

## Original Article

## Repeated probing of hosts: an important component of superparasitism

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Parasitoids that encounter a previously parasitized host inspect it externally and internally, sometimes eventually laying additional eggs (superparasitism). The fitness effects of increased clutch sizes generated through superparasitism are widely studied, whereas the consequences of multiple host probings during the inspection received less attention. To address this issue, we offered a host to 1–5 females of the encyrtid wasp *Copidosoma koehleri* consecutively, or presented it 1–5 times to a single female. We noted whether the hosts died before pupation of either host or wasp, produced parasitoid pupae, or developed into moth pupae. Additional hosts were dissected after varying numbers of probings to determine their parasitoid egg loads. Host rejection rates prior to ovipositor insertion did not differ between treatments. Host rejections after ovipositor insertion, characterized by brief (<10 s) probing durations, were more common in the single- than in the multiple-female treatment. This could reflect avoidance of self-superparasitism, or increased selectivity by host-experienced females. Egg number per host increased with the number of prolonged probings in both treatments. Some hosts that received 1–2 probings (brief or prolonged) yielded moth pupae, while no hosts with five probings survived to pupation. Hosts probed three times (corresponding to <1 and 2.2 eggs in the single- and multiple-female treatments, respectively) produced the largest proportion of parasitoid pupae. The parasitoids' success is thus strongly affected by the number of host probings. Overcoming host defenses through repeated probings is a previously overlooked potential benefit of superparasitism. *Key words*: conspecific parasitism, *Copidosoma*, host handling, host inspection, host mutilation, parasitoid, self-superparasitism. [*Behav Ecol*]

## INTRODUCTION

Parasitoids lay their eggs in or on an arthropod host, which is later consumed by their larvae. Many parasitoids also regularly superparasitize their hosts, that is, oviposit in hosts that had been previously parasitized by the same individual (self-superparasitism) or by a different one (conspecific superparasitism). The adaptive value of superparasitism is not obvious, because it is associated with many costs. In solitary parasitoids, only one of the eggs laid in a host completes development, so that any additional oviposited eggs are apparently wasted (van Alphen and Visser 1990). In gregarious species, several offspring can emerge from a single host, but larvae from superparasitized hosts may develop more slowly (Harvey et al. 1993; Gu et al. 2003) and reach a smaller adult body size (Potting et al. 1997). In addition, superparasitism in gregarious species can increase mortality of developing larvae when host carrying capacity is exceeded. For example, the mortality of *Cotesia flavipes* immatures inside their moth host *Chilo partellus* is threefold higher in superparasitized hosts than in singly-parasitized ones (Potting et al. 1997). Superparasitism also increased the mortality of host larvae in *Pieris rapae* butterflies parasitized by *Cotesia glomerata* (Gu et al. 2003). Similarly, the survival of *Cotesia congregata* offspring within the moth *Manduca sexta* increases at moderate levels of superparasitism, but decreases to zero when the parasitoid load approaches 800 eggs (Dorn and Beckage 2007).

In both solitary and gregarious species, destruction of the eggs of the first clutch by the superparasitizing female (ovicide) contributes to parasitoid mortality as well (van Alphen and Visser 1990). In addition, superparasitism can lead to increased premature mortality of hosts through repeated injection of venom, or excessive feeding on host hemolymph by the ovipositing females (Bernardo et al. 2006 on the solitary ectoparasitoid *Pnigalio soemius*; Kaspi et al. 2011 on the gregarious ectoparasitoid *Diglyphus isaea*). In such cases, none of the parasitoid larvae survive.

What benefits could compensate for the above costs, and account for the ubiquity of superparasitism? Some advantages to superparasitism were suggested when competition for hosts is high. In solitary parasitoids, conspecific superparasitism under high competition creates an opportunity for eliminating a competitor's offspring, and can be more profitable than searching for rare empty hosts (van Alphen and Visser 1990). Self-superparasitism increases the survival prospects of at least some of the offspring, if conspecific parasitism of the same host is likely to occur later (Ito and Yamada 2005 for the ectoparasitoid *Echthrodolphax fairchildii*). Even if hosts are abundant, superparasitism may ensure that at least one of the parasitoid's offspring reaches adulthood, when mortality of immature stages is frequent (Rosenheim and Hongkham 1996 for the endoparasitoid *Comperiella bifasciata*). Furthermore, in some gregarious endoparasitoids, superparasitized hosts exhibit increased food consumption and weight gain, thus increasing resource availability for the parasitoid larvae (Mackauer and Chau 2001; Gu et al. 2003). Finally, superparasitism by gregarious species can inhibit the hosts' immune reaction and thereby reduce parasitoid mortality through encapsulation: superparasitism reduced

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encapsulation of the eggs of the endoparasitoid, *Metaphycus flavus*, by their scale insect host (Tena et al. 2008). Similarly, superparasitism of late-instar cotton leafworm (*Spodoptera littoralis*) larvae increased the emergence success of their endoparasitoids, *Microplitis rufiventris* wasps (Khafagi and Hegazi 2008).

Interestingly, many of the putative advantages of superparasitism studied to-date derive from an increased clutch size, rather than from repeated ovipositions in the host. This implies that laying large clutches in a single oviposition could improve offspring competitive prospects, increase host feeding, and overcome the host's immune system, even when no superparasitism occurs. Thus, the advantage of self-superparasitism, compared to generation of large clutches per oviposition, is not yet sufficiently explained. Rosenheim and Hongkham (1996) point out that self-superparasitism differs from producing multiple-egg clutches in increasing inter-oviposition time intervals within broods, and in requiring identification of hosts that are self-parasitized. Here we hypothesize that repeated probing of the host with the parasitoid's ovipositor is another important feature of superparasitism, which is not involved in producing multiple-egg clutches. We predict that the repeated host probing that occurs during superparasitism generates additional benefits to parasitoids. Furthermore, parasitoids frequently inspect previously parasitized potential hosts internally by probing them with their ovipositor, but subsequently reject the hosts and do not oviposit in them. Probing is often preceded by external inspection of the host for pheromone marks left by the previous female. We predict that such probings affect the hosts' developmental prospects, and hence also the survival of the developing parasitoids.

To address these predictions, we studied repeated host probing by the polyembryonic wasp, *Copidosoma koehleri* (Encyrtidae: Hymenoptera), an egg-larval parasitoid of the potato tuber moth *Phthorimaea operculella*. This parasitoid generally releases a single egg per ovipositor insertion (Keasar et al. 2006), and frequently superparasitizes its hosts, both in the field and in insectary cultures (Segoli et al. 2009). Females discriminate empty from parasitized hosts based on external cues (Segoli et al. 2010), but the role of in-host probing in the parasitism process is not known. Each of *C. koehleri*'s eggs proliferates clonally within the host to form a clutch of ca. 40 genetically identical embryos. Thus, large offspring broods develop even in the absence of superparasitism in this species. *C. koehleri*'s polyembryonic lifestyle therefore helps to dissociate the effects of multiple host probings from those of multiple individuals per brood.

We introduced hosts to parasitoids for 1–5 times and recorded the wasps' host-probing behavior. We then followed the hosts' development to estimate the consequences of different number of probings by the wasps. In subsequent experiments, we distinguished between brief probings that preceded host rejection and prolonged probings associated with oviposition. Because the mechanisms and consequences of self and conspecific superparasitism are often different, we studied the effects of repeated probing by the same female, as well as by different females.

## METHODS

### Insect rearing

Laboratory-reared hosts and parasitoids were used for the experiments. Their rearing followed a standard protocol (Berlinger and Lebiush-Mordechi 1997). The temperature during rearing and throughout the experiment was  $27 \pm 2$  °C,

and relative humidity was ~60%. Hosts used for the experiment were 0- to 12-h-old moth eggs. Wasps were 0- to 24-h-old virgin females, thus all of their offspring were males. This simplified the interpretation of developmental results, because male larvae develop gregariously and nonaggressively in superparasitized hosts (Segoli et al. 2009). Each individual was presented to one host.

## EXPERIMENTAL DESIGN

### Experiment 1: Host development following repeated presentation to parasitoids

The aims of this experiment were to record the frequencies of internal probings of fresh versus parasitized hosts by parasitoids, and to determine how the number of probings affects host development. A host, placed in a 60-mm-diameter Petri dish, was presented to a parasitoid wasp 1–5 times in succession. In the single-wasp treatment, each host was sequentially presented to a single-wasp female. In the multiple-wasp treatment, each host was presented to up to five different wasps, each from a different clone. The wasps' behavior was observed under a dissecting microscope. The wasp was allowed one ovipositor insertion into the host at each presentation. Encounters with no ovipositor insertion within 2 min of antennal contact with the host were scored as external rejections before probing. One hundred hosts were used for each treatment, that is, 20 for each of 1–5 presentations to parasitoids. However, rejections before ovipositor insertion reduced the number of hosts that were eventually probed multiple times, and increased the number of hosts probed only once. The mean  $\pm$  standard deviation (SD) number of wasps per clone used in this experiment was  $4.05 \pm 1.94$ . Hosts in the experiment were reared on potatoes until they died as immatures (eggs or larvae), pupated, or produced a mummy containing parasitoid pupae. Some hosts were lost because of fungal infestation of the potatoes, reducing sample sizes. We also reared one fresh unparasitized host in parallel to each host that was presented to the parasitoids, on a different potato, and recorded whether it survived to pupation. This was done to quantify premature host death that is not related to parasitism. In this control group, we used 100 hosts for each of the 2 treatments.

We classified each host presentation as leading to either external rejection or nonrejection (a dichotomous response variable). We then used logistic regression, with the Marascuilo procedure as a post hoc test, to determine whether this variable is affected by treatment (single-female vs. multiple-female) and by the number of previous host probings.

To analyze the effects of treatment and number of previous probings on the hosts' developmental fate, we took a 2-step approach. First, each host was scored as having either reached pupation or died prematurely. Next, we classified the pupated hosts as either wasp or moth pupae. Thus, the response variable for each step was dichotomous and was analyzed by logistic regression, with treatment and number of probings as the independent variables.

### EXPERIMENT 2: CHARACTERIZATION OF BRIEF AND PROLONGED OVIPOSITOR INSERTIONS

During Experiment 1, we noticed that probing durations of the hosts varied widely between successive presentations. We repeated the host presentation protocol described earlier with an additional sample of hosts to quantify this variation. In this sample, we recorded the time from the insertion of

the wasp ovipositor into the host until its withdrawal. Unlike Experiment 1, when hosts were rejected before probing, they were presented again (to the same individual in the single-wasp treatment, but to a different one in the multiple-female treatment) until probed. After recording the durations of ovipositor insertions, we dissected the hosts by crushing them under a cover slide in insect Ringer's solution, and inspected them under a phase-contrast microscope for the presence of wasp eggs. Seventy-five hosts were used for each treatment, 15 for each of 1–5 presentations to parasitoids. A total of  $3.76 \pm 1.33$  wasps were used per clone,  $n = 30$  clones.

Probing durations showed a bimodal frequency distribution, with peaks at 5 and 35 s. We calculated the proportion of ovipositor insertions that were shorter than 10 s as a function of the number of previous host probings. We hypothesized that such brief probings indicate internal host rejections. As an indirect test of this hypothesis, we regressed the number of parasitoid eggs recorded within the hosts on the number of brief and prolonged probings they had received. We expected the number of prolonged probings, but not the number of brief ones, to significantly affect the number of eggs observed in the dissected hosts.

As a further indirect test of whether brief (<10 s) ovipositor insertions are associated with host rejection after probing, we allowed each of 20 additional females to probe a host twice in succession. We recorded the durations of ovipositor insertions and determined the number of wasp eggs in dissected hosts as above. The first ovipositor insertion into a host was generally prolonged, whereas the second insertion by the same wasp was often brief. In these cases, dissected hosts are expected to contain only 1 parasitoid egg, if eggs are not released during brief probings. Two parasitoid eggs are expected, if both brief and prolonged probings are associated with egg-laying.

### EXPERIMENT 3: HOST ACCEPTANCE BY NAÏVE VERSUS EXPERIENCED WASPS

All parasitoids in the multiple-wasp treatment had no previous host encounters, whereas most females in the single-wasp treatment encountered the host more than once, hence were not naïve from the second encounter on. To test whether previous host encounters can account for any differences between the single- and multiple-wasp treatments, we offered previously probed hosts to a group of nonnaïve females. Forty wasps, each from a different clone, made a prolonged probing in a host, and were then offered a second host (the focal host), which had been probed by a different individual up to 10 min earlier. Thus, this control resembles the single-wasp treatment in that non-naïve wasps encountered the focal host. It resembles the multiple-wasp treatment in that the focal host was offered to 2 different individuals. We recorded the duration of probing of the focal host, and dissected it to determine how many wasp eggs it contained. We expected similar frequencies of long probings and ovipositions in the focal hosts as in multiple-wasp treatment of Experiment 2, if host acceptance is not affected by the parasitoids' previous experience. However, if experience affects host acceptance then focal hosts are expected to resemble hosts probed twice in the single-female treatment of Experiment 2, with respect to number of probings and eggs.

## RESULTS

### Experiment 1: Host development following repeated presentation to parasitoids

Hosts were scored as externally rejected if the wasp did not probe them with her ovipositor within 2 min of antennal

contact. Hosts that had been previously probed 1–4 times received more external rejections than hosts that had not been previously probed (Marascuilo procedure:  $P = 0.05$  for single-wasp,  $P < 0.05$  for multiple-wasp). The frequency of external rejections did not differ significantly between the single- and multiple-wasp treatments (logistic regression: Wald statistic=0.66,  $df = 1$ ,  $P = 0.41$ ), but was affected by the number of previous probings of the host (Wald statistic=23.37,  $df = 4$ ,  $P < 0.001$ ). This effect reflects the fact that no rejections occurred on the fifth presentation of hosts in the multiple-wasp treatment (Fig. 1). In the single-wasp treatment, however, previously-probed hosts were rejected in about 30% of the cases, regardless of the number of previous probings.

The hosts' developmental fate is summarized in Fig. 2. Up to 55% of hosts that were not presented to parasitoids died during their egg or larval stages, and almost all remaining ones developed into moth pupae. Larval mortality was often caused by fungal infestation of the potato that served as food source. A single parasitized mummy developed in this control treatment, apparently a contamination. None of the hosts that were probed 5 times survived to pupation (of either host or wasps). The proportion of hosts that died before the pupal stage was not significantly affected by treatment (Wald statistic = 0.27,  $df = 1$ ,  $P = 0.60$ ) or by number of previous probings (Wald statistic = 1.49,  $df = 1$ ,  $P = 0.22$ ). Mummies containing parasitoid pupae developed from some of the hosts that received 1–4 probings, and their proportion was highest after 3 ovipositor insertions in both treatments. The number of previous probings (Wald statistic = 4.57,  $df = 1$ ,  $P = 0.03$ ), but not treatment (Wald statistic = 0.01,  $df = 1$ ,  $P = 0.94$ ), significantly affected the proportion of parasitized hosts out of the surviving ones.

### EXPERIMENT 2: CHARACTERIZATION OF BRIEF AND PROLONGED OVIPOSITOR INSERTIONS

The proportions of brief ovipositor insertions were significantly higher in the single-wasp than in the multiple-wasp treatment (Wald statistic = 87.38,  $df = 1$ ,  $P < 0.001$ , test for the effect of treatment). They also varied among successive probings (Wald statistic = 36.16,  $df = 1$ ,  $P < 0.001$ , test for the effect of number of probings). Post hoc tests showed that the effect of the number of probings varied between treatments: brief ovipositor insertions were less common during a host's first probing than during successive probings in the single-female treatment. The frequency of brief insertions did not

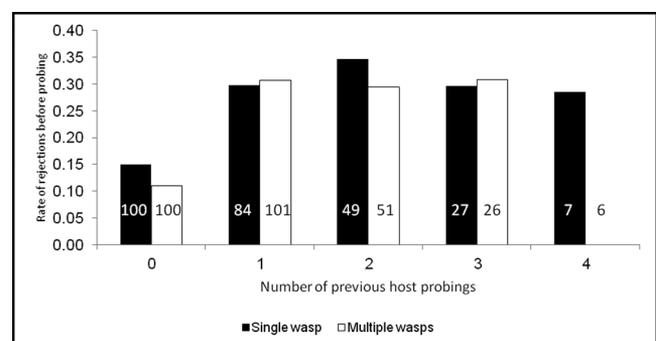
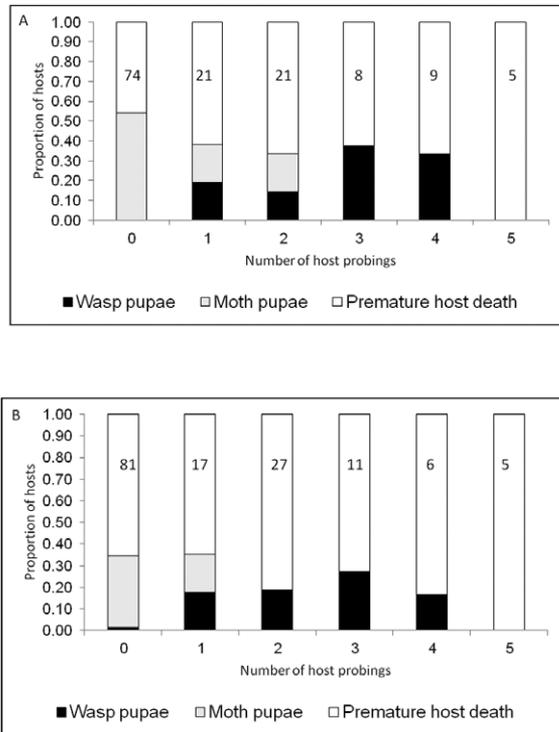


Figure 1

Frequency of external host rejection before probing, in the single-wasp (black bars) and multiple-wasp (empty bars) treatments. Numbers within bars indicate sample sizes.

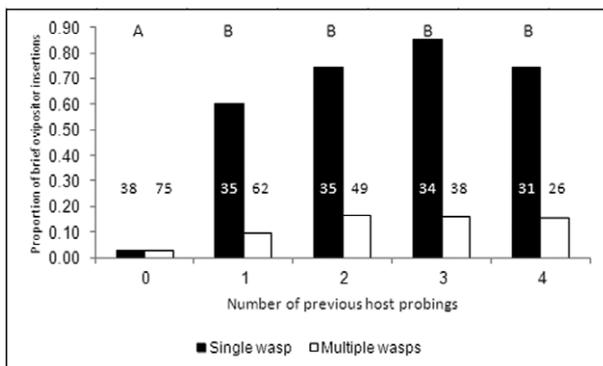


**Figure 2**

The developmental fate of hosts that received 0–5 ovipositor probings by a single (A) or multiple (B) females. Brief probings were not distinguished from prolonged ones in this experiment. Numbers within bars indicate sample sizes.

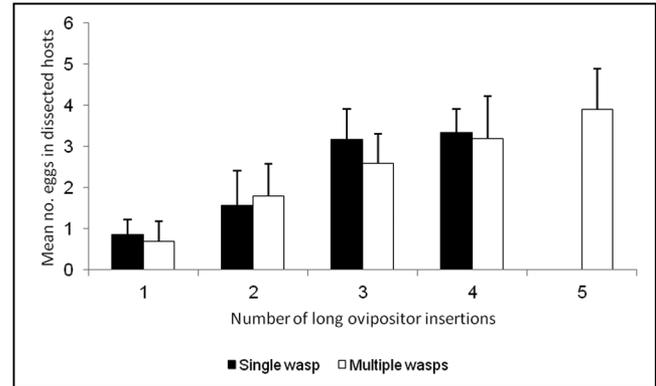
vary among successive probings in the multiple-female treatment (Fig. 3).

We next considered the effect of repeated brief and prolonged probings on the number of eggs laid per host. Females in the multiple-wasp treatment readily performed repeated prolonged probings of hosts. Females in the single-wasp treatment, however, avoided prolonged ovipositor insertions into hosts that they had previously probed. Consequently, we observed only 3 hosts that received 4 prolonged probings in this treatment, and none of the hosts received 5 prolonged probings. The number of parasitoid eggs in dissected hosts significantly increased with the number of prolonged ovipositor insertions, but was not



**Figure 3**

Proportions of brief (<10 s) ovipositor insertion as a function of the number of previous host probings. Numbers within the figure indicate sample sizes. Different letters indicate significant differences in Marascuilo post hoc tests,  $P < 0.05$  (see text for details).



**Figure 4**

Number of wasp eggs in dissected hosts as a function of the number of prolonged (>10 s) ovipositor insertions. The number of prolonged probings significantly affected the number of eggs in both treatments (multiple regressions,  $P < 0.001$ , see text for details).

affected by the number of brief probings (Fig. 4, multiple regression for single-female treatment:  $r^2 = 0.65$ ,  $P < 0.001$  for the whole model,  $P = 0.32$  for brief probings,  $P < 0.001$  for prolonged probings; regression for multiple-female treatment:  $r^2 = 0.69$ ,  $P < 0.001$  for the whole model,  $P = 0.27$  for brief probings,  $P < 0.001$  for prolonged probings). This supports the hypothesis that prolonged probings, but not brief ones, were associated with oviposition. The sample of 20 additional hosts probed twice by a single wasp provided further support for this hypothesis: 17 of the hosts received 1 prolonged and 1 brief probing. The mean  $\pm$  SD number of wasp eggs in these hosts was  $0.94 \pm 0.56$  (12 hosts with 1 egg, 3 with none, 2 with 2 eggs). This mean did not differ significantly from 1 ( $t_{16} = 0.44$ ,  $P = 0.67$ ).

### EXPERIMENT 3: HOST ACCEPTANCE BY NAÏVE VERSUS EXPERIENCED WASPS

The focal host received a prolonged probing by an experienced wasp in 17 of 40 cases. Ten of these hosts contained 2 parasitoid eggs upon dissection (probably 1 egg in each probing), 6 hosts contained 3 eggs (probably 1 egg in one probing, 2 eggs in the other), and 1 host contained 1 egg. This indicates that almost all 17 hosts were indeed parasitized twice, and that a prolonged probing corresponded to 1 egg in most cases. This frequency of prolonged probings (17/40) differs significantly from that observed for 2 naïve females (36/40 in the multiple-female treatment, Fisher's Exact test,  $P < 0.001$ ). It does not differ from the proportion of prolonged ovipositions by a single female presented twice with the same host (12/40, Fisher's Exact test,  $P = 0.35$ ). We conclude that oviposition experience increases the wasps' avoidance of previously parasitized hosts.

### OVERALL EFFECT OF HOST PROBINGS ON PARASITOID DEVELOPMENTAL SUCCESS

The parameters measured above can be combined to generate an estimate of wasp developmental success after different numbers of host probings (both brief and prolonged). These estimates, summarized in Table 1, show that the parasitoids attain the highest survival to pupation with 3 host probings. The approximate corresponding mean clutch sizes were 2.2 in the multiple-female treatment, and 0.8 in the single-female treatment. Of these eggs, 1.79 and 0.57 are expected to have reached pupation in the multiple- and

**Table 1**  
**The effects of 1–5 host probings (both brief and prolonged) on the number of eggs laid and the clutch developmental prospects**

Parameter	Treatment	Probing no.				
		1	2	3	4	5
Proportion of prolonged probings <sup>a</sup>	Single-wasp	0.97	0.40	0.26	0.15	0.26
	Multiple-wasp	0.97	0.90	0.84	0.84	0.85
Mean $\pm$ SD number of eggs laid in prolonged probings <sup>b</sup>	Single-wasp	0.87 $\pm$ 0.35	1.57 $\pm$ 0.85	3.17 $\pm$ 0.75	3.33 $\pm$ 0.58	3.90 $\pm$ 0.99
	Multiple-wasp	0.70 $\pm$ 0.48	1.80 $\pm$ 0.79	2.60 $\pm$ 0.70	3.20 $\pm$ 1.03	
Estimated no. eggs/host <sup>c</sup>	Single-wasp	0.84	0.63	0.82	0.50	3.32
	Multiple-wasp	0.68	1.62	2.18	2.69	
Proportion of clutches reaching pupation <sup>d</sup>	Single-wasp	0.35	0.26	0.70	0.61	00
	Multiple-wasp	0.55	0.58	0.82	0.52	
Estimated no. of eggs reaching pupation/host <sup>c</sup>	Single-wasp	0.29	0.16	0.57	0.31	00
	Multiple-wasp	0.37	0.94	1.79	1.40	

The proportions of clutches reaching pupation were divided by the proportion of unparasitized hosts that pupated in the single- and multiple-wasp treatments. This was done to correct for differences in mortality of unparasitized hosts between the treatments.

<sup>a</sup>Data from Fig. 3. <sup>b</sup>Data from Fig. 4. <sup>c</sup>Proportion prolonged probings  $\times$  number of eggs laid per prolonged probing. <sup>d</sup>Data from Fig. 2, corrected for mortality of unparasitized hosts. <sup>e</sup>Number of eggs per host  $\times$  proportion of clutches reaching pupation.

single-female treatments, respectively, whereas the remaining eggs were lost because of premature host death. Thus, 3 probings per host may have been more beneficial to females in the multiple-wasp treatment than to females in the single-wasp treatment.

## DISCUSSION

The number of parasitoid eggs in a host (a) increased with the number of prolonged ovipositor insertions; (b) was not significantly affected by the number of brief ovipositor insertions; and (c) was close to 1 after one brief and one prolonged insertion. Observation (c) is compatible with 2 interpretations, because the wasp egg could have been laid either during the prolonged probing or during the brief one. Combined with observations (a) and (b), however, it seems that brief probings of hosts by *C. koehleri* are not associated with egg-laying, whereas prolonged probings generally lead to oviposition of a 1-egg clutch. Nevertheless, the total number of probings (both brief and prolonged) had a greater effect on the parasitoids' reproductive prospects than the total number of eggs laid. This conclusion is supported by the finding that maximal parasitoid survival to pupation occurred in hosts that are probed 3 times, in both the single-wasp and multiple-wasp treatments. The estimated corresponding number of eggs per host, however, varied almost 3-fold between the treatments. Moreover, some hosts that received 1–2 probings (either short or long) developed into moth pupae, whereas none of the hosts that were probed 3–5 times did so (Fig. 2). Thus, repeated probings of hosts affected their development even when the probings did not involve oviposition.

Probing might affect host development through chemicals transferred in the process of stinging. These could include venom proteins (Asgari and Rivers 2011) and genetic symbionts such as polydnviruses (Beckage 2008). The venom and calyx proteins are injected into hosts during ovipositor insertion, and their amounts may increase after repeated probing. These substances are involved in the digestion of host tissues to produce nutrients for parasitoid development. It has therefore been suggested that superparasitized hosts may have higher nutritional value for the wasp larvae, compared to singly parasitized hosts (Khafagi and Hegazi 2008). In the present experiment, many wasps in the single-female experiment

probed their host several times but oviposited only once. Such hosts, although not superparasitized, may be more nutritious to the parasitoid larvae than hosts probed only once, if they received multiple doses of proteins that promote host digestion. Polydnvirus transfer during stinging also suppresses the host immune response, reducing encapsulation of parasitoid eggs (Beckage 2008). This effect may be enhanced in multiply probed hosts, consistent with the fact that no moth pupae developed from hosts probed 3 times or more in this study. This interpretation assumes that the amounts of venom and calyx protein injected during an ovipositor insertion are limited, and can only be increased through repeated probing. In addition, repeated probing may inflict multiple mechanical injuries on the host tissues (unpublished observations), which could further reduce its resistance to the parasitoids' development.

The proportion of hosts that reached pupation was rather low, even in individuals that were not parasitized (Fig. 2). Similar low developmental success rates of unparasitized *P. operculella* were recorded in previous laboratory and field tests (Kesar & Steinberg 2008). Fungal infestation of potatoes was an important cause of larval mortality in the present experiment. Such mortality is not expected to indicate poor host quality at the egg stage at which parasitism occurred, and therefore unlikely to have biased the results. Three probings per host were associated with higher developmental success than 1–2 probings, and were also more successful than 4–5 probings. This was because of an increase in premature host death after 4–5 probings (Fig. 2). Premature host mortality could indicate that host carrying capacity was exceeded in the multiple-wasp treatment, where the estimated number of eggs per host increased with the number of probings (Table 1). In the single-wasp treatment, however, we estimate that repeated probings of hosts did not systematically increase the number of oviposited parasitoid eggs (Table 1), because most probings were brief. Additional factors probably contributed to premature mortality of hosts probed 4–5 times, such as mechanical mutilation by the multiple ovipositor insertions, or injection of lethal doses of venom. Notably, the wasps did not restrict their probings to 3 per host, although this reduced the hosts' future survival. This may reflect a limitation on their host evaluation ability, or reflect the fact that the wasps were constrained to a single host in a small dish. This might have artificially increased their rates of revisits and repeated probings of the host.

Females in the single-wasp treatment avoided repeated prolonged probings in their host, whereas females in the multiple-wasp treatment readily performed prolonged probings of previously parasitized hosts. Because we found that prolonged probings were usually ovipositions, this implies that females avoided self-superparasitism more than conspecific superparasitism, in agreement with previous findings in *C. koehleri* (Segoli et al. 2010). Superparasitism by conspecifics is preferred over self-superparasitism in other species as well (e.g., Ueno 1994 on *Itopectis naranyae*; Darrouzet et al. 2007 on *Eupelmus vuilleti*). Preference of conspecific superparasitism was suggested to provide a selective benefit to parasitoids by reducing competition among sibling larvae (van Alphen and Visser 1990). A second explanation for the difference in prolonged probings between treatments in our study involves previous exposure to hosts. All of the females in the multiple-wasp treatment were host-naïve, whereas most individuals in the single-wasp treatment encountered their host more than once during the experiment. Experienced females are less likely to superparasitize than naïve individuals in other parasitoid species (e.g., Henneman et al. 1995; Potting et al. 1997; Santolamazza-Carbone et al. 2004). Experiment 3 concurs with these findings, as wasps with a previous host encounter performed fewer prolonged probings of hosts parasitized by conspecifics than naïve females. We conclude that both avoidance of self-superparasitism and previous experience can account for the low frequencies of oviposition in the single-female treatment. An additional explanation for the increased host selectivity in the single-female treatment is that wasps with previous host encounters were more egg-limited than naïve females, and hence more selective toward hosts. We consider this hypothesis unlikely, because females in both treatments were far from exhausting the 100-egg complement reported for *C. koehleri* (Kfir 1981).

Our results highlight the fitness implications of multiple probings of hosts by parasitoids as a possible selective advantage of superparasitism. These findings were obtained from virgin females, which produced only male offspring, and their applicability to mated females should be tested in additional experiments. Existing evolutionary hypotheses, especially for self-superparasitism, focus on potential fitness advantages arising from larger clutches. However, these hypotheses do not suggest a benefit to self-superparasitism over production of multiple-egg clutches. For example, they do not account for *C. koehleri*'s tendency to lay mostly single-egg clutches, yet to probe hosts repeatedly. We propose that repeated insertions of the parasitoid's ovipositor may improve its likelihood to overcome host defenses. Thus, probing a self-parasitized host before rejecting it (as in this study; Ueno 1994; Tena et al. 2008) may provide a double benefit: assessing the host's parasitism state, and improving the prospects of the previously laid brood.

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