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Ovicide in the whitefly parasitoid, *Encarsia formosa*

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The oviposition decisions made by insect parasitoids when encountering hosts of variable quality have been the subject of extensive theoretical and experimental investigation. For parasitoids that lay their eggs inside the host, the possible outcomes of encounters with parasitized hosts have been assumed to include only oviposition (superparasitism), rejection, or in some cases feeding on host haemolymph. We document another outcome in *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), a species that has been a model system for the study of oviposition behaviour. In *E. formosa*, females may kill eggs previously laid within the host by jabbing them with their ovipositor before ovipositing themselves. (1) Our observations indicated that jabbed eggs were indeed killed. (2) In experimental arenas in the laboratory, ovicide occurred in the majority of encounters with parasitized hosts and at highest frequency in encounters resulting in oviposition. (3) There was no significant difference in the handling time associated with oviposition+ovicide in parasitized hosts in comparison with oviposition alone, suggesting that there is no time cost to ovicide. (4) Ovicide did not appear to be incidental to normal probing within a host. Radial analysis of the direction of ovipositor movement with respect to the centre of the previously laid egg within the host showed that females engaged in ovicidal bouts probed most often in the direction of the egg. This is the first well-documented study of ovicide in an endoparasitoid. We suggest ovicide may be under-reported in other endoparasitoid species due to the difficulty of observing it.

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The optimality approach in behavioural ecology has been used successfully in increasing our understanding of the reproductive behaviour of insect parasitoids (see Godfray 1994, for a review). One of the areas most thoroughly explored is the decisions a parasitoid makes when confronted with hosts of variable quality, and in particular when confronted with previously parasitized hosts. Superparasitism (laying another egg or clutch of eggs) was thought to occur because of a parasite's inability to discriminate between parasitized and unparasitized hosts. This earlier interpretation has now given way to a more sophisticated iterative process of estimating fitness returns from different responses to these hosts, and then developing and testing models that predict the circumstances under which superparasitism is adaptive (van Alphen & Visser 1990). Models have varied in the currency identified as limiting (reviews in van Alphen & Visser 1990; Godfray 1994), but have generally converged on two or three behaviours that may be performed by a female parasitoid when encountering a parasitized host: she may reject the host, oviposit in (superparasitize) the host, or in some species, she may feed on the haemolymph of the host to gain nutrients for future egg development. One basic but critical assumption of optimality models is that the set of possible behaviours (the 'strategy set') of the animal has been correctly identified (Grafen 1991). In this study we focus on a parasitoid that has been a model species for understanding oviposition behaviour, *Encarsia formosa*, and document that females encountering already parasitized hosts may kill previously laid eggs within the host. Recognition that ovicide is an option in the strategy set of this species changes our assessment of the quality of parasitized hosts as oviposition sites, and raises the possiblity that ovicide in other endoparasitoids may have been overlooked.

Much of the current theory relating to parasitoid encounters with parasitized hosts concerns whether a female should reject the host or lay another egg or clutch of eggs in the parasitized host (superparasitize). The probability that a female will superparasitize the host is predicted to depend on the probability of a second egg or clutch winning the competition with the first and surviving, as well as factors such as patch quality as assessed by the female, and her estimation of the density of other foraging females (Griffiths 1977; Hubbard et al. 1987;

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Mangel 1992; Visser et al. 1992; Visser 1993, 1995). A less frequently observed behaviour is ovicide, when a parasitoid kills the previously laid eggs of another female. Killing eggs laid previously may shift the competitive balance in favour of the ovicidal female, to some extent negating a situation of diminishing returns described in the marginal value theorem (Charnov 1976).

The phenomenon of ovicide can be viewed in the larger context of the decision to superparasitize. Superparasitism has been documented in representatives of many of the major families of the parasitic Hymenoptera (Salt 1961) and may occur even when a second female's egg or eggs have less than a 50% chance of winning the contest with the first female's eggs (van Alphen & Visser 1990). Superparasitism rather than rejection of the host has been shown to occur when females are not egg limited, the density of searching females is high, and many hosts are parasitized (van Alphen & Visser 1990; Visser 1993). Ovicide followed by oviposition may be considered a special case of superparasitism in which a female increases the probability of her offspring's survival by killing a previous female's eggs. Ovicide is predicted to evolve when the competitive advantage of the first female's offspring is great (Strand & Godfray 1989), and should depend on the time and energy costs to the female of performing this behaviour, the probability of the egg being kin, and the quality of the host for the development of subsequently laid eggs following ovicide (Smith & Lessells 1985; Strand & Godfray 1989). Furthermore, ovicide may only evolve when parasitoid females can discriminate between parasitized and unparasitized hosts, and find and kill previously laid eggs.

Perhaps due to constraints on the ability of parasitoids to find previously laid eggs, the occurrence of ovicide is far less well documented than superparasitism and has been mentioned as an ovipositional strategy in only a few parasitoids. Of these, all but one are ectoparasitoids (review in Godfray 1994). Ovicide by an endoparasitoid has only been observed in the solitary, parthenogenetic parasitoid of whiteflies, *E. formosa* (Arakawa 1987). In this species, ovicide was observed in the process of another experiment and was described but not quantified (Arakawa 1987). To our knowledge no further study has followed this observation and subsequent authors have suggested that the females observed may have only shifted the position of the egg (Hoddle et al. 1998).

Other aspects of *E. formosa* host finding and oviposition behaviour have been well studied (van Lenteren 1976; van Lenteren et al. 1976, 1980, 1987; Nell et al. 1976; van Roermund et al. 1994; van Roermund & van Lenteren 1995a, b). When a wasp encounters a host, she first examines it externally by drumming her antennae on the host dorsum. At this point, she may reject the host, or may insert her ovipositor for an internal examination (van Lenteren et al. 1980; van Roermund & van Lenteren 1995a). Host stage, host quality and the physiological state of the parasitoid may influence the female's decision to reject, oviposit, or feed on the host (van Lenteren et al. 1980, 1987; van Vianen & van Lenteren 1986; van Roermund & van Lenteren 1995a). To feed on the host, the female removes her ovipositor from the host after probing inside it and drinks haemolymph from the wound. Among destructive host feeders such as *E. formosa*, this usually results in the death of the whitefly nymph and eliminates that host from future use as an oviposition resource. Many host-feeding parasitoids receive nutrients from the host haemolymph that they cannot obtain by feeding on nectar or host honeydew (Heimpel & Collier 1996). *Encarsia formosa* is synovigenic, meaning it matures eggs continuously in adulthood. For synovigenic parasitoids like *E. formosa*, host feeding is essential for egg maturation and is therefore an investment of time and energy in future reproduction.

METHODS

Experimental Insects

Encarsia formosa is a parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). The first specimens were found in 1926 on tomato plants in a greenhouse in England. By the 1930s this species was being mass reared and shipped to other countries for regular use as an agent for biological control of whiteflies in greenhouses, and it is in wide commercial use today (Hoddle et al. 1998).

We obtained experimental *E. formosa* females from a culture initiated in the laboratory in the winter of 1997 and maintained on a 16:8 h light:dark cycle at 27°C and with 35% relative humidity until the time of this study in 1998. We obtained wasps for the culture from a commercial insectary (Ciba Bunting, Colchester, U.K.). Wasps were cultured on the nymphs of the greenhouse whitefly, *Trialeurodes vaporariorum*, raised on green bean and cotton plants. In cultures and experiments, we placed the females on early fourth-instar nymphs, a preferred stage for oviposition (Nechols & Tauber 1977). We harvested leaves from the plants 11–15 days following exposure to ovipositing female wasps, or upon the appearance of the blackened whitefly 'mummies', indicating parasitism by *E. formosa*. We placed the harvested leaves in emergence jars or segregated them into individual petri dishes, from which we collected the emerged wasps.

Experimental Arenas

We conducted behavioural observations in circular arenas measuring 3 × 16 mm (height × interior diameter). Each arena consisted of an adhesive, annular, latex foam pad (Callous Cushions, Schering-Plough Inc., Memphis, Tennessee), 36 mm in exterior diameter, which we affixed to a glass slide or petri dish. The open top of the arena was covered with a glass coverslip and a triangular slit cut in the side of the pad allowed for the introduction of wasps with an aspirator. *Encarsia formosa* females are minute (<1 mm in body length) and wasps housed in these arenas appeared to forage, feed, examine hosts and groom naturally. Following the methods of Arakawa (1987), we removed early fourth-instar *T. vaporariorum* nymphs from leaves and affixed them, ventral-side-down to the underside of the coverslip with drops of a dilute honey

solution. Whiteflies are phloem-feeding insects and nymphs removed in this manner cannot complete development but will remain alive in a humidified environment for about 8 h.

We placed the arenas under a dissecting microscope at 50 × magnification, illuminated by transmitted light delivered by a two-armed fibre optic light source. Early fourth-instar *T. vaporariorum* nymphs are a flattened ovoid shape and the interiors of the nymphs could be easily visualized through the wax-free, translucent venter. After we introduced a wasp into the arena, it was possible to observe the actions of the wasp's ovipositor within the host as well as the wasp's activities while not in contact with hosts.

Egg Development Experiment

To determine whether eggs that appeared to have been jabbed by the wasp's ovipositor were actually killed, we conducted an experiment in which we compared punctured and unpunctured eggs ca. 1 day after oviposition for evidence of embryogenesis. We exposed arrays of five third- to early fourth-instar hosts to groups of *E. formosa* in the same type of arena described previously. The wasps were allowed to oviposit inside the hosts and probe within hosts after they had been parasitized. Some hosts received two or more eggs from different females, some eggs were punctured, and some were left intact. We observed activities of the wasps continuously while they were exposed to the hosts and recorded the ultimate condition (punctured or intact) of all of the eggs obtained. For this analysis, we only included hosts that contained one or two eggs, the condition of which we were certain.

Following observation, we removed the hosts from the arena and transferred each host to a single drop of a Ringer's solution on a separate, labelled slide. After 19–24 h of incubation at room temperature in a box lined with damp paper towels, we dissected the eggs from the hosts in an insect Ringer's solution, then removed the saline and added a drop of fixative (2.5:3:1; chloroform:95% ethanol:glacial acetic acid). After 3 min, we added a drop of lacto-aceto-orcein (0.2% w/v synthetic orcein in a 1:1:1 mixture of lactic acid, acetic acid and distilled water). We then placed a coverslip on the stained egg and examined it under a compound microscope using phase contrast illumination at 200–400 × .

Behavioural Observations: Experiments 1 and 2

For behavioural observations we introduced individual naïve female wasps, 24–48 h old, into an arena containing parasitized or unparasitized nymphs. We arranged five nymphs in the centre of the coverslip in a pentagonal array. For the arrays used in the 'parasitized' treatment, we had previously allowed nonexperimental females to lay single eggs inside hosts. We prevented ovicide and superparasitism of these hosts by observing the entire session and gently brushing females off hosts that contained an egg.

To begin an observational session, we introduced a wasp into an experimental arena, and then placed a coverslip with a host array over the arena. At the moment the wasp contacted the first host with her antennae, we initiated behavioural recording. We recorded behavioural sequences of interactions with all hosts using the Observer 3.0 software program (Noldus Information Technology b.v. 1995) loaded into a Psion Workabout hand-held computer. These behaviours included those that took place within the body of the host. We distinguished between a host 'encounter', which was considered to include all of the activities of the wasp on a host at a given time, including possibly multiple ovipositor insertions, and a probing 'bout', which included all of the behaviours performed following a single ovipositor insertion. Wasps could then perform more than one bout within a given host encounter, as well as encounter a particular host more than once over the course of an observation. 'Ovicidal bouts' were those bouts during which ovicide occurred, while 'nonovicidal bouts' were those that did not result in ovicide, nor were preceded by ovicide in a previous bout during the same encounter with a host. An observation was terminated when the wasp left a host and did not return to situate herself on another host within 5 min.

Our observations of *E. formosa* oviposition behaviour outside the host are in agreement with van Lenteren et al. (1980). Wasps typically examine a leaf or other surface by drumming their antennae on the surface while walking. They seem to perform this behaviour normally as they explore the interior of an arena. Upon contacting a host with her antennae, a wasp will either continue walking or will pause to drum the dorsum of the host with her antennae ('antennating'). Often she will further investigate the host with a behaviour we called 'situating', which includes walking over the host's dorsal surface, turning and antennating frequently. A wasp that remains on the host will then lower her abdomen and attempt to puncture the host cuticle with her ovipositor. We called these efforts 'trying', and they may continue for several seconds to a few minutes. During this behaviour, the interior surface of the nymph's dorsal integument may be seen to buckle from the pressure of the ovipositor, but the ovipositor tip cannot be observed within the body of the host. We recorded the initiation of 'probing' behaviour as soon as the tip of the ovipositor appeared within the host. From this point forward, the movements of the ovipositor within the host could be clearly distinguished. We recorded the probing behaviour within the hosts with different degrees of detail in the first and second experiments.

In the first experiment, 'probing' described all movements of both extension and retraction of the ovipositor within the host. We created a more detailed record of the probing behaviour in the second experiment by modifying the Observer program. An effect of this modification was the separate coding of the extension ('probe') and retraction ('retract') movements of the ovipositor, so that that the frequency of these movements could be recorded. We also recorded the direction of each ovipositor probing movement in relation to the head of the

whitefly host. We assigned movements towards the head of the host a probing angle of zero, with subsequent angle divisions spaced at 45° intervals around a 360° circle. We also recorded probes that were directed straight towards the venter of the host.

In the second experiment, we recorded additional descriptive data that could not be recorded with the Observer program: (1) the position of each oviposition puncture; (2) the position of the original egg (for parasitized hosts) and (3) the final position of any eggs laid by the experimental wasp.

In both experiments, we recorded behaviours that typically follow probing behaviour. Prior to oviposition, the valves of the ovipositor move past each other rapidly in a rhythmic behaviour we called 'vibrating', which is visible inside the host. This movement is actually indicative of the movement of the egg through the ovipositor (Quicke et al. 1994). There is a pause of ca. 9 s in ovipositor movement prior to the appearance of the egg. When the egg swelled into view we recorded the initiation of 'oviposition'. A wasp probing inside a host does not always lay an egg, but instead may just leave the host. Alternatively, she may remove her ovipositor and turn around on the host to feed on the haemolymph as it wells up from the puncture she has made. We also recorded host feeding.

Lastly, any behaviours that involved the ovipositor piercing a previously laid egg we termed 'jabbing'. The female pierces the chorion of the egg, moving the tip of her ovipositor into the cloudy, white contents of the egg, sometimes moving it sideways in a tearing motion or all the way through the egg so that the tip can be seen emerging from the opposite side. Our results indicated that jabbing behaviour inevitably leads to the death of the egg and this behaviour is referred to as ovicide in other sections of this paper. Other behaviours that the female performed while off of the host such as walking, grooming, or feeding on honeydew, were recorded collectively as 'off host' behaviour.

Analysis

We compared the relative frequency of encounters with parasitized and unparasitized hosts leading to oviposition, host feeding and host rejection using a *G* test (Sokal & Rohlf 1981). We compared the duration of probing in host encounters that included ovicide with those that did not include ovicide using a *t* test. Lastly, in experiment 2, we compared the radial analyses of the mean directions of ovipositor extension ('probing') to a uniform radial distribution using Rayleigh's test (Zar 1984). We examined the mean direction of ovipositor extension for all bouts in parasitized arenas with respect to the head of the whitefly (set at 0°) to evaluate the possibility that females regularly extend their ovipositor in one direction. In a separate analysis, we compared the mean direction of ovipositor extension with respect to the centre of the egg (set at 0°) during ovicidal and nonovicidal bouts. Here, we tested the null hypothesis that ovicidal behaviour occurs at random and does not involve repeated extensions of the ovipositor towards the egg.

RESULTS

Consequences of Egg Puncture

Fixation and staining of unpunctured eggs 1 day after being laid indicated that they had all reached the blastula stage of embryogenesis. In each of the 10 unpunctured eggs examined, tens of dark red staining syncytial nuclei were apparent. In contrast, none of the 10 eggs that received at least one puncture revealed any stained nuclei. Also, punctured eggs that had been alone inside the host were indistinguishable from those that had been incubated with a second intact egg, indicating that we were not simply viewing the developmental arrest of one egg in the presence of another. Furthermore, we found that we could generally distinguish between developing and dead eggs under the dissecting microscope (at 50 ×) immediately after dissection. Live eggs often slipped out of the host easily and appeared translucent around the perimeter but opaque white in the centre. Killed eggs, in contrast, were uniformly opaque white and were often embedded in host tissue.

Host Use

Female *E. formosa* spent similar amounts of time on parasitized and unparasitized patches ($\bar{X} \pm$ SE time: experiment 1: unparasitized: 41.0 ± 4.4 min; parasitized: 43.0 ± 5.9 min; $t_{0.05,9}$=0.33, NS; experiment 2: unparasitized: 29.4 ± 3.6 min; parasitized: 34.3 ± 5.29 min; $t_{0.05,9}$= 0.49, NS). Wasps in unparasitized arenas tended to have a greater number of host encounters than those in parasitized arenas, but the difference was not significant ($\bar{X} \pm$ SE number: experiment 1: unparasitized: 5.9 ± 0.84; parasitized: 4.6 ± 0.83; $t_{0.05,9}$=1.03, NS; experiment 2: unparasitized: 4.7 ± 0.47; parasitized: 3.6 ± 0.48; $t_{0.05,9}$= 1.64, NS).

In experiment 1, there was a significant difference in the host use by wasps in parasitized and unparasitized arenas (*G* test: G_2=8.94, P< 0.05; Fig. 1a). More than 75% of host encounters in unparasitized arenas resulted in oviposition rather than host feeding or rejection after probing only (Fig. 1a). In contrast, wasps in parasitized arenas oviposited in roughly half of their encounters with hosts, and in the remainder of encounters, fed on hosts or rejected probed hosts. The same tendency was seen in experiment 2 (Fig. 1b), but these differences were not significant (G_2=5.49, NS).

The frequency of ovicide across all encounters in the parasitized arenas was the same in the two experiments. Wasps in the parasitized arenas of each experiment killed a previously laid egg in 53% of their encounters with hosts (experiment 1: N=43; experiment 2: N=32). Similarly, in both experiments, ovicide was most common in encounters that resulted in oviposition (experiment 1: 75%, N=22; experiment 2: 63%, N=17; Fig. 1a, b), followed by those in which the host was ultimately

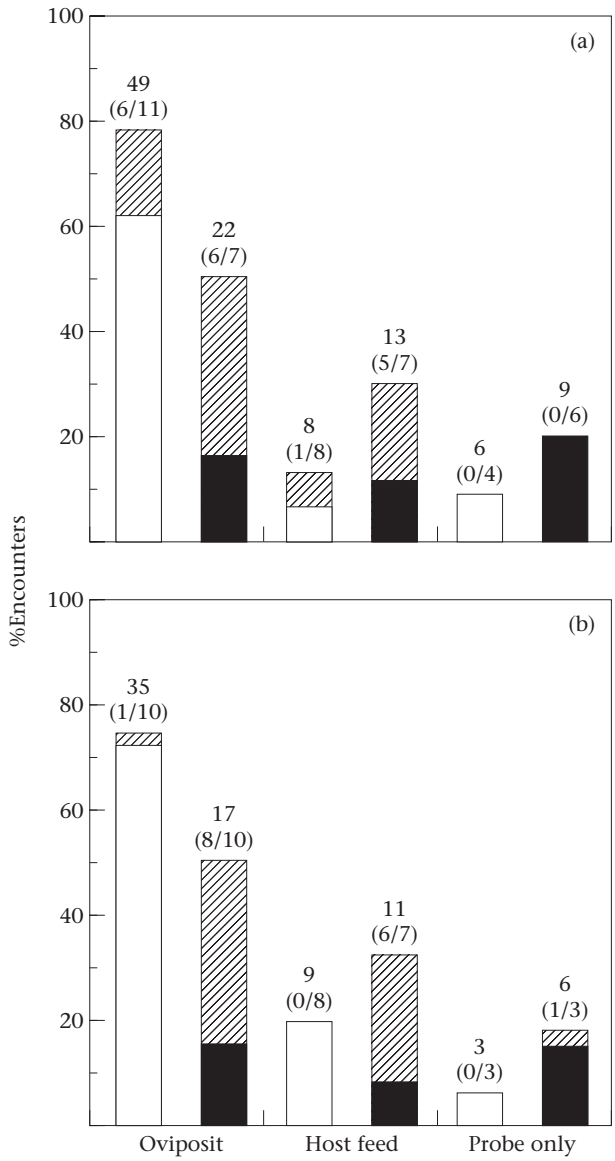

Figure 1. (a) Experiment 1. (b) Experiment 2. Total percentage of encounters with hosts resulting in oviposition, host feeding, or probing only. Encounters that occurred in 'unparasitized' (□) and 'parasitized' (■) arenas. ▨: The proportion of the encounters that included ovicide. Ovicide in 'unparasitized' arenas was possible when females re-encountered hosts in which they had already laid an egg. In the oviposition category, the solid black portions of the bars indicate superparasitism. The numbers over the bars are the number of encounters (*N*), and in parentheses, the number of females that were observed to commit ovicide during a particular behaviour/the total number of females observed to perform the behaviour. The ultimate use of the host (oviposition, host feeding, or rejection) differed significantly between parasitized and unparasitized arenas in experiment 1 (G_2=8.94, P<0.05), but not in experiment 2.

fed upon (experiment 1: 61%, N=13; experiment 2: 46%, N=11; Fig. 1a, b). Ovicide was least frequent when the host was rejected, occurring only twice in experiment 2 and not at all in experiment 1.

There were also incidents of ovicide in the 'unparasitized' arenas of both experiments. These occurred when a female encountered a host into which she had already laid an egg and then killed her own egg. Wasps in the unparasitized arenas of the first experiment re-encountered and probed hosts containing their own eggs 18 times; 12 of these encounters resulted in ovicide (66%). Wasps in the unparasitized arenas in experiment 2 re-encountered their own eggs only twice and only one of these two wasps killed her own eggs. Some wasps in the parasitized arenas also killed their own eggs, although these numbers were not high. For example, in experiment 2, three females encountered a total of four of their own eggs, and two of the females each killed one egg.

Probing Duration

For the wasps in the parasitized arenas, handling time was not lengthened by the act of killing an egg (Fig. 2a, b). In fact, in both experiments the mean probing duration was longer in oviposition encounters that did not involve ovicide than in those that did, but the difference was not significant (experiment 1: $t_{0.05,14}$= 1.33, NS; experiment 2: $t_{0.05,22}$=1.24, NS). This result suggests that killing an egg does not confer a time cost on ovipositing wasps that engage in this behaviour.

Host-feeding encounters, in contrast, were longer when ovicide occurred (Fig. 2a, b). This difference was statistically significant in experiment 1 ($t_{0.05,9}$=2.66, P<0.05), but not in experiment 2 ($t_{0.05,12}$=0.45, NS).

Ovipositor Insertion Points and Radial Distribution of Probing Angles: Experiment 2

Ovipositor insertion points for all bouts in the parasitized arenas were distributed across the dorsal surface of the whitefly (Fig. 3). Although there was a concentration of insertion points along the midline, the females showed no consistent preference for a particular point at which to insert their ovipositor.

We compared the probing directions of wasps that were engaged in ovicidal probing bouts with respect to two points: the head of the whitefly and the centre of the egg. When the head of the whitefly was used as a reference point (0°), wasps that engaged in ovicidal bouts did not consistently probe in a particular direction (Fig. 4). In contrast, when the centre of the egg was used as a reference point, wasps that committed ovicide tended to probe in the direction of the egg (Fig. 5a). When pooled, the orientation of the mean probing angles in relation to the egg was statistically significant (Rayleigh's test: Z=8.03, N=22, P<0.05; grand mean angle across females=2.35°; Zar 1984). Note that because these data are means, a significant direction does not necessarily indicate that the wasps oriented to the egg prior to contact.

Females that were not engaged in ovicidal bouts did not show any orientation to the centre of the egg (Fig. 5b). The radial distribution of mean probing angles by these females did not differ significantly from a uniform circular distribution (Z=0.17, N=14, NS; Fig. 5b).

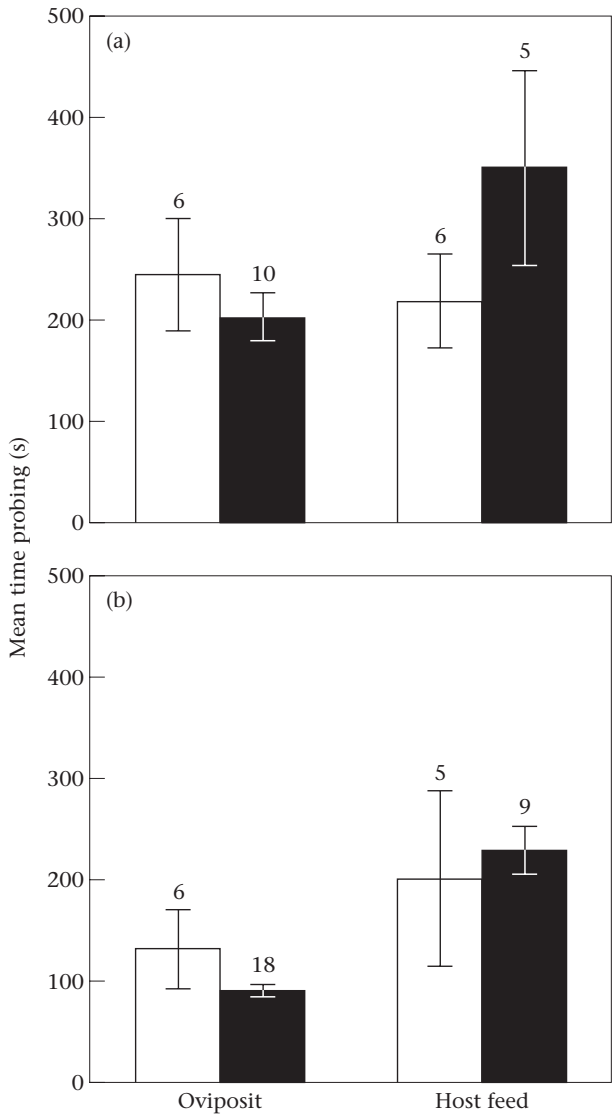

Figure 2. (a) Experiment 1 (b) Experiment 2. Mean±SE time (s) spent probing during host encounters that led to oviposition or host feeding in the parasitized arenas. □: Encounters in which ovicide did not occur; ■: encounters in which ovicide did occur. The probing duration in oviposition encounters was not significantly lengthened (or shortened) by the act of ovicide in either experiment. The probing duration in host-feeding encounters was significantly longer when ovicide occurred in experiment 1 ($t_{0.05,9}$=2.66, P<0.05), but not in experiment 2.

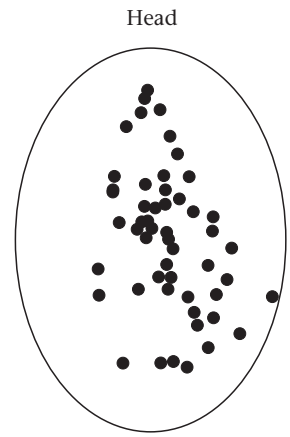

Figure 3. Schematic diagram of a whitefly nymph from above. Dots indicate the ovipositor insertion points for all bouts in the parasitized arenas in experiment 2.

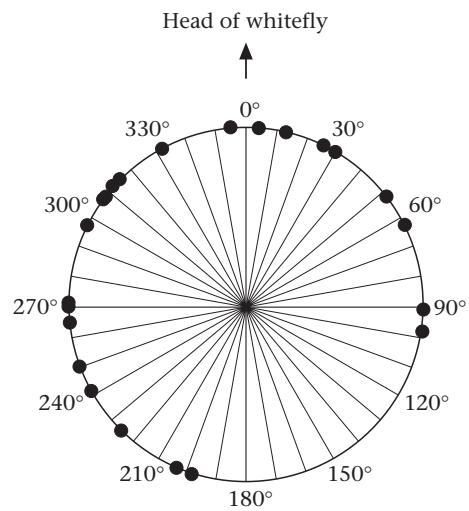

Figure 4. Direction of ovipositor probing in parasitized arenas in experiment 2. Zero degrees designates the end point of a vector from the ovipositor insertion point to the head of the whitefly. Points around the circle designate means of the angles probed in ovicidal bouts. The distribution of the mean angles of probing in ovicidal probing bouts did not differ significantly from uniform.

In summary, our observations suggest that females do not systematically insert their ovipositors at a particular point, nor do they consistently probe in one direction within the host, but females that engage in ovicidal bouts tend to probe repeatedly in the direction of the egg.

DISCUSSION

Frequency and Consequences of Ovicide in *E. formosa*

Our research indicates that *E. formosa* do damage previously laid eggs inside the host with their ovipositors, and that this damage, even when only a single puncture, results in the death of the egg. While the death of the egg may result from direct damage to the early embryo, minor breaches of the egg membranes may also leave the egg more vulnerable to immune responses of the host. Although evidence of whitefly physiological defence against parasitism is lacking (Gerling 1990), many insect species subject to parasitism do have defensive responses to the presence of a parasitoid in the body, such as encapsulation, in which haemocytes form an airtight capsule around the parasitoid egg (Salt 1963; Godfray 1994). Our finding that punctured eggs were embedded in host tissue to which they adhered suggests that the physiological response of whitefly nymphs to killed versus healthy eggs is different.

Ovicide in *E. formosa* appears not to be the accidental consequence of probing behaviour within the host. Our

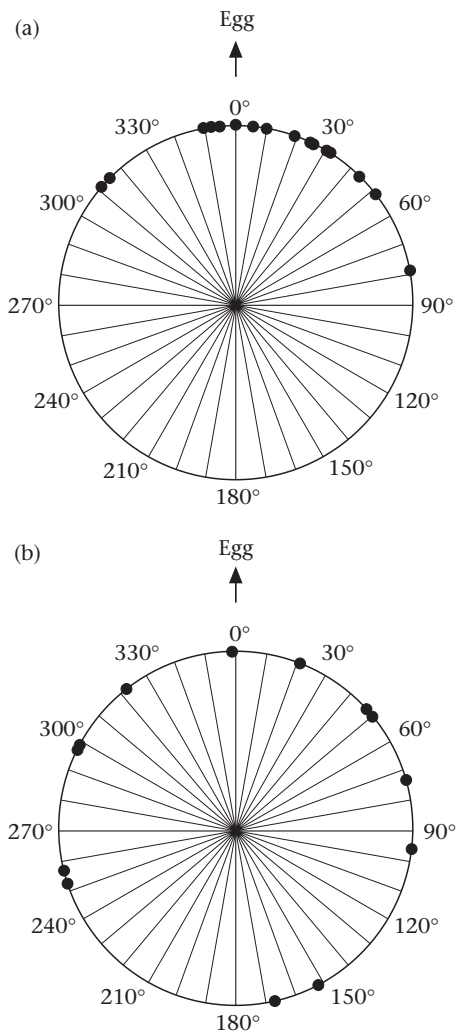

Figure 5. Direction of ovipositor probing in parasitized arenas in experiment 2. Zero degrees designates the end point of a vector from the ovipositor insertion point to the centre of the egg. Points around the circle designate the means of the angles probed in (a) ovicidal probing bouts, and in (b) nonovicidal probing bouts (those that did not include ovicide nor were preceded by ovicidal bouts). The distribution of the mean angles probed in ovicidal probing bouts differed significantly from uniform (Z_{21}=8.02, P<0.05). The grand angular mean of probes in the ovicidal bouts was 2.34°. The distribution of the mean angles of probing in nonovicidal probing bouts did not differ significantly from uniform.

observations suggest that females cannot accurately predict the location of a previously laid egg in the host, and do not simply pierce the egg in the course of their normal probing. Rather, ovicidal females show directed movement of their ovipositor towards the egg. The orientation of the ovipositor towards the egg could possibly be in response to a chemical gradient produced by the egg, but we believe it is more likely that females simply repeatedly extend their ovipositor in the direction in which the first contact with the egg is made.

Wasps in both experiments 1 and 2 committed ovicide frequently, killing an egg in just over half of all encounters in parasitized arenas that resulted in oviposition, host feeding, or probing with rejection of the host by the female. It is perhaps not surprising that this behaviour is most common during encounters that result in oviposition, as ovicide eliminates the offspring's direct competitor. The relatively high incidence of ovicide during host-feeding encounters is more curious. Because host feeding in this species results in the death of the host, host feeding alone is sufficient to kill a competitor's egg. We can suggest three possible explanations for this behaviour. It is possible that an ovicidal female gains an additional nutritional benefit from ingesting both host haemolymph and egg contents. Our experiments did not allow assessment of this, but interestingly, a typical method of ovicide for the bethylid *Gonozius platynotae* is to eat the eggs (Venkatramen & Chako 1961). Alternatively, a female's intended host use may change after she has already located and killed the egg, either due to a reassessment of host quality or the actual reduction of host quality by her own extensive probing performed in search of the egg. Lastly, females that engage in lengthy probing behaviour in preparation for host feeding may increase the odds of accidentally killing an egg that is present. The finding from the first experiment that wasps spent a significantly longer time host feeding when ovicide occurred is consistent with the last explanation.

The high frequency of ovicide during oviposition encounters on parasitized hosts has interesting implications for understanding certain aspects of oviposition behaviour in *E. formosa*. The oviposition behaviour of this species in the presence of parasitized hosts has largely been assessed in terms of the female's ability to discriminate between parasitized and unparasitized hosts and the frequency of superparasitism (van Lenteren et al. 1976, 1980; van Roermund & van Lenteren 1995a). However, it appears that *E. formosa* females do not just assess host quality, but actively improve the quality of parasitized hosts by eliminating competitors. The existence of ovicide fundamentally changes our understanding of intraspecific competition and oviposition behaviour in *E. formosa*.

Because experimental protocols in previous studies differed from ours, we cannot interpret the level of ovicide that may have occurred in these studies. Clearly, our experiments with few hosts and some arenas in which all hosts were parasitized were designed to create the conditions promoting ovicide. However, our data suggest ovicide occurs frequently and is likely to affect the interpretation of certain results. *Encarsia formosa* has been reported to avoid superparasitism in hosts containing conspecific eggs with 86% or greater efficiency, increasing to 100% efficiency in the avoidance of self-superparasitism, or killing her own eggs (van Roermund & van Lenteren 1995a). These assessments were based on observations of the females' behaviour (outside of the host) and on postobservation dissections. The parasitoids were observed from above as they foraged on a leaf or in an arena, so the actions of the females' ovipositors within the host could not be observed directly. Dissections following observations of oviposition behaviour from above the host may erroneously record oviposition following

ovicide as superparasitism, if the killed egg remains intact. Alternatively, when the killed egg is damaged beyond recognition by an ovicidal female, an observer may record a rejection by the second female, when actually it is the second female's egg that is left within the host. Clearly, it is neither practical nor desirable to conduct all studies of oviposition behaviour by observing the activities of the parasitoid ovipositor within the host. This would preclude the study of behaviour while on the leaf and any in which the wasps are allowed to develop. However, our observations suggest that killed eggs could be distinguished from healthy eggs after a period of incubation, and, in a blind test, killed eggs were not significantly less detectable upon dissection of the host (unpublished data). Thus, dissection protocols may be corrected for the occurrence of ovicide.

Our observations indicated that female *E. formosa* may kill their own eggs, as the rate of ovicide was fairly high when females probed hosts containing their own eggs. However, we have not examined the possibility that females ordinarily reject self-parasitized hosts before they insert their ovipositors, but once probing has begun no further discrimination occurs. Our analyses did not include the frequency of contacts with particular hosts, without ovipositor probing, that were followed by rejections. Van Roermund & van Lenteren's (1995a) measurement of encounter and rejection frequency also included antennal rejection, which is host contact and rejection without probing. They found that naïve females rejected 95% of self-parasitized hosts, and 77% of conspecific-parasitized hosts. Other differences in the experimental protocols may in part explain the differences in the results between the van Roermund & van Lenteren (1995a) study and this one.

Theory predicts that ovicide will evolve more readily when females can discriminate between their own eggs and those of conspecifics (Smith & Lessells 1985). For this reason it would be especially valuable to examine further the behaviour of *E. formosa* in response to encounters with self-parasitized and conspecific-parasitized hosts.

Factors Favouring the Evolution of Ovicide

Strand & Godfray (1989) examined ovicide in the gregarious parasitoid *Bracon hebetor*, and they constructed a general model to predict the conditions favouring the evolution of ovicide or superparasitism. According to their predictions, ovicide will evolve (1) when the time to kill eggs is short; (2) when travel times between hosts increases; (3) when the proportion of parasitized hosts increases and (4) when the competitive advantage of the first clutch is large. These predictions assume a significant time cost to ovicide, but one that is short relative to travel time and host handling time without ovicide. Indeed *B. hebetor* requires 30–90 min to find and kill the eggs of a previous clutch, a significant cost. However, because the entire oviposition period may last 3–8 h, time for ovicide is still relatively short (Strand & Godfray 1989).

In contrast, host handling time and oviposition by *E. formosa* females occurs within minutes, and ovicide, within seconds. The total probing time involved in oviposition was not significantly increased in encounters in which females also located and killed an egg, suggesting that there is no time cost to ovicide in *E. formosa*. In *E. formosa* then, one might ask why a female should refrain from, rather than commit, ovicide.

There are other potential costs to ovicide (Smith & Lessells 1985). In animals that cannot discriminate between their own eggs and conspecific eggs, ovicidal behaviour may result in the death of their own offspring. If, as seems possible, *E. formosa* does not discriminate between self-parasitized and conspecific-parasitized hosts once the ovipositor has been inserted, ovicide may be favoured when a female arrives at a new, parasitized patch. For some period, the female's net payoff should be positive for each egg killed prior to oviposition. The timing of the wasp's departure from the patch should depend on the variation in quality of hosts within and among patches, the travel time between patches, and the degree to which eggs are limiting. In general, a female that is relatively egg limited should leave the patch earlier, while a female that is neither limited by eggs or time should remain in the patch until all hosts are parasitized, regardless of the number of her own eggs she has killed.

Even if *E. formosa* females can avoid ovicide of their own eggs, one might predict that they would still kill competitors' eggs and oviposit in parasitized hosts at a lower frequency than in unparasitized hosts if the host quality is lower. Our findings on the differences in host use by females in parasitized and unparasitized arenas suggest that wasps do discriminate between hosts and treat parasitized hosts as less suitable for oviposition. Females usually oviposited in unparasitized hosts, while they were as likely to feed upon or reject parasitized hosts as they were to oviposit.

The quality of a parasitized host may be reduced and the host rejected for oviposition simply because of a failure of the female to find the first egg. In solitary species the second larva is likely to be at a competitive disadvantage to the first, either through resource monopolization by the first larva or active aggression (McBrien & Mackauer 1991; Godfray 1994). Only one offspring can develop per host, so the risk of losing the offspring if ovicide is unsuccessful is fairly high. Even when ovicide can ensure the victory of the second egg over the first, however, the act of parasitism may alter the internal environment of the host in such a way that it is less suitable for the second egg's development than an unparasitized host. The mechanical damage inflicted on host tissues by the ovipositor movement of the first female during probing may also reduce host quality for other females such that further exploration inside the host makes it less valuable.

Frequency of Ovicide in Endoparasitoids

Other than *E. formosa*, ovicide has only been documented among ectoparasitoids. In addition to the braconid *B. hebetor* (Strand & Godfray 1989; Antolin et al. 1995), other ovicidal species include several of the

bethylids (Goertzen & Doutt 1975; Griffiths & Godfray 1988; Hardy & Blackburn 1991; Mayhew 1997), the pteromalid *Eupelmus vuilleti* (van Alebeek et al. 1993), and the ichneumonid *Pleolophus indistinctus* (Price 1970). *Bracon hebetor* commits ovicide only occasionally (Strand & Godfray 1989). In one experiment 20% of *B. hebetor* females killed eggs during their encounters with their phycitine moth hosts (Antolin et al. 1995). *Eupelmus vuilleti* females, in contrast, not only regularly kill eggs of their heterospecific competitors, *Dinarmus basalis*, but actively concentrate their ovipositions on bruchid hosts already parasitized by the other species (van Alebeek et al. 1993). The frequency of ovicide can be very high among the Bethylidae, ovicide being the typical response to discovery of an unguarded, parasitized host (Griffiths & Godfray 1988). In 94% of observations on the bethylid *Goniozus platynotae*, the female destroyed at least part of the previous brood within 72 h after encountering the host, whether or not she oviposited herself.

The occurrence of ovicide among endoparasitoids may be more widespread than our observations of *E. formosa*. Using the same experimental technique, we observed ovicide at similar frequencies in *E. luteola* Howard (M. R. McElween & M.S.H., unpublished data), a species closely related to *E. formosa* (Polaszek et al. 1992). Our observations of the frequency of ovicide indicate that, at least for some *Encarsia* species, eggs may be no 'more difficult and costly to locate and destroy' than those of ectoparasitoids, as was suggested as an explanation of why the behaviour is more commonly observed in ectoparasitoids (Mayhew 1997). The dorso-ventrally flattened shape and the transparency of whitefly hosts may afford a unique opportunity to observe parasitoid ovipositor movement within a host. For virtually all other endoparasitoids, ovicide can only be inferred from careful observations of whether eggs dissected from hosts are alive or dead. We are not aware of any study of this kind, and thus ovicide in endoparasitoids may be under-reported.

However, there are reasons why the evolution of ovicide may be especially likely to evolve in a parasitoid–host system like that of *E. formosa* and its whitefly hosts. Mayhew (1997) speculated that ovicide should be favoured in ectoparasitoids with large, easily located eggs that can be found quickly and killed. Although small relative to the eggs of many ectoparasitoids, the eggs of *E. formosa* are fairly large (ca. 0.13 mm in length) relative to the whitefly nymphs they parasitize (length × width: 0.51–0.73 × 0.31–0.48 mm; Nechols & Tauber 1977). Additionally, the flattened shape of whitefly nymphs provides a limited three-dimensional volume to explore with the ovipositor. One might speculate that ovicide could also occur either in other endoparasitoids that lay their eggs in a precise location within their host, even when the difference in volume between the egg and the host is great. Also, one might look for ovicide when an external cue identifies the location of the egg(s) within the host (e.g. some Encyrtidae, where egg stalks protrude from the host cuticle; Clausen 1940). Ovicide would probably be less likely to evolve in systems in which a small egg is laid in the haemolymph of a comparatively large host, as are the hydropic eggs of some aphid parasitoids (Clausen 1940).

Lastly, the unusual reproductive biology of many *Encarsia* species may preadapt this group for efficient egg location within the host. *Encarsia luteola*, a close sexual relative of *E. formosa*, is an autoparasitoid, as are almost all sexual *Encarsia*. Autoparasitoid females lay male eggs in or on female larvae of their own or other species, and must regularly locate immature wasps within the body of the parasitized whitefly. This requires the ability to find an immature wasp inside the whitefly, and differentiate it from host tissue before laying an egg inside. We often observed an *E. formosa* female kill an egg and, without removing the ovipositor from the previous egg, lay her own egg directly inside the first. This behaviour is reminiscent of autoparasitoid oviposition within conspecific or heterospecific parasitoid larvae or pupae. Conceivably a sensory mechanism capable of these functions and associated with the ovipositor could have been co-opted by the ancestral *E. formosa* to search out and kill conspecific eggs inside the host. Thus, the presence of vulnerable eggs, combined with the propensity to seek out and oviposit within wasp tissue within the primary host, may have promoted the evolution of ovicide. Rapid selection for such a behaviour might only require a small fitness advantage in a behaviour so closely linked with reproduction.

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