The effect of stinging: aphidiid parasitoids (Hymenoptera) "prefer" pseudoparasitized pea aphids

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Females of the hymenopteran parasitoids *Aphidius smithi* SHARMA & SUBBA RAO and *Ephedrus californicus* BAKER showed partial preference for third-instar nymphs of the pea aphid that had been stung, but not parasitized, by the other species. Wasps parasitized a greater proportion of such pseudoparasitized aphids than of unparasitized or parasitized hosts, in that order. Preference declined with time after stinging. *E. californicus* females required longer to oviposit in unparasitized (mean = 10.5 sec) than parasitized or pseudoparasitized hosts (mean = 7.8 sec). The observed preference patterns reflect both qualitative differences between parasitized and unparasitized aphids and differential costs incurred by the parasitoids in terms of handling time.

INTRODUCTION

Stinging is a common behaviour among Hymenoptera, including parasitoid wasps (STEINER, 1986). Especially for parasitoids of large and active hosts, injection of a venom that causes partial or complete paralysis is an effective strategy against host escape and defense tactics. However, a wasp can still reject a potential host after she has stung and probed it with her ovipositor. Such stung hosts may show various pathologies normally associated with successful parasitism and, for that reason, were called pseudoparasitized by JONES (1985).

Although females of Aphidiidae possess a venom apparatus (EDSON & VINSON, 1979), only *Monoctonus paulensis* ASHMEAD has been reported to paralyze aphids by stinging (CALVERT & VAN DEN BOSCH, 1972). We noted that pea aphids, *Acyrthosiphon pisum* (HARRIS) (Homoptera: Aphididae), which had been stung but not parasitized by the aphidiid *Ephedrus californicus* BAKER, were often preferred by other wasps over their unparasitized counterparts during a subsequent attack.

A wasp's oviposition decisions should be based on the costs of depositing an egg and on the value of a particular host relative to others in the environment (CHARNOV & SKINNER, 1985; MANGEL, 1989). Consequently, a lower-quality host may be preferred over a higher-quality host if the attack incurs low costs and has a high probability of succeeding. Because pseudoparasitized aphids are in fact unparasitized, the observed preference would be explained if the stung aphids differed in some subtle way that made them either (1) more vulnerable to parasitism (= lower cost), or (2) more suitable for parasitoid development (= higher quality), or (3) both.

We tested these hypotheses, using the pea aphid and two species of aphidiid parasitoids, *Aphidius smithi* SHARMA & SUBBA RAO and *E. californicus,* as a model system. The results are discussed with regard to the evolution of stinging behaviour in aphidiid wasps.

Insects

All species of Aphidiidae are solitary, internal parasitoids of aphids (MACKAUER & CHOW 1986; STARY, 1988). Females usually lay a single egg in attacked aphids. Host discrimination has been demonstrated in several species, including *A. smithi* and *E. californicus* (CHOW & MACKAUER, 1984, 1986; MACKAUER, 1990). If wasps encounter few unparasitized aphids, they may superparasitize; however, only one parasitoid larva in each host normally completes development.

We reared colonies of *A. smithi* and *E. californicus* in the laboratory on pea aphids that fed on broad beans, *Vicia faba* L. cv. "Broad Windsor". The colonies were maintained at $21-24\,^{\circ}\mathrm{C}$ and continous light. In all trials, we used third-instar nymphs of the pea aphid and unmated, 2- to 4-day-old parasitoid females which had been caged with aphids to gain experience in handling hosts.

Experiment 1

Wasps should selectively attack pseudoparasitized aphids if stinging causes short-term paralysis or otherwise affects an aphid's defensive behaviours, such as kicking and walking. To test this hypothesis, we placed 5 parasitoid females (species B = searching wasps) for 15 min in a screened paper cup containing a total of 15 aphids: 5 aphids were unparasitized and able to move freely; 5 aphids had been stung once by parasitoid species A; and 5 aphids were unparasitized but had been anaesthetized with CO_2; the latter were included as a control to evaluate the influence of aphid defenses on parasitoid oviposition success. To determine which, if any, of the stung aphids were parasitized (i. e. contained a parasitoid egg or larva) and which had been pseudoparasitized, we used two parasitoid species (A and B) that could be distinguished in all immature stages. Also, we marked all initially unparasitized aphids by amputating the distal third of one antenna (MACKAUER, 1972). At the end of a trial, aphids were sorted into 3 groups (unparasitized, anaesthetized, and stung) and transferred to bean plants that were kept in a growth chamber at 21 °C. After 4 days, the surviving aphids in each class were counted and dissected: N_{un} = initially unparasitized; N_{ps} = pseudoparasitized by species A; N_{sup} = parasitized by species A; and N_{CO_2} = initially unparasitized but anaesthetized wit CO_2. In addition, we counted, separately for each class, the numbers of aphids parasitized by species B and the numbers of parasitoid eggs and larvae present in all aphids.

Expt 1 included two sets of 20 cages, with 15 aphids in each cage. We reversed the oviposition sequence in Expt 1 a (A = *E. californicus* and B = *A. smithi)* and Expt 1 b (species A = *A. smithi* and B = *E. californicus)*. The interval between the attack by A and the aphids' exposure to B was ≤ 10 min.

Experiment 2

The second experiment was designed to detect changes over time in the effect of stinging, if any, on pea aphid behaviour and vulnerability to parasitoid attack. We used the same design as for Expt 1, except that we did not include any anaesthetized aphids in the trials.

Expt 2 included four sets of 20 (or more) cages, with 10 aphids in each cage, as follows: species A = *E. californicus* and B = *A. smithi,* with an interval of 3 h

between attacks (Expt 2 a); species A = *A. smithi* and B = *E. californicus,* with intervals of 3 h, 8 h, and 24 h between attacks (Expt 2 b−d).

Statistical analysis

The numbers of hosts parasitized and the numbers of eggs laid by species B were pooled over all replicates, separately for each host class. We used the G-test with Williams' correction (SOKAL & ROHLF, 1981, p. 728) to test for homogeneity among replicates.

We estimated species B's preference for pseudoparasitized aphids as the deviance between the proportion of aphids parasitized (as determined by dissection) and the proportion available in the environment (COCK, 1978; MACKAUER, 1983 a):

$$I = (H_{ps}/N_{ps}) \times ([N_{un} + N_{sup} + N_{CO_2}]/[H_{un} + H_{sup} + H_{CO_2}])$$

where N and H are, respectively, the total numbers of hosts available and parasitized in each class (as above). If there is appreciable superparasitism that varies among host classes, it may be useful to estimate preference (I') on the basis of the numbers of eggs (E) laid rather than the numbers of hosts attacked. Under these conditions, the two estimators, I and I', are not the same, the difference reflecting variations in superparasitism between host classes.

RESULTS AND DISCUSSION

Females of *A. smithi* and *E. californicus* showed partial preference for pseudoparasitized pea aphids, which they attacked with equal or higher frequency than unparasitized aphids; already parasitized aphids were least preferred (Tab. 1). Anaesthetized aphids were more vulnerable to successful parasitism than their unparasitized but free-moving counterparts. Superparasitism varied among the different host classes, with anaesthetized and pseudoparasitized aphids showing the highest proportions of multiple ovipositions (Tab. 2).

The index of preference, which reflects differences in host availability, varied with the time T after the attack by species A. Preference was highest for T = 3 h and declined with increased interval length, regardless of whether preference was assessed on the numbers of hosts parasitized (I, see Tab. 1) or of eggs laid (I', see Tab. 2).

The observed patterns of host selection by *A. smithi* and *E. californicus* reveal, on one level, qualitative differences between unparasitized and parasitized aphids and, on another level, behavioural differences between the various classes of unparasitized aphids. Aphidiid females normally avoid oviposition in aphids that are already parasitized when unparasitized hosts are available (CHOW & MACKAUER, 1986; MACKAUER, 1990; MCBRIEN & MACKAUER, 1990). However, in both experiments, wasps were provided a choice of parasitized as well as of dissimilar kinds of unparasitized aphids that presumably varied in their interactions with the wasps. While anaesthetized aphids were completely immobilized and hence unable to express any defensive behaviours, unparasitized aphids were able to avoid parasitism by kicking or walking away (HAMILTON, 1973; ROITBERG *et al.,* 1979; GARDNER *et al.,* 1984; GERLING *et al.,* 1990). Pseudoparasitized pea aphids showed no visible evidence of paralysis or trauma; but wasps attacked them with nearly as much success as anaesthetized aphids (Tab. 1, 2). The high vulnerability of pseudoparasitized aphids to subsequent parasitism indicates that

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Table 1. Numbers (and proportions) of pea aphids in different classes parasitized by *Aphidius smithi* and *Ephedrus californicus* (T, interval between first [by species A] and subsequent [by species B] attacks; n, total number of aphids dissected; H, numbers of aphids parasitized by species B among hosts that were initially unparasitized [H_{un}], pseudoparasitized by species A [H_{ps}], parasitized by species A [H_{sup}], and anaesthetized with CO_2 [H_{CO2}]; I, index of preference for pseudoparasitized aphids based on proportions of aphids parasitized in each class. Proportions sharing the same letter[s] are not statistically different [$P > 0.05$] by G-test with Williams' correction for homogeneity of replicates [SOKAL & ROHLF, 1981, p. 728].

T	n	H_{un}	H_{ps}	H_{sup}	H_{CO2}	I
Oviposition sequence: A = E. californicus; B = A. smithi						
10 min	283	64(.660)[b]	26(.788)[ab]	34(.540)[b]	81(.900)[a]	1.100
3 h	144	59(.831)[a]	11(1.00)[a]	18(.290)[b]		1.727
Oviposition sequence: A = A. smithi; B = E. californicus						
10 min	288	38(.388)[c]	18(.783)[a]	31(.449)[bc]	58(.592)[ab]	1.633
3 h	191	33(.333)[b]	22(.815)[a]	19(.292)[b]	--	2.570
8 h	191	65(.699)[a]	8(.571)[ab]	31(.369)[b]	--	1.054
24 h	381	95(.490)[a]	7(.412)[ab]	30(.176)[b]	--	1.199

Table 2. Numbers of eggs laid in (and rate of superparasitism of) different classes of pea aphids by *A. smithi* and *E. californicus* (T, interval between first [by species A] and subsequent [by species B] attacks; e, total number of eggs laid in all dissected aphids; E, numbers of eggs laid by species B in hosts that were initially unparasitized [E_{un}], pseudoparasitized by species A [E_{ps}], parasitized by species A [E_{sup}], and anaesthetized with CO_2 [E_{CO2}]; I', index of preference for pseudoparasitized aphids based on proportions of eggs laid in each host class).

T	e	E_{un}	E_{ps}	E_{sup}	E_{CO2}	I'
Oviposition sequence: A = E. californicus; B = A. smithi						
10 min	432	101(1.04)	53(1.61)	55(0.87)	223(2.48)	1.059
3 h	149	100(1.41)	26(2.36)	23(0.37)	--	2.556
Oviposition sequence: A = A. smithi; B = E. californicus						
10 min	171	42(1.11)	22(1.22)	36(1.16)	71(1.22)	1.701
3 h	122	50(0.51)	47(1.74)	25(0.38)	--	3.806
8 h	125	83(0.89)	8(0.57)	34(0.40)	--	0.864
24 h	159	117(0.60)	9(0.53)	33(0.19)	--	1.285

stinging by species A had a subtle influence on aphid behaviour, especially escape and defensive behaviours.

The results suggest that *A. smithi* and *E. californicus* inject a substance, possibly a venom (STOLTZ, 1986), into the host when probing it with the ovipositor. This action appears to be independent of oviposition. Injection of a venom causing temporary or permanent host paralysis or even death, especially after repeated attacks, has been reported in other insect parasitoids (STEINER, 1986). For example, CALVERT & VAN DEN BOSCH (1972) observed that aphids stung by *M. paulensis* became paralyzed and were unable to move for several minutes; pea aphid nymphs required 3–11 min to recover from paralysis. The authors noted that not all paralyzed aphids contained parasitoid eggs, i. e. they were pseudoparasitized. In contrast to aphidiid wasps, *Aphelinus* females (Hymenoptera: Aphelinidae) are well known to immobilize aphids prior to host feeding and oviposition (WILBERT, 1964; BOYLE & BARROWS, 1978).

However, GARDNER *et al.* (1984) reported that the defensive behaviour of the aphid *Metopolophium dirhodum* (WALKER) was increased for a short period after a stinging attack by *Aphidius rhopalosiphi* DESTEFANI-PEREZ. Aphids stung once (a first attack had a 45% probability of resulting in oviposition) became more agitated, with the consequence that successful parasitism declined during subsequent attacks. Using the same parasitoid species, ANKERSMIT *et al.* (1986) found that parasitism of the "Brown" form of *Sitobion avenae* (F.) increased with the number of stings but was independent of it in the "Green" form. As the objectives of both studies were very different from those of our research, the results cannot be directly compared. In particular, the use of a single parasitoid species (rather than two as in our work) made it virtually impossible to determine by aphid dissection which of two wasps had deposited an egg during successive attacks, especially for short intervals.

It is worth noting that oviposition behaviour differs widely among aphidiid parasitoids. Whereas some species, including *A. smithi* (MACKAUER, 1983 b), complete an attack and oviposition sequence in less than 1 sec, other species require considerably longer to do so, occasionally using the forelegs to prevent an aphid's escaping (CHOW & MACKAUER, unpublished). Females of *E. californicus* needed on average 11.8 sec to probe and deposit an egg in a third-nymphal instar of the pea aphid; oviposition was rarely successful if the female withdrew her ovipositor after 6 sec or less (CHOW & MACKAUER, 1986). Consequently, oviposition success in this species should increase if the host is unable to express defensive behaviours. We confirmed this supposition by comparing oviposition times, which differed significantly (1-way ANOVA; F = 9.27; df = 1, 44; P = 0.004) between unparasitized ($\bar{x} \pm 1$ SEM = 10.5 ± 0.6 sec) and previously parasitized or pseudoparasitized pea aphids (7.8 ± 0.5 sec). Interestingly, mean oviposition times were the same for parasitized and pseudoparasitized aphids, a fact indicating that stinging affected both groups in a similar manner. Although *A. smithi* females parasitized relatively more pseudoparasitized than unparasitized pea aphids, the difference was less pronounced than in *E. californicus* (Tab. 1, 2). A possible explanation is *A. smithi*'s rapid strike and oviposition, a behaviour limiting the females' exposure to aphid defenses.

The importance of stinging as a defensive strategy against mainly mammalian predators is well recognized in the social Hymenoptera (HERMANN & BLUM, 1981). By contrast, in insect parasitoids including Aphidiidae, stinging presumably evolved as an anti-host-defense strategy that made it possible for a wasp to

probe a mobile host with her ovipositor. Whereas the injection of a paralyzing venom and the deposition of an egg are two separate and functionally independent events, host marking appears to be dependent on successful oviposition in *A. smithi* and *E. californicus* (CHOW & MACKAUER, 1986; MACKAUER, 1990). Wasps should therefore not discriminate between unparasitized and pseudoparasitized aphids; but they should be more successful at depositing an egg in the latter, especially those species with long probing and oviposition times, such as *E. californicus*. This is what we observed. Changes in the index of preference indicate that the increased vulnerability of pseudoparasitized aphids was limited to a brief period after a stinging attack (Tab. 1, 2), a finding consistent with the actions of a venom (BEARD, 1978; STOLTZ, 1986).

Although we have demonstrated a "preference" by *A. smithi* and *E. californicus* females for pseudoparasitized pea aphids, we have not identified the mechanism(s) underlying this numerical difference. In particular, the possibility cannot be excluded that stinging (in the absence of oviposition) may also cause physiological changes, a condition that could increase a host's relative value for parasitoid development. However, the weight of the evidence favours the explanation that the parasitoids' oviposition decisions were influenced by behavioural differences between stung and unparasitized pea aphids. As our results show, a parasitoid female may accept a previously rejected aphid if the attack incurs low costs (in terms of handling time) and has a high probability of succeeding (as measured in lost opportunity time if the attack fails). It is interesting that, in the absence of oviposition and host marking by the first-attacking wasp, the injection of a paralyzing venom can produce benefits that are available to other searching females. Such behaviour provides a new and different perspective on the dynamics of parasitoid oviposition decisions.

ZUSAMMENFASSUNG

Die Wirtswahl der beiden Blattlausschlupfwespen-Arten *Aphidius smithi* SHARMA & SUBBA RAO und *Ephedrus californicus* BAKER wurde in Laborversuchen untersucht. Wenn unparasitierte, scheinparasitierte («pseudoparasitized») und von der anderen Art bereits parasitierte Erbsenblattläuse *Acyrthosiphon pisum* (HARRIS) zur gleichen Zeit angeboten wurden, dann wurden die schein-parasitierten Blattläuse zu einem höheren Prozentsatz parasitiert. Diese relative Präferenz wurde mit fortschreitender Zeit nach einem Anstrich geringer. Weibchen von *E. californicus* benötigten durchschnittlich 10,5 sec zur Eiablage in unparasitierte, aber nur 7,8 sec in schein-parasitierte oder bereits parasitierte Blattläuse. Zwei Faktoren sind entscheidend für das beobachtete Wirtswahlverhalten der beiden Schlupfwespen-Arten: einerseits die qualitativen Unterschiede zwischen unparasitierten und parasitierten Wirten und andererseits die unterschiedlichen Kosten («handling time»), welche für die Weibchen bei der Eiablage in die verschiedenen Wirtskategorien entstehen.

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