time. Most previous work considered the degradation of deterring pheromone marks as a likely mechanism for time-dependent decreases in host rejection (Núñez and Papaj 2001; Mehrejad and Copland 2007). Our results show similar acceptance rates of hosts that had been parasitized either 2 min or 2 h previously (experiment 4). This could indicate either that *C. koehleri* females do not mark their hosts with short-term pheromones or that factors beyond such pheromones guide their host choices. Our findings do not rule out the possibility that longer-term pheromones are used for host marking, which did not degrade within the duration of our experiments. Indeed, many host-marking pheromones of insects persist for several days (Liu et al. 2012). Research on the existence and chemistry of *C. koehleri*'s host-marking pheromones is needed to evaluate these possibilities.

The results of experiment 5 suggest that the decrease in host selectivity can be attributed to changes in the wasps' history of parasitism. A similar interpretation was proposed for the time-dependent decrease in self-rejection observed in *P. nipponica* (Ueno and Tanaka 1996). This parasitoid does not seem to use chemical cues for host discrimination. Moreover, manipulations that interfere with short-term memory (encounters with additional hosts and cooling) also reduced its rejection of self-parasitized hosts. *P. nipponica* females were suggested to memorize the morphology or scent of their last host as a means of self-discrimination. Similarly, the wasp *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae) may avoid superparasitism by learning the location of the hosts it had recently parasitized (McKay and Broce 2004).

Short-term memory of a specific host cannot explain the time-dependent increase in acceptance of hosts parasitized by

conspecifics in our experiment 5. This is because the wasps rejected hosts they had not encountered before, in dishes that they had not occupied before. An alternative interpretation, which is consistent with our results, is that the wasps modified their host selectivity with time since their last oviposition: only high-quality (healthy) hosts were accepted immediately after a previous parasitism event. With time, the acceptance threshold decreased so that lower-quality (parasitized) hosts were accepted as well.

Dynamic, state-dependent host selectivity has been previously proposed to account for elevated superparasitism rates as female age and egg load increase (Sirost et al. 1997; Islam and Copland 2000). However, these variables do not explain the different superparasitism levels in the present study because female age and oviposition experience were similar in treatments G and H, but host acceptance rates differed. Instead, our results suggest time since the last oviposition, a component of foraging experience, as an additional state variable that affects host acceptance. Host encounter rates were found to affect superparasitism levels in additional parasitoids. *Aptesis nigrocincta* (Ichneumonidae) superparasitized their sawfly hosts more often when encountering them at a reduced frequency (Babendreier and Hoffmeister 2002). In *Venturia canescens* (Ichneumonidae), superparasitism rates were also increased when presentation frequencies of the hosts (larvae of the Indian meal moth) were experimentally reduced (Hubbard et al. 1999).

Our data also show that *C. koehleri* females with one oviposition experience avoided parasitized hosts more than naïve individuals. A similar increase in host rejection rates following an oviposition experience is known from other parasitic wasps (Chow and Mackauer 1986; Santolamazza-Carbone et al. 2004; Ardeh et al. 2005). A related effect of experience on host evaluation was described in the wasp *Anaphes victus* (Mymaridae): naïve females probe their hosts internally with their ovipositors to assess their parasitism status. As the parasitoids gain experience, they learn to associate external pheromones with the presence of a conspecific in a host and shift to assessing host quality through external inspection. They revert to probing the hosts internally with their ovipositors if deprived of hosts for 2 h or longer, suggesting that the learned cues are forgotten during host deprivation (van Baaren and Boivin 1998).

The combined conclusion from our results is that females with a recent oviposition experience reject parasitized hosts more often than individuals with a less recent experience or with no previous host encounter at all. This can be plausibly interpreted within the learning perspective proposed by van Baaren and Boivin (1998): the wasps learned cues associated with parasitized hosts while ovipositing in them. These cues were retained in a short-term memory and account for the high rejection rates of parasitized hosts that were presented 5 s later. Host acceptance increased as the short-term memory decayed,

which could have happened in treatments with longer host encounter intervals. Naïve wasps have not yet learned the cues associated with parasitized hosts, hence showed low avoidance of such hosts as well.

The possible adaptive value of superparasitism in parasitic wasps has been widely discussed (van Alphen and Visser 1990; Dorn and Beckage 2007). Conspecific superparasitism generally yields lower fitness benefits than parasitizing a healthy host. The benefits of self-superparasitism are even lower because they involve competition among siblings. Nevertheless, superparasitism was suggested to be advantageous when hosts are scarce or when interspecific competition with other parasitoids is high (van Alphen and Visser 1990). In agreement with these predictions, *C. koehleri* generally avoids superparasitism and especially self-superparasitism (Keinan et al. 2012). Heightened host selectivity immediately after oviposition may reduce the risk of self-superparasitism, as females are likely to reject hosts that they had just parasitized. On the other hand, increasing the intervals between host presentations (a possible cue for low host density) enhances superparasitism by reducing the wasps' host selectivity. Learning of parasitism-associated cues during oviposition, and short-term memory retention of such cues, is a simple mechanism that could mediate this behavioral flexibility.

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