Influence of egg load and host size on host-feeding behaviour of the parasitoid *Aphytis lingnanensis*

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Abstract. 1. Direct behavioural assays were used to investigate the influences of host size and parasitoid egg load on the decision to host feed versus oviposit made by the parasitoid *Aphytis lingnanensis* Compere. Egg load was manipulated without concurrent influences on the history of host contact by exploiting size-related variation in fecundity and by holding parasitoids at different temperatures to vary the rate of oocyte maturation.

2. Host feeding comprised a series of feeding bouts, separated by renewed probing of the scale insect body. Successive feeding bouts were progressively shorter, suggesting that hosts represent 'patches' yielding resources at a decelerating rate.

3. Parasitoids were significantly more likely to host feed on smaller hosts and oviposit on larger hosts.

4. Neither egg load nor the treatment variables (parasitoid size and holding temperature) exerted significant influences on the decision to host feed versus oviposit on second instar (low quality) hosts.

5. The failure to observe an effect of egg load on host-feeding decisions was not simply a reflection of the parasitoids being entirely insensitive to egg load; significant effects of egg load on parasitoid search intensity and clutch size decisions were observed.

6. Parasitoids developing on second instar (low quality) hosts experienced high levels of mortality late during development and yielded very small adults.

7. The discord between these experimental results and predictions regarding the importance of egg load underscores the need for additional work on the proximate basis for host-feeding decisions and the nutritional ecology of insect parasitoids.

Key words. Hymenoptera, Aphelinidae, *Aphytis lingnanensis*, host feeding, egg load, oviposition, clutch size, parasitoid.

Introduction

The oviposition behaviour of insect parasitoids has been intensively studied for its role in the population dynamics of host–parasitoid systems and because of the utility of parasitoids as models for investigating the evolution of animal life history strategies. Attention has focused primarily on the issues of host acceptance for oviposition, clutch size decisions, and sex allocation (van Alphen & Vet, 1986; Waage, 1986; Mangel, 1987; Antolin, 1992).

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However, as highlighted by the seminal paper by Jervis & Kidd (1986), many parasitoids are also regularly faced with a qualitatively different decision: whether to use an encountered host for oviposition or as a source of nutrition for the foraging adult parasitoid, i.e. 'host feeding'.

Although host feeding contributes substantially to the overall mortality imposed by parasitoids on host populations and has been documented for a large number of insect parasitoids (Jervis & Kidd, 1986; Kidd & Jervis, 1989), little is known about the factors that influence host-feeding decisions. Host feeding is, however, clearly linked to the need to secure nutrients for the continued production of oocytes; adult parasitoids that emerge

with their full complement of oocytes already matured (proovigenic species) do not exhibit host feeding, while many (but not all) adult parasitoids that continue to mature oocytes (synovigenic species) do host feed (Jervis & Kidd, 1986). Furthermore, comparative evidence suggests that species producing nutrient-rich, fully-yolked eggs ('anhydropic eggs') often host feed, while species producing nutrient-poor, incompletely-yolked eggs that must absorb nutrients from the host's haemolymph to complete embryogenesis ('hydropic eggs') generally do not host feed (Dowell, 1978; Jervis & Kidd, 1986). Although there are many exceptions to these generalizations, in the form of synovigenic species with both hydropic and anhydropic eggs that do not host feed (Dowell, 1978; Donaldson & Walter, 1988), the role of host feeding in securing nutrients for egg production is clear (Leius, 1961a; Sandlan, 1979). Indeed, some synovigenic parasitoids are completely unable to mature oocytes without first host feeding ('anautogenous' parasitoids; Leius, 1961b; Jervis & Kidd, 1986; van Lenteren *et al.*, 1987; Wardle & Borden, 1990). This link between host feeding and oocyte maturation suggests a hitherto untested hypothesis, that the decision to feed on a host rather than oviposit may be linked to the number of mature oocytes present in a parasitoid's ovaries (henceforth 'egg load').

The potentially central role of egg load in modulating insect oviposition behaviour has been emphasized by a recent class of behavioural models that employs dynamic state variables to describe the changing state of an insect over time (Iwasa *et al.*, 1984; Mangel, 1987, 1989). These models predict that parasitoids with larger egg loads should express broader host ranges by accepting lower quality hosts for oviposition and should deposit larger clutches than insects with smaller egg loads. Although tests of these hypotheses have been hindered by the difficulty of experimentally manipulating egg load without concurrent influences on other potentially important variables, such as experience with host contact (Rosenheim, 1992), empirical support for these key hypotheses is mounting (Minkenberg *et al.*, 1992).

Chan (1991) has recently developed dynamic state variable models to analyse parasitoid decisions to oviposit versus host feed on hosts of different qualities. Her most detailed class of models was constructed with the following fundamental assumptions: (i) host feeding provides nutrients only for the maturation of oocytes, and does not contribute to parasitoid maintenance, which is supported by non-host foods; (ii) multiple host types exist that vary in their ability to support the development of parasitoid offspring; and (iii) a time-lag occurs in the use of nutrients obtained through host feeding to mature additional oocytes. These models yield a clear prediction that parasitoids with smaller egg loads will host feed rather than oviposit on lower quality hosts, whereas parasitoids with larger egg loads will oviposit.

Here we test this prediction with direct behavioural assays of *Aphytis lingnanensis* Compere (Hymenoptera: Aphelinidae), a gregarious ectoparasitoid of armoured scale insects (Homoptera: Diaspididae). The assumptions

of Chan's (1991) aforementioned models appear to be appropriate for this species. First, laboratory experiments have shown that *A.lingnanensis* and a closely related species, *Aphytis melinus* DeBach, rely on carbohydrate-rich non-host foods for maintenance, and cannot survive by host feeding alone (DeBach & White, 1960; G. E. Heimpel, personal communication). Second, hosts of various sizes may be attacked, and, as shown by several workers (Abdelrahman, 1974; Opp & Luck, 1986; Hare & Luck, 1991) and confirmed in the current study, smaller hosts are less suitable for parasitoid development. Third, the assumption of a time lag in the maturation of oocytes is probably valid for all host-feeding parasitoids, and is suported for *A.lingnanensis* by the observation of developing oocytes of a range of sizes in the ovarioles of reproductively active parasitoids (J. A. Rosenheim, personal observation). This parasitoid was selected in part because previous work has demonstrated a strong effect of egg load on other aspects of behaviour, including search intensity, clutch size, and host handling time (Rosenheim & Rosen, 1991). In addition, host feeding by *Aphytis* species has been reported to be 'non-concurrent', i.e. parasitoids either host feed or oviposit on a host, but generally not both (Fisher, 1952; Abdelrahman, 1974; Gulmahamad & DeBach, 1978; Reeve, 1987; Walde *et al.*, 1989; Yu *et al.*, 1990); thus, the choices available to *Aphytis* are simpler than those available to other parasitoids that may oviposit and feed to varying extents on a single host (e.g. Sandlan, 1979).

We employ an experimental protocol that allows us to manipulate egg load without concurrent effects on the history of host contact ('experience'). This is important, because experimental designs based upon changes in parasitoid behaviour associated with (i) periods of host deprivation (e.g. Flanders, 1953; Sandlan, 1979; Bai & Mackauer, 1990) or (ii) sequential host contacts (e.g. Collins *et al.*, 1981; Reeve, 1987) cannot generally isolate the effect of egg load from the effect of experience. Experience may influence parasitoid oviposition behaviour by changing the parasitoid's perception of host availability, which in turn may alter the parasitoid's perceived likelihood of becoming host-limited versus egg-limited (Rosenheim & Rosen, 1991). Our behavioural assays also quantify the importance of host size in host-feeding decisions.

Materials and Methods

Biology and insect cultures. *Aphytis* spp. are gregarious ectoparasitoids of armoured scale insects (Homoptera: Diaspididae), a group of sessile sucking insects that secrete wax filaments to form a protective covering, the scale cover, into which the moulted exuviae are also incorporated (Rosen & DeBach, 1979). *Aphytis* use the ovipositor to drill through the scale cover, puncture and probe the scale insect body, and may then either deposit one or more eggs or feed on host haemolymph. Haemolymph is conducted from the punctured scale insect body to the

surface of the scale cover through a feeding tube, which is produced by viscous secretions exuded from the tip of the ovipositor and moulded into 'a straw by rhythmic movements of the ovipositor (Fisher, 1952).

All assays were performed during 1990 with a uniparental population of *A.lingnanensis* that had been maintained in the laboratory since October 1987, when it was founded from wasps emerging from *Aonidiella* sp. hosts growing on mandarin, *Citrus* sp., in the Philippines. Use of a uniparental strain simplified the interpretation of the assays by decoupling sex allocation decisions from other oviposition decisions. Parasitoids were reared at $24 \pm 1°C$ and a photoperiod of 16:8 L:D on a biparental population of the oleander scale, *Aspidiotus nerii* Bouché, grown on butternut squash, *Cucurbita moschata* (Duchesne). Host scale insects used in behaviour assays were second instar (sexes indistinguishable) and third instar female California red scale, *Aonidiella aurantii* (Maskell), grown on butternut squash. A series of squash were used sequentially during the assays, and squash number was recorded to test for an influence of the squash fruit on parasitoid behaviour. Because third instar scale insects become unsuitable as hosts soon after mating, third instars were maintained as virgins by caging them individually beneath one end of a gelatin capsule (size 02) affixed to the squash with a ring of adhesive gum ('Blu-Tack', Bostik, Leicester) to prevent male access.

Behaviour assay. To test the influence of egg load on host-feeding decisions, we presented hosts of known size to parasitoids with a uniform history of host encounter but varying egg loads. Variation in egg load was obtained both by exploiting size-related variation in fecundity (Opp & Luck, 1986; Luck, 1990) and by holding parasitoids at different temperatures to modulate the rate of oocyte maturation (Rosenheim & Rosen, 1991). Parasitoid pupae were collected from hosts and held singly in 1.5 ml glass vials provisioned with honey. Abundant size variation was obtained by selecting pupae that had developed on hosts of different sizes and in different clutch sizes. Newly emerged adults (0–12 h old) were collected in the morning, sorted by approximate size, and each size-class then divided into two groups to be held for an additional day at $18 \pm 1°C$ and $26.5 \pm 1°C$, respectively, $70 \pm 5\%$ r.h., a 14.5:9.5 L:D photoperiod, and with access to honey.

Behavioural assays were performed 08.00–17.00 hours on the day following the temperature treatments. Parasitoids were brought to the temperature at which the assays were performed $(26.5 \pm 1°C)$ at least 30 min before testing. Within each day we attempted to alternate tests of parasitoids from the two temperature treatments and to include parasitoids from a broad range of sizes. Each assay consisted of two stages; in the first stage the parasitoid was confined with a single second instar (low quality) host to provide a choice between host feeding and oviposition; in the second stage the parasitoid was confined with a single third instar (high quality) host to obtain oviposition. Only those replicates in which parasitoids oviposited on the second host (96/98 replicates) were included in the analysis; in this way we precluded the possibility that

parasitoid decisions on the first host were constrained by the availability of oocytes to deposit.

In the first stage of the assay, parasitoids were transferred into the small end of a gelatin capsule (size 02; diameter 6.5 mm, height 9 mm), which was affixed to the squash with adhesive gum to enclose one second instar scale insect. The squash was positioned beneath a stereoscope, illuminated with fibre optic lighting, and parasitoid behaviour was observed continuously under $10\times$ magnification. The following observations were recorded, with all times measured to the nearest second. (1) The time required to 'discover' the host scale insect, as evidenced by parasitoid arrestment and the initiation of host investigation with the antennae; we used this time as a measure of parasitoid search intensity. In 95/98 replicates, the second instar host scale insect was discovered upon the first contact with the host. Trials were terminated after 30 min if the host had not yet been discovered. (2) The parasitoid's decision to either (i) reject the host, (ii) host feed only, (iii) oviposit only, or (iv) host feed and oviposit. Previous studies (Rosenheim & Rosen, 1991) had demonstrated that egg deposition was reliably signalled by two series of rhythmic pumping movements. Host feeding was signalled by the parasitoid placing its mouthparts over the point where the ovipositor had pierced the scale insect cover. (3) In replicates where host feeding occurred, (i) the times of initiation and termination of all periods during which host haemolymph was actively imbibed, and (ii) the times when attempts to feed were unsuccessful, due either to the failure to locate the puncture location and/or the inability to drink there. (4) The time when the host was abandoned to reinitiate searching activity; the period between this time and the time of host discovery defined 'total host handling time'.

To begin the second stage of the assay, parasitoids were immediately transferred to a third instar host upon quitting the first host. To execute the transfer, we lifted one edge of the gelatin capsule, touched the parasitoid gently with a fine brush, thereby causing the parasitoid to jump off the squash surface and onto the gelatin capsule, and then quickly repositioned the capsule over the second host on a separate squash fruit. The same observations were then repeated. Parasitoids were given 60 min to discover the second host, and the number of eggs deposited on the host was recorded.

Immediately following the assay two perpendicular diameters of the ellipsoid scale covers of both scale insect hosts, including the longest diameter if one was apparent, were measured with an ocular micrometer to the nearest 0.02 mm. Scale cover areas were estimated as $(\pi/4) \times$ (diameter 1) $\times$ (diameter 2), the area of an ellipse. Parasitoids were frozen immediately following the assays and dissected later the same day. The ovaries were dissected from the abdomen in saline by grasping the tip of the abdomen with forceps and pulling distally while holding the thorax with a fine probe. Full-sized, mature-form oocytes (i.e. smooth shaped elongate-oval eggs with dark cytoplasm, without associated trophocytes, and located at the bases of the ovarioles) were then counted. To produce

an index of parasitoid size, parasitoids were slide mounted and the lengths of both hind tibiae measured with an ocular micrometer to the nearest 0.0015 mm and averaged. Parasitoid egg load at any time during the assay was calculated as the sum of eggs deposited from that time until the end of the assay and the number of oocytes counted during the dissections. We performed a total of 111 replicates.

Parasitoid development on second instar hosts. To estimate the quality of second instar scale insects as hosts, we monitored the development of eggs deposited during the behavioural assays. Parasitized hosts were covered with a gelatin capsule as before and held for at least 30 days at 26.5 ± 1°C, 60 ± 10% r.h., and a 14.5:9.5 L:D photoperiod for development to be completed. Hind tibiae of parasitoids reaching the adult stage were measured as before. Developmental mortality was partitioned into two classes: (i) that occurring in the egg or early larval stages, before a substantial portion of the host had been consumed, and (ii) that occurring in the final larval instar, prepupal, pupal, or pre-emergence adult stages, after most or all of the host had been consumed.

Parasitoid size and oocyte size. To interpret the relationship between parasitoid size and egg load, we investigated the relationship between adult parasitoid size and mature oocyte size. Newly emerged adults, reared and collected as described above, were held singly in 1.5 ml glass vials provisioned with honey for 1 day at 24 ± 1°C and a photoperiod of 16:8 L:D. Parasitoids were then frozen and dissected the same day. Mature oocytes were teased from the ovaries, counted, and slide-mounted with an elevated coverslip. Slides were examined with phase-contrast microscopy and oocyte length was measured with an ocular micrometer to the nearest 0.0015 mm. Oocytes damaged during the dissection were excluded. For this analysis we needed an objective definition of a mature oocyte; the dissections suggested that oocytes that were mature by our previous criteria (form, location within the ovarioles; see above) were those that were >80.5% the length of the largest oocyte present.

Results and Discussion

Treatment effects on egg load and oocyte size

Egg load was strongly correlated with parasitoid size for parasitoids in both temperature treatments (Fig. 1). Regressions of egg load on hind tibia length were significantly different for the high and low temperature groups ($F_{2,107} = 95.4$, $P < 0.001$); parasitoids held at 18°C matured 2.1–5.5 fewer oocytes on average than comparably sized parasitoids held at 26.5°C. One factor apparently contributing to the linear rather than potentially exponential relationship between parasitoid hind tibia length and egg load was a positive relationship between parasitoid size and mean mature oocyte length (mean mature oocyte length = 0.118 + 0.394 (hind tibia length); both lengths expressed in mm, $r^2 = 0.64$, $N = 21$, $P < 0.001$).
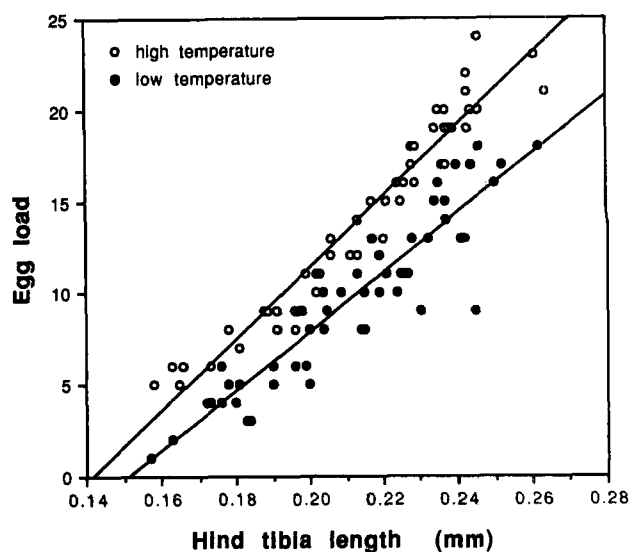

Fig. 1. Influences of parasitoid size and the holding temperature treatment on egg load in *A.lingnanensis*. Linear regression for the high temperature group ($n = 53$): egg load = 194.4(hind tibia length) − 27.6; $r^2 = 0.94$, $P < 0.001$; for the low temperature group ($n = 58$): egg load = 161.0(hind tibia length) − 24.4; $r^2 = 0.85$, $P < 0.001$.

Host-feeding behaviour

Host feeding on the second instar scale insect was observed in 44/96 complete replicates. Preparation for host feeding was as described for other *Aphytis* spp. (Fisher, 1952; Abdelrahman, 1974; Gulmahamad & DeBach, 1978) and involved examination of the scale cover with the antennae while walking across the scale, drilling through the scale cover, and extensive probing of the scale insect body with the ovipositor. A train of rhythmic body movements immediately preceded the withdrawal of the ovipositor from under the scale cover and the parasitoid's search for the haemolymph flow with the antennae and mouthparts. These body movements apparently correspond to the rhythmic movements of the ovipositor reported by Fisher (1952) to be associated with the production of the feeding tube.

Unlike host-feeding behaviour previously described for *Aphytis* spp., host feeding by *A.lingnanensis* frequently comprised a series of feeding bouts, separated by 'interbouts' comprising the renewed probing of the scale insect body with the ovipositor at the original drilling site and an additional train of rhythmic body movements. Parasitoids feeding on second instar hosts were never observed to feed from more than a single location. Parasitoids made an average of 2.89 ± 1.63 (SD) successful feeding bouts per host, and a significant trend for successive feeding bouts to be progressively shorter was observed (Fig. 2; Spearman's rank correlation: $r_s = -0.49$, d.f. = 125, $P < 0.001$). Preparatory behaviour was not universally successful in generating a flow of haemolymph; parasitoids made 0.89 ± 0.99 (SD) unsuccessful feeding bouts
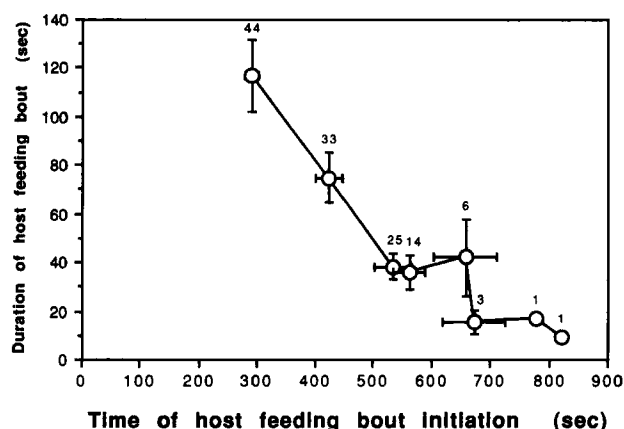
Fig. 2. Timing and duration of successive host-feeding bouts on second instar hosts by *A.lingnanensis*. Shown are the mean (±SE) time that the first through eighth feeding bouts were initiated (time 0 = the time of host discovery) and their mean (±SE) duration. Numbers above SE bars are sample sizes.

per host. Interbout times, including time spent on unsuccessful feeding attempts, did not show a significant trend towards progressive shortening (Fig. 3; $r_s = -0.13$, d.f. = 81, $P > 0.05$). The total host handling time for parasitoids host feeding on second instar scale insects averaged 10.38 ± 2.81 (SD) min ($n = 43$).

We interpret the overall temporal pattern of feeding as indicating that parasitoids encountered increasing difficulty in producing sustained haemolymph flows as the turgor of host scale insects decreased with progressive feeding; thus, hosts can be interpreted as 'patches' yielding resources at a decelerating rate. Two lines of evidence argue against an alternative interpretation of the data, namely that successive feeding bouts were shortening because of parasitoid satiation. First, a reanalysis of bout duration data after excluding the final feeding bout of each replicate again revealed a trend towards progressive bout
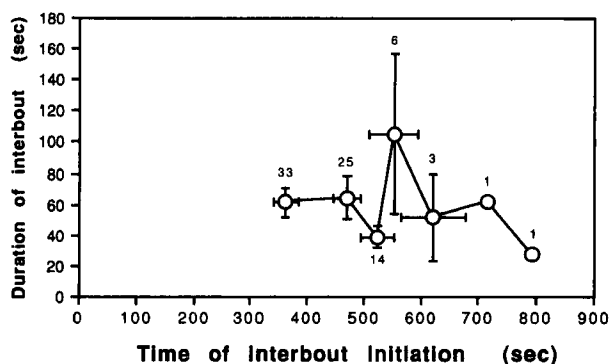

Fig. 3. Timing and duration of successive interbouts for *A.lingnanensis* feeding on second instar hosts. Shown are the mean (±SE) time that the first through seventh interbouts were initiated (time 0 = the time of host discovery) and their mean (±SE) duration. Numbers above or below SE bars are sample sizes.

abbreviation ($r_s = -0.39$, d.f. = 81, $P < 0.01$); the fact that these bouts were followed by additional feeding suggests that they were terminated for reasons other than satiation. Second, observations of host feeding on third instar host scale insects made during this experiment and previous experiments (J. A. Rosenheim and D. Rosen, unpublished data) demonstrated that parasitoids made a larger number of feeding bouts on these much larger hosts (mean number of bouts = 8.3 ± 5.4, $n = 8$).

Decisions to host feed versus oviposit on second instar hosts

Parasitoids ($n = 96$) discovering the second instar host showed four classes of responses: deposit a single egg ($n = 48$), host feed ($n = 53$), reject the host without host feeding ($n = 3$), or deposit a single egg and host feed ($n = 2$). Stepwise logistic regression was used to identify variables exerting significant influences on the decision to host feed versus oviposit; replicates in which parasitoids rejected the host or host fed and oviposited were excluded. The analysis revealed that only host size and squash number exerted significant effects (Table 1). Parasitoids were significantly more likely to host feed on smaller hosts (scale cover area $\leq 0.40\,\text{mm}^2$) and oviposit on large hosts (scale cover area $> 0.40\,\text{mm}^2$; Fig. 4). Because the squash number variable refers to the six squash that were used sequentially during the assays as substrates for the second instar hosts, the significant effect of squash number indicates either that the squash differed in some way that influenced parasitoid behaviour, or that some other unmeasured

Table 1. Stepwise logistic regression of factors influencing the decision to host feed versus oviposit on second instar host scale insects by *A.lingnanensis*.

Step no.	Variable entered	Coefficient (±SE)[a]	d.f.	Improvement χ^2	P
1	Scale cover area	-28.4 ± 7.8	1	26.4	<0.001
2	Squash number	$-$[b]	5	21.1	0.001

Variables not entered	d.f.	Approximate F to enter	P
Temperature	1	0.49	0.48
Hind tibia length	1	0.57	0.45
Egg load	1	1.51	0.22

C. C. Brown goodness of fit $\chi^2 = 0.27$, d.f. = 2, $P = 0.88$, indicating that the logistic model is adequate for these data.

[a] Coefficients (b_i) of the logistic equation; predicted probability of parasitoids ovipositing, $P = e^u/(1 + e^u)$, where $u = b_0 + b_1 x_1 + b_2 x_2 + \ldots + b_n x_n$, and x_i is the independent variable. Scale cover area coded in mm^2.

[b] The analysis produces a separate coefficient for each of the five design variables generated for the categorical variable of squash number.
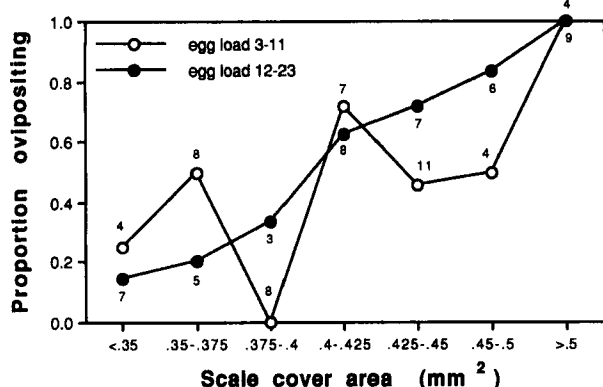
Fig. 4. Influences of host size and parasitoid egg load on the decision to host feed versus oviposit on second instar hosts by *A. lingnanensis*. Shown are the proportion of parasitoids that chose to oviposit; 1.0 − this proportion = the proportion that host fed. Numbers above (low egg load group) or below (high egg load group) symbols are sample sizes. Host size classes: '.35−.375' indicates $0.35 <$ host size ≤ 0.375.

variable changed over the course of the experiment and influenced the parasitoids.

Neither egg load nor the treatment variables (hind tibia length and holding temperature) exerted significant influences on the decision to host feed versus oviposit (Table 1). Thus, our 'egg load hypothesis' (i.e. that parasitoids with smaller egg loads would choose to host feed rather than oviposit on low quality hosts) was not supported. For the egg load hypothesis to have been supported, the curve for parasitoids with smaller egg loads in Fig. 4 should have been shifted to the right of the curve for parasitoids with larger egg loads; such a shift was not apparent. The larger fluctuations in the proportion ovipositing for parasitoids with smaller egg loads (Fig. 4) is apparently just a reflection of small sample sizes; regardless of egg load, in encounters with all but the very largest hosts (scale cover area $>0.50 \, mm^2$) some parasitoids chose to host feed and others to oviposit.

Influences of egg load on other aspects of behaviour

The result that egg load was not influencing the decision to host feed versus oviposit on second instar hosts leads to the question of whether egg load was influencing any aspect of the behaviour of parasitoids studied in these assays. Two analyses suggest that it was.

First, parasitoids with larger egg loads exhibited higher search intensities. Parasitoids that successfully located the second instar host during the 30 min assay period had significantly larger egg loads (mean $= 12.22 \pm 5.16$ (SD), $n = 99$) than parasitoids that failed to locate hosts (mean $= 7.17 \pm 5.06$ (SD), $n = 12$); a stepwise logistic regression of factors influencing host discovery identified egg load as the sole significant variable (improvement $\chi^2 = 11.2$, $P = 0.001$), with temperature, parasitoid hind

tibia length, scale cover area, and squash number all non-significant. The time required to locate hosts was, however, not found to be significantly influenced by egg load for parasitoids searching for either the second or third instar hosts (stepwise multiple regressions, all variables n.s. in both analyses).

Second, clutch sizes on the third instar host were significantly influenced by egg load (Table 2). Parasitoids with smaller egg loads deposited clutches of predominantly two eggs, whereas parasitoids with larger egg loads deposited clutches of predominantly three eggs (Fig. 5). Host size was again found to be a critical factor influencing behaviour (Table 2); larger hosts elicited the oviposition of larger clutches (Fig. 6). Clutch size decisions were not significantly influenced by either the treatment variables (hind tibia length and holding temperature) or the outcome of the encounter with the second instar host (i.e., oviposit, host feed, reject, or oviposit plus host feed).

Finally, in contrast to the results of a previous study (Rosenheim & Rosen, 1991), stepwise multiple regression analyses failed to identify any consistent influences on total host handling time for the two hosts encountered. For the second instar host, none of the variables tested (hind tibia length, holding temperature, egg load, scale cover area, squash number) had a significant influence on the time required to complete host feeding, whereas only the temperature treatment influenced the time required to oviposit ($r = -0.53$, d.f. $= 46$, $P < 0.001$); parasitoids held at the lower temperature required longer to handle hosts.

Table 2. Stepwise polychotomous logistic regression of factors influencing clutch size decisions on third instar hosts by *A. lingnanensis*[a].

Step no.	Variable entered	Coefficient $(\pm SE)$[b]	d.f.	Improvement χ^2	P
1	Scale cover area	3.75 ± 0.94	1	29.4	<0.001
2	Egg load	0.29 ± 0.07	1	22.6	<0.001
3	Squash number	$-$[c]	3	16.1	0.001

Variables not entered	d.f.	Approximate χ^2 to enter	P
Temperature	1	0.91	0.34
Hind tibia length	1	0.04	0.85
Previous decision on first host	3	3.26	0.35

[a] To minimize the possibility that clutch size decisions were constrained by the availability of oocytes to deposit, parasitoids with <4 eggs (the largest clutch size observed) were excluded from the analysis.

[b] Coefficients (b_i) of the logistic equation; probability of clutches greater than size i, $P(\text{clutch} >i) = e^u/(1 + e^u)$, where $u = b_0 + b_1x_1 + b_2x_2 + \ldots + b_nx_n$, and x_i is the independent variable. Scale cover area coded in mm^2.

[c] The analysis produces a separate coefficient for each of the three design variables generated for the categorical variable of squash number.
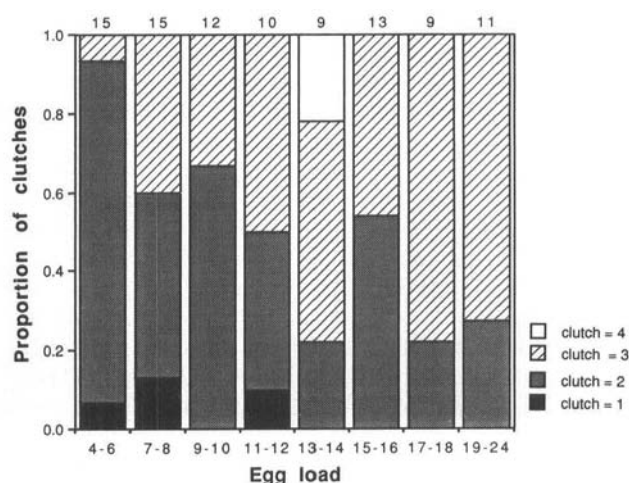
Fig. 5. Influence of egg load on clutch size decisions by *A.lingnanensis* ovipositing on third instar hosts. Numbers above columns are sample sizes.

Total host handling time for parasitoids that oviposited on the second instar host averaged 2.76 ± 1.10 (SD) min ($n = 48$), only 26% of the time required for host feeding. For the third instar host, only clutch size (partial correlation coefficient, $r = 0.59$, d.f. $= 87$, $P < 0.001$) and parasitoid hind tibia length (partial correlation coefficient, $r = -0.55$, d.f. $= 86$, $P < 0.001$) had significant influences on total handling time; smaller parasitoids and parasitoids depositing larger clutches required longer to handle hosts.

Parasitoid development on second instar hosts

Our experimental design rests upon the assumption that second instar scale insects represent low quality hosts.
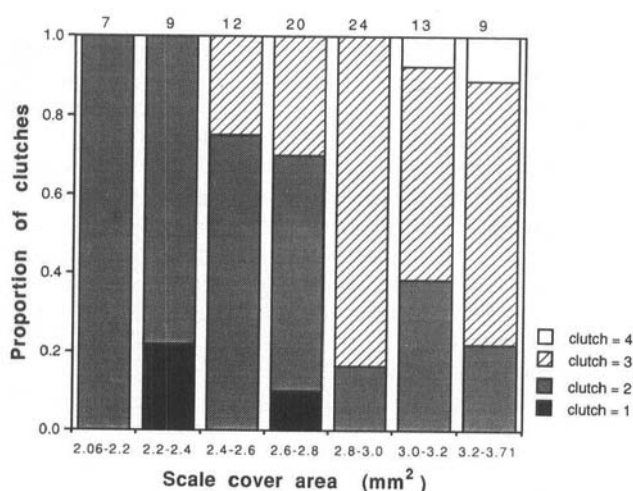

Fig. 6. Influence of host size on clutch size decisions by *A.lingnanensis* ovipositing on third instar hosts. Numbers above columns are sample sizes. Host size classes: '2.2–2.4' indicates $2.2 <$ host size ≤ 2.4.

This assumption is supported by two lines of evidence: first, high parasitoid mortality occurring late in development and second, the small size of the parasitoid offspring that developed successfully to adult emergence.

Of fifty eggs deposited singly on second instar hosts without concurrent host feeding, only seventeen (34%) emerged successfully; twelve (24%) died in the egg or early instar stages, and perhaps most importantly twenty-one (42%) died during the late larval, pupal, or adult (pre-emergence) stages. Stepwise logistic regression demonstrated that the probability of developing and emerging successfully was independent of host size and squash number ($P > 0.5$ for both variables). Moderate to high mortality (23.8–63.3% across different squash) in the egg or early instar stages had previously been found to be characteristic of this population of *A.lingnanensis* developing on large third instar hosts, apparently reflecting in part differences between squash fruits used to grow the scale insect hosts (Rosenheim & Rosen, 1991). Mortality during the late larval, pupal, or adult (pre-emergence) stages had, however, been consistently low on third instar hosts (0–9.5% across different squash, overall mean $= 3.9\%$; Rosenheim & Rosen, 1991). The high mortality (42%) during these late developmental stages for parasitoids developing on second instar hosts was apparently a result of small host size or some qualitative difference between second and third instar hosts. The more than ten-fold increase in mortality during late developmental stages was statistically significant ($G = 45.2$, $P < 0.001$).

Parasitoids developing successfully to emergence on second instar hosts were extremely small (mean hind tibia length $= 0.150 \pm 0.014$ (SD) mm). These adults were significantly smaller ($P < 0.001$ for all contrasts) than adults developing in clutches of one, two or three on large third instar hosts (mean hind tibia length $= 0.221 \pm 0.019$ (SD), 0.202 ± 0.016 (SD), and 0.175 ± 0.024 (SD) mm, respectively; Rosenheim & Rosen, 1991). Regression analysis suggested that adult offspring hind tibia length was independent of second instar host cover area ($r = 0.22$, d.f. $= 21$, $P > 0.05$). The relationship between adult size and fecundity consistently observed for *Aphytis* spp. (Fig. 1; Opp & Luck, 1986; Luck, 1990; Rosenheim & Rosen, 1991) suggests that these extremely small females would have only limited abilities to produce eggs.

General Discussion

We tested the prediction (Chan, 1991) that host quality and parasitoid egg load should both influence a parasitoid's decision to host feed or oviposit on an encountered host. Our experimental results support only an effect of host quality: parasitoids predominantly host fed on the smaller hosts and predominantly oviposited on the larger hosts (Fig. 4). This result mirrors the conclusion of Walde *et al.* (1989), who showed using group assays of *A.melinus* that parasitoids preferred to host feed on smaller third instar California red scale and oviposit on larger third instars. Other parasitoids that display non-concurrent host-feeding

(i.e. hosts are either fed upon or oviposited on, but not both) have also been shown to respond to host size (van Alphen et al., 1976; Sandlan, 1979; Löhr et al., 1988; Heinz & Parrella, 1989; Barrett & Brunner, 1990; Kidd & Jervis, 1991); in all cases smaller hosts, which are less valuable as resources for supporting the development of parasitoid progeny, were more likely to be host fed upon. This pattern is consistent with behaviour that maximizes reproductive success given competing demands for oviposition and adult parasitoid nutrition (Chan, 1991).

Parasitoids did not, however, alter their choice between host feeding and oviposition in response to variation in egg load (Table 1, Fig. 4). This negative result was not simply a reflection of the parasitoids being entirely insensitive to egg load; before encountering the first host, parasitoid search intensity was dependent on egg load, and after encountering the first host, parasitoid clutch size decisions were dependent on egg load (Table 2, Fig. 5). Thus, parasitoids appeared to be capable of modulating behaviour in response to variation in egg load, but did not do so when making host-feeding decisions.

Why might the behaviour of A.lingnanensis fail to conform to the predictions of Chan's (1991) models? One possibility is that the assumptions upon which the models were built are not appropriate for this parasitoid. In addition to the assumptions discussed in the introduction (i.e. host feeding functions only to mature oocytes; hosts vary in quality; egg maturation requires time), at least one additional assumption is critical in supporting the predicted influence of egg load on the host feed versus oviposit decision, and warrants discussion here. This is the assumption that current egg load does not influence the value of host feeding as a means of maturing additional oocytes; Chan (1991) assumes that host feeding allows some constant number of additional oocytes to be matured for parasitoids of any current egg load, subject to a ceiling set by the maximum egg storage capacity of the parasitoid. Is there some scenario under which the violation of this assumption could negate the prediction that parasitoids with larger egg loads should show a greater likelihood of ovipositing rather than host feeding on low quality hosts? One such scenario would occur if host feeding makes a larger contribution to oocyte maturation for individuals with larger egg loads than individuals with smaller egg loads. Under this scenario parasitoids with larger egg lods might view hosts both as more valuable for oviposition and for host feeding; if these effects were of approximately equal magnitudes, the result might be no effect of egg load on host feeding versus oviposition decisions (as we have seen here).

Although direct tests of the value of host feeding to parasitoids with different egg loads have not been performed for any system, we feel that the results of our study suggest that A.lingnanensis with large egg loads will not have more to gain by host feeding, and that instead just the reverse may be true. To arrive at this conclusion, we first assume that current egg load (or, for our experiment, the treatment variables of parasitoid size and holding temperature) has no effect on the amount of material extracted from a host during feeding. This assumption is supported by two lines of evidence. First, the data in Figs 2 and 3 and the comparison of host feeding on second instar hosts and third instar hosts suggest that parasitoids were not able to feed to satiation, and that therefore the amount of material ingested probably reflected characteristics of the host scale insect rather than the attacking parasitoid. Second, although we did not quantify the amount of material ingested during feeding, we did measure the total time spent actively drinking haemolymph; this total feeding time was independent of parasitoid egg load ($r = -0.25$, $n = 43$, $P > 0.05$), independent of parasitoid hind tibia length ($r = -0.27$, $n = 43$, $P > 0.05$), and did not differ significantly for parasitoids held at 18°C (mean = 217 ± 73 (SD) s, $n = 23$) or 26.5°C (mean = 207 ± 103 (SD) s, $n = 20$; $t = 0.37$, $P > 0.05$). These observations support the supposition that host feeding on second instar hosts enables A.lingnanensis to mature some number of additional oocytes that is constant across individuals with varying egg loads. The observation that larger parasitoids produce larger oocytes (see also O'Neill & Skinner, 1990) suggests in fact that these larger individuals might require more exogenous nutrients to mature a fixed number of oocytes, but ignoring this possible effect makes our argument more conservative.

We are left, then, with the paradox of what appears to be a theoretical prediction for an egg load effect (Chan, 1991) that is not supported empirically, despite the fact that egg load influences other aspects of A.lingnanensis behaviour. The predicted egg load effect appears to stem from two basic processes. First, parasitoids with low egg loads are more likely to have their lifetime reproductive success constrained by egg availability, and are therefore selected to oviposit only on high quality hosts to maximize the fitness gained per egg. Second, we suggest that parasitoids with a larger egg load will expect to benefit less from the opportunity to mature additional oocytes through host feeding than parasitoids with a smaller egg load, and support this conclusion with two arguments. First, parasitoids with larger egg loads may eventually reach the limit of their oocyte storage capacity. Second, and more importantly, the value of producing additional oocytes depends critically upon the parasitoid having an opportunity to deposit them on suitable hosts. This probability will in general be <1.0, and will decrease as current egg load increases, because an increasingly large number of hosts must be encountered over the parasitoid's lifetime to exhaust the supply of already-matured oocytes. In short, additional oocytes are not very valuable to a parasitoid that has little chance of finding enough hosts to deposit the oocytes that it is already carrying.

We feel that the observed discord between theory and our experimental results highlights the dearth of empirical and theoretical studies of host-feeding behaviour. The lack of concordance could reflect either incorrect assumptions in our theoretical arguments or unknown behavioural constraints acting on A.lingnanensis. Additional work both with A.lingnanensis and other parasitoids is clearly needed to elucidate the proximate control of host-feeding

decisions as well as the nutritional ecology of host feeding and oocyte maturation.

Acknowledgments

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