Eur. J. Entomol. **97**: 347–353, 2000
ISSN 1210-5759

Host-instar selection in the aphid parasitoid *Monoctonus paulensis* (Hymenoptera: Braconidae, Aphidiinae): a preference for small pea aphids

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Key words. *Acyrthosiphon pisum*, Aphidiinae, aphids, Braconidae, host choice, host size, *Monoctonus paulensis*, parasitoid, pea aphid, specificity

Abstract. *Monoctonus paulensis* is a solitary parasitoid of several species of aphids, including the pea aphid, *Acyrthosiphon pisum*. We evaluated host-instar selection by comparing the parasitoid's preference for the four nymphal instars of the pea aphid, presented two at a time in dichotomous choice tests. Females parasitized more, and laid more eggs in, the relatively smaller aphids among those available. This preference was independent of aphid instar: L1 > L2 > L3 > L4. Preference was not influenced by female size or age. Normal and anaesthetized aphids were accepted equally. The total time needed by a female to capture, position, and parasitize an aphid varied among host instars, with fourth instars requiring nearly twice as much time as first, second, and third instars. The probability of an attacked aphid escaping or avoiding parasitism increased with aphid instar, from ~10% in first and second instars to ~50% in fourth instars. Although fourth-instar pea aphids contain more resources for offspring development than smaller counterparts, it may not be profitable for a female to invest opportunity time in attacks on large aphids.

INTRODUCTION

Most species of aphidiine parasitoids (Hymenoptera: Braconidae, Aphidiinae) attack and successfully develop in all four nymphal instars of their aphid hosts (Mackauer, 1973; Liu et al., 1984; Sequeira & Mackauer, 1987; Weisser, 1994). Although second and third instars are often preferred, aphid embryos and adult aphids may also be suitable hosts (Mackauer & Kambhampati, 1988; Völkl & Mackauer, 1996). Patterns of preference are not fixed, however, but often vary with the availability and relative abundance of different host types (Chow & Mackauer, 1991) and the female's physiological state, including her egg load, previous reproductive success, and age (Weisser, 1994; Michaud & Mackauer, 1995; Mackauer et al., 1996). Older instars and adult aphids are larger than first and second instars and, potentially, contain more nutritional resources; however, these resources may not always be accessible by the parasitoid (Sequeira & Mackauer, 1992). Moreover, large aphids can better defend themselves against attacks and therefore may be more costly to the female than small aphids (Gerling et al., 1990; Chau & Mackauer, 1997; Losey & Denno, 1998). Direct costs to the female include the risk of injury due to host defenses and lost opportunity time if an attack fails. Ecological theory predicts that a female's reproductive success reaches a maximum if host choice is based on the optimal balance between her costs in finding and capturing a host and the latter's quality for the development and growth of her offspring (reviewed in Godfray, 1994).

Monoctonus paulensis (Ashmead) (Hymenoptera: Braconidae, Aphidiinae) is a solitary parasitoid of several species of aphids, including the pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphidoidea: Aphididae) (Calvert & van den Bosch, 1972a; Calvert, 1973). Oviposition behaviour differs in several aspects from that of most other Aphidiinae. Females use the fore legs to hold and position an aphid for oviposition. The host is envenomed, which results in a transient paralysis. Eggs are deposited in the aphid's fused thoracic ganglia between the first and second pair of legs (Calvert & van den Bosch, 1972b). Although only one larva survives in each host, females may deposit several eggs during a single oviposition bout, which can last several minutes (Chau & Mackauer, 1999). Anecdotal observations by Calvert & van den Bosch (1972b) and Calvert (1973) suggest that *M. paulensis* selectively parasitizes small first and second nymphal instars, as was also observed by Griffiths (1960) in *Monoctonus crepidis* (Haliday). Chau & Mackauer (1997) provided a possible explanation of this apparent preference for small rather than large hosts. They showed that parasitoid offspring were most likely to survive in first instars of the pea aphid, which had the lowest mortality risk due to dropping from the feeding site after parasitism as compared with other instars. However, Chau & Mackauer (1997) did not examine host choice in *M. paulensis* and determine if observed preferences could be accounted for by attributes of the female, differences in host quality, or a combination of both.

This study is divided in several parts. First, we describe host selection in females of *M. paulensis* provided with different instars of the pea aphid in dichotomous choice tests. Next, we test several alternative hypotheses that could account for the observed preference pattern. We test if preference for the relatively smaller, as opposed to the relatively larger, pea aphids is dependent on female

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size, female age, or host mobility. We determine if a female's costs in terms of opportunity time differ between aphid instars.

MATERIAL AND METHODS

Insect colonies

Pea aphids were reared on potted broad bean plants, *Vicia faba* L. cv. "Broad Windsor", in growth chambers at 20 ± 1°C, 50–55% R.H., and continuous light. To obtain aphids of the same age and size, we caged apterous virginoparae on bean plants. After 6 h, any offspring produced were transferred to fresh plants and reared as synchronous cohorts until they reached the desired instar for experimentation: first (L1, 24 h old), second (L2, 48 h old), third (L3, 72 h old), and fourth (L4, 120 h old) instar. We estimated aphid body size in terms of dry mass (Table 1). For each cohort, 25 nymphs were killed with CO_2, dried in an oven at 77°C for 3 days, and individually weighed on a Cahn 21 electronic microbalance (sensitivity, 0.001 mg).

The stock colony of *M. paulensis* was reared on early third instars of the pea aphid under the same conditions as above. After parasitized aphids had mummified, they were removed from the plants and placed in waxed paper cups. Eclosed males and females were left in the cups so that they could mate; however, we did not verify if experimental females had, in fact, mated. Mating can influence a female's offspring allocation to host patches (Michaud & Mackauer, 1995) but should not influence host specificity. Parasitoids were provided with a solution of equal parts of honey and water as food but did not have access to aphids. Females used in the experiments were 48 h old and similar in size, excepting those used in Experiments 2 and 3 (see below).

Host-instar preference

Using dichotomous choice tests, we tested the hypothesis that females of *M. paulensis* do not distinguish between the four instars of the pea aphid (Experiment 1). The four instars were compared two at a time, for a total of six sets of replicated choice tests: L1 vs L2 (n = 15), L1 vs L3 (n = 15), L1 vs L4 (n = 14), L2 vs L3 (n = 13), L2 vs L4 (n = 12), and L3 vs L4 (n = 15). A convenient index of preference is the ratio of the proportions of available and accepted host types, or the ratio of eggs laid in different host types (Cock, 1978; Mackauer, 1983a). Both indices yield similar results if the number of eggs laid is correlated with the number of hosts parasitized (Mackauer, 1983a). Females of *M. paulensis* often deposit multiple egg clutches during a single ovipositor insertion, however. Because the probability of more than one egg being laid varies with host size (Chau & Mackauer, 1999), egg numbers may give a biassed estimate of preference. We used, therefore, the numbers of aphids parasitized, rather than eggs laid, as a measure of preference.

TABLE 1. Age and dry mass at parasitization of pea aphids, *Acyrthosiphon pisum*, used as hosts of *Monoctonus paulensis* in dichotomous choice tests.

Choice	"Young" host			"Old" host		
	Instar	Age (h)	Dry mass (mg)	Instar	Age (h)	Dry mass (mg)
(1)	L1	22	0.037 ± 0.001	L2	46	0.067 ± 0.003
(2)	L1	22	0.029 ± 0.001	L3	70	0.063 ± 0.004
(3)	L1	23	0.030 ± 0.001	L4	119	0.238 ± 0.008
(4)	L2	45	0.062 ± 0.004	L3	69	0.112 ± 0.007
(5)	L2	47	0.047 ± 0.002	L4	119	0.203 ± 0.005
(6)	L3	71	0.077 ± 0.005	L4	118	0.222 ± 0.013

Abbreviations: L1, L2, L3, L4 – apteriform first, second, third, fourth nymphal instar. Age (range ± 3.75 h) and dry mass (mean ± SE) of hosts at parasitization based on samples of 15 aphids per instar.

If parasitized hosts are not replaced in choice tests, the probability increases that the less preferred host type is accepted as the preferred hosts become parasitized and are rejected by the female to avoid superparasitism; relative preference eventually can no longer be detected (Mackauer, 1983a). In preliminary tests, we determined that discrimination between host types would be maximized if each parasitoid female was provided with 30 aphids, 15 of each instar, and allowed to forage for approximately 1 h. Older instars in each test had the distal end of one antenna amputated as a biomarker (Mackauer, 1972). Females were placed individually in a plastic Petri dish (5.5 cm in diameter × 1 cm high), which was lined with a Whatman #1 filter paper. At the end of a trial, the aphids were transferred to a screened cage containing a fresh bean shoot, as above. After 4 days of rearing, 10 individuals from each instar were dissected, and the parasitoid eggs and first-instar larvae in each aphid were counted. In a few cages, less than 10 aphids survived until they could be dissected, especially among the small first and second instars (see below). Both live and dead parasitoid offspring were counted in superparasitized aphids. Because of the large number of trials involved, different cohorts of aphids and parasitoids were used for the six choice combinations; however, the sequence of trials involving different instar-combinations was arbitrary, with trials interspersed in time.

Influence of female size and age

Experiment 1 showed that females of *M. paulensis* accepted all four pea aphid instars as hosts but preferred the relatively smaller aphids among those available. Because all experimental females were standardized for size and age, we tested in two additional experiments the hypotheses that host choice is determined by female size (Experiment 2) and female age (Experiment 3). If a preference for small hosts simply reflects some physical constraint of the female, such as ovipositor length (Brandl & Vidal, 1987; Gerling et al., 1990), large females, as opposed to small females, should be more successful attacking large hosts, whereas small females should be more (or equally) successful as large females attacking small hosts. We tested this proposition by rearing two cohorts of *M. paulensis*; one cohort was reared on first instars and the other on fourth instars of the pea aphid. Because parasitoid size is a positive function of host size, parasitoids developing in small aphids are significantly smaller on average than counterparts developing in large aphids (Nicol & Mackauer, 1999). Using the same design as for Experiment 1, we provided both small (n = 15) and large females (n = 15) with a choice between 15 second and 15 fourth instars in a Petri dish; females were allowed to forage for 70 min. At the end of a trial, the female was killed with CO_2, oven-dried, and her dry mass determined. The aphids were reared for 4 days on bean plants, separately by replicate. Ten aphids of each instar per replicate were dissected, and the parasitoid eggs and larvae in each aphid were counted.

Old, as opposed to young, females can maximize their lifetime reproductive success by searching host patches more thoroughly and accepting a broader range of host types (Roitberg et al., 1993; Weisser & Houston, 1993; Weisser, 1994; Michaud & Mackauer, 1995). We tested the hypothesis that host choice in *M. paulensis* varies with female age, predicting that older females lacking reproductive success and approaching death would be less selective than younger females. Two cohorts of females differing in age were given a choice between 15 first-and 15 third-instar pea aphids in a Petri dish; one cohort of females was 2 days old (n = 20) and the other cohort was 7 days old (n = 15); females rarely live longer than 8–10 days in the laboratory. All other procedures were the same as in Experiment 1.

Influence of host mobility

We tested the hypothesis that host choice in *M. paulensis* is influenced by aphid mobility and defensive behaviour (Experiment 4). Especially first instars are generally less mobile and have fewer effective anti-parasitoid defenses than older instars, which are larger and more active (Losey & Denno, 1998). If large pea aphids are difficult to capture and subdue by females of *M. paulensis*, anaesthetized aphids that are unable to move and escape should be accepted equally, independent of their size (small vs large). Aphids were anaesthetized with CO_2 for 5 min (L2) and 10 min (L4), respectively. A cohort of females was divided into two groups; females in the treatment group (n = 19) were given a choice between anaesthetized aphids, while those in the control group (n = 20) were given a choice between unanaesthetized aphids of the same size and age. Each trial included 16 aphids, eight second and eight fourth instars, and lasted 30 min. After 4 days of rearing, six aphids of each instar per trial were dissected, and the parasitoid eggs and larvae in each aphid were counted.

Time costs

We hypothesized that a female's costs in terms of opportunity time increase with host size (Experiment 5). Opportunity time (T) is defined as the total time invested by a female to capture an aphid, position it for oviposition, and deposit one or several eggs during a single oviposition bout; T is the sum of handling time and oviposition time as defined by Chau & Mackauer (1999). Because some aphids were able to free themselves from a female's grasp or, for unknown reasons, were released by the female without stinging, we hypothesized further that the proportion of aphids escaping parasitism increases with host size or instar. We divided a cohort of females into four groups, one group for each aphid instar: L1 (n = 19), L2 (n = 20), L3 (n = 20), and L4 (n = 10). Females were placed singly in a Petri dish with five aphids and allowed to attack each aphid once. For each aphid, we measured T with a stopwatch to the nearest second. An attacked aphid was removed immediately and replaced with one of the same kind until each female had stung a total of 10 aphids in sequence. Stung and potentially parasitized aphids were transferred to numbered clip cages (Adams & van Emden, 1972) attached to bean leaves and reared individually. After 4 days of rearing, we dissected all aphids and counted the parasitoid eggs and larvae in each.

Statistical analysis

For statistical analysis, we used the BIOMstat version 3.2 (Rohlf & Slice, 1996) and the SPSS version 9.0 for Windows packages of programs. Hochberg's GT2 method is recommended for unplanned comparisons between pairs of means based on unequal sample sizes (Sokal & Rohlf, 1995, pp. 246–252). Preference in dichotomous choice tests was estimated as the ratio of the relative proportion of younger hosts accepted as compared with the proportion of older hosts accepted among those available:

$$J_{young/old} = [a / (a + b)] / [c / (c + d)],$$

where a and b are the total numbers of accepted and rejected hosts among the younger instar, and c and d are the total numbers of accepted and rejected hosts among the older instar, pooled between trials. The error variance of J in terms of its natural logarithm is (Bliss, 1967, p. 73):

$$\mathrm{var}\,(J) = (1/a) - (1/a + b) + (1/c) - (1/c + d).$$

The 95% confidence interval of ln (J) is given by $\{\ln (J) - [\sqrt{\mathrm{var}\,(J)} \cdot 1.96]\}$ and $\{\ln (J) + [\sqrt{\mathrm{var}\,(J)} \cdot 1.96]\}$. An absence of preference is indicated if the 95% confidence interval includes the value of 1.

RESULTS

Host-instar preference

The numbers of aphids parasitized ($F_{5,\,78} = 6.309$, $P = 0.0001$) and eggs laid in total by females of *M. paulensis* ($F_{5,\,78} = 6.581$, $P < 0.001$) varied between the six instar combinations (Table 2). Pooling within instars across combinations, we found that the mean number of aphids parasitized by each female declined with host instar: L1 ($\bar{x}$ ± SE = 8.05 ± 0.25 aphids, n = 44), L2 (7.45 ± 0.42 aphids, n = 40), L3 (6.86 ± 0.32 aphids, n = 43), and L4 (4.22 ± 0.41, n = 41), in that order. Parasitism was not instar-specific, however. Females laid the most eggs in trials including L1 and L2 hosts and the fewest eggs in trials including L4 hosts (Table 2). When given a choice between two equally available host types, wasps accepted more smaller than larger instars, regardless of the combination (paired-*t* test, L1 vs L2: $t_{14} = 3.434$, $P = 0.004$; L1 vs L3: $t_{14} = 3.055$, $P = 0.009$; L1 vs L4: $t_{13} = 10.724$, $P < 0.001$; L2 vs L3: $t_{12} = 3.959$, $P = 0.002$; L2 vs L4: $t_{11} = 7.707$, $P < 0.001$; L3 vs L4: $t_{14} = 3.449$, $P = 0.004$). For all six combinations, the index of preference J was significant, indicating the following ranked order of preference: L1 > L2 > L3 > L4 (Table 2).

Female size

Females developing in L4 aphids were approximately twice as large in terms of dry mass ($\bar{x}$ ± SE = 0.209 ± 0.010 mg; n = 15) as their counterparts developing in L1 aphids ($\bar{x}$ ± SE = 0.096 ± 0.003 mg; n = 15) (one-way

TABLE 2. Preference pattern of *Monoctonus paulensis* for different instars of the aphid in dichotomous choice tests.

Choice	Instar	n	n_{dis}	n_{par}	n_{eggs}	J (95% CI)
(1)	L1	15	150	7.67 ± 0.45	9.27 ± 0.61	1.22 (1.05–1.42)
	L2		145	6.07 ± 0.73	7.00 ± 0.86	
(2)	L1	15	148	7.67 ± 0.40	8.53 ± 0.76	1.20 (1.04–1.39)
	L3		150	6.47 ± 0.60	7.00 ± 0.72	
(3)	L1	14	133	8.86 ± 0.40	15.43 ± 1.72	1.61 (1.39–1.87)
	L4		140	5.79 ± 0.47	7.64 ± 0.65	
(4)	L2	13	130	9.38 ± 0.31	11.85 ± 0.65	1.28 (1.15–1.44)
	L3		130	7.31 ± 0.47	8.69 ± 0.73	
(5)	L2	12	120	7.08 ± 0.70	9.58 ± 0.95	2.74 (1.98–3.79)
	L4		120	2.58 ± 0.82	3.08 ± 1.01	
(6)	L3	15	150	6.87 ± 0.58	9.40 ± 0.92	1.69 (1.35–2.11)
	L4		150	4.07 ± 0.63	5.53 ± 0.85	

Abbreviations: L1, L2, L3, L4 – apteriform first, second, third, fourth nymphal instar. n – number of replicates (females); n_{dis} – total number of aphids dissected pooled between replicates; n_{par} – total number of aphids parasitized pooled between replicates; n_{eggs} – total number of eggs laid pooled between replicates; J – index of preference (95% confidence interval) for the younger over the older host instar. Values for which variation is shown are means ± SE. All differences between means within choice combinations and columns are statistically significant by paired *t*-test ($P < 0.05$).

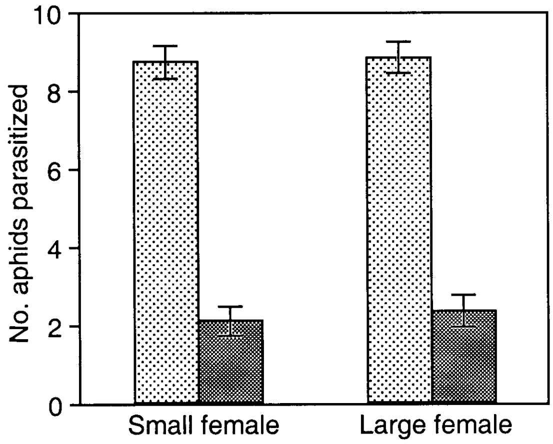

Fig. 1. Influence of body size as measured by dry mass on host choice in naive females of *Monoctonus paulensis*. Females were 2 days old. Large (0.209 mg) and small females (0.096 mg) were given a choice between 15 second- and 15 fourth-instar pea aphids for 70 min. Columns represent mean numbers (bars ± 1 SE) of aphids parasitized by each female based on dissection of 10 individuals per instar and trial. (Second and fourth instars are indicated by light- and dark-shaded columns, respectively).

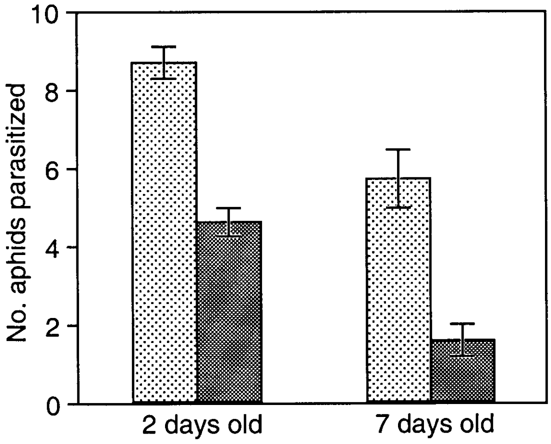

Fig. 2. Influence of age on host choice in naive females of *Monoctonus paulensis*. Young (2 days old) and old females (7 days old) were given a choice between 15 first- and 15 third-instar pea aphids for 60 min. Columns represent mean numbers (bars ± 1 SE) of aphids parasitized by each female based on dissection of 10 individuals per instar and trial. (First and third instars are indicated by light- and dark-shaded columns, respectively).

ANOVA, $F_{1,28}$ = 118.37, $P < 0.001$). Large and small females did not differ in the total number of aphids parasitized (large females, 11.13 ± 0.60 aphids; small females, 10.80 ± 0.63 aphids; one-way ANOVA, $F_{1,28}$ = 0.146, P = 0.71) and eggs laid (large females, 19.53 ± 2.04 eggs; small females, 18.67 ± 1.18; one-way ANOVA, $F_{1,28}$ = 0.136, P = 0.72). Also, host preference did not vary with body size in that both large and small females parasitized significantly more L2 than L4 aphids (paired *t*-test, large females, t_{14} = 15.598, $P < 0.001$; small females, t_{14} = 17.26, $P < 0.001$) (Fig. 1), with the two preference indices overlapping in their 95% confidence intervals (large females, $J_{L2/L4}$ = 3.91; 95% CI, 2.89–5.29; small females, $J_{L2/L4}$ = 4.23; 95% CI, 3.59–4.97).

Female age

Two-day-old females parasitized more aphids ($\bar{x}$ ± SE = 13.30 ± 0.57 aphids) than 7-day-old females (7.20 ± 1.05 aphids) that had no prior host experience (one-way ANOVA, $F_{1,33}$ = 29.52, $P < 0.001$); they also laid more eggs than older females (2-day-old females, 20.55 ± 1.14 eggs; 7-day-old females, 9.07 ± 1.09 eggs; $F_{1,33}$ = 50.01, $P < 0.001$). Although both groups of females parasitized significantly more L1 than L3 aphids that were equally available (paired *t*-test, young females, t_{19} = 9.086, $P <$ 0.001; old females, t_{14} = 6.469, $P < 0.001$) (Fig. 2), old rather than young females were more selective (young females, $J_{L1/L3}$ = 1.92; 95% CI, 1.64–2.26; old females, $J_{L1/L3}$ = 3.70; 95% CI, 2.47–5.52).

Host mobility

Naive females of *M. paulensis* accepted both anaesthetized aphids and normal aphids able to move and defend themselves. Slightly more anaesthetized aphids than normal aphids were parasitized in total (anaesthetized aphids, $\bar{x}$ ± SE = 7.47 ± 0.46 aphids; normal aphids, 6.25 ± 0.39 aphids; one-way ANOVA, $F_{1,37}$ = 4.137, P = 0.049); however, the total number of eggs laid by each female did not differ between these hosts (anaesthetized aphids, 12.16 ± 1.09 eggs; normal aphids, 10.15 ± 0.77 eggs; $F_{1,37}$ = 2.296, P = 0.14). Also, aphid behaviour did not affect the direction of preference in that both groups of females parasitized more L2 than L4 hosts (Fig. 3); but a relatively smaller proportion of L2 were parasitized among anaesthetized hosts (anaesthetized aphids, $J_{L2/L4}$ = 2.84, 95% CI = 2.49–3.24; normal aphids, $J_{L2/L4}$ = 4.21, 95% CI = 3.49–5.07).

Time costs

Not all the pea aphids encountered by females of *M. paulensis* were also parasitized. A large percentage of aphids (23.5%; n = 895) either escaped or were released by the parasitoid without stinging, with more L4 (47.6%) than L3 (29.1%), and more L3 than L1 (10.4%) and L2 (7.4%) instars escaping ($F_{3,65}$ = 22.10, $P < 0.001$). Of 684 aphids that were, in fact, captured and stung, some died before they could be dissected. Mortality was highest among L1 (21.7%) and lowest among L4 (6%). Among 592 aphids dissected, 34 (5.7%) contained no parasitoid eggs or larvae; these aphids were excluded from further analysis. The total time (T) needed to position an aphid and deposit an egg varied with host size as measured by dry mass ($F_{3,554}$ = 18.44, $P < 0.001$). Females needed almost twice the time to parasitize L4 ($\bar{x}$ ± SE = 36.67 ± 4.15 sec; n = 94) than L1 (19.15 ± 0.67 sec; n = 137), L2 (23.03 ± 0.87; n = 163) and L3 aphids (22.08 ± 0.67; n = 164); differences in T between the three younger instars were not significant (by Hochberg's GT2 method).

DISCUSSION

Females of *M. paulensis* attacked and laid eggs in all four nymphal instars of the pea aphid; however, when given a choice, they always accepted the relatively

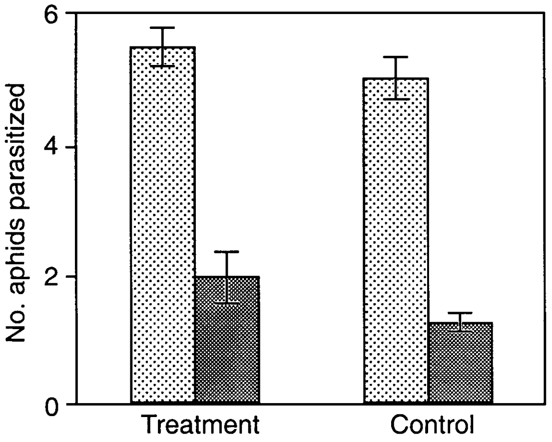

Fig. 3. Influence of host mobility on host choice in *Monoctonus paulensis*. Two-day-old, naive females were given a choice between 8 second- and 8 fourth-instar pea aphids for 30 min. Females in the treatment group received anaesthetized aphids, whereas those in the control group received normal aphids capable of movement and defense. Columns represent mean numbers (bars ± 1 SE) of aphids parasitized by each female based on dissection of 6 individuals per instar and trial. (Second and fourth instars are indicated by light- and dark-shaded columns, respectively).

younger and smaller instars over equally available older and larger instars: L1 > L2 > L3 > L4 (Table 2). The magnitude of preference for smaller aphids was influenced, apparently, by the relative difference in size between the available host types (Tables 1, 2). Although large hosts contain more nutritional resources for offspring development and growth, they may be more difficult to capture than small hosts. Especially in parasitoids that physically attack and subdue their hosts, host choice is expected to reflect a trade-off between the female's risk of injury or loss of opportunity time (if a large host escapes) and her gain in fitness from offspring growing to a larger size and/or developing faster. Early instars are generally more abundant than older instars and adult aphids in natural populations (Hughes, 1963). The relatively high mortality observed in our experiments among first instars could indicate that small aphids are susceptible to wasp venom; however, pea aphids stung by *M. paulensis* recover relatively quickly from the paralysis, including first instars (A. Chau, unpubl.). If hosts are equally suitable but vary in size, females should favour medium-sized hosts and avoid both very small hosts (which may be suboptimal for the immature parasitoid) and very large hosts (which may be risky to attack for the female). A preference for the smallest available hosts, as observed in *M. paulensis*, is unexpected and counter to this prediction. We tested three alternative hypotheses that could explain the observed pattern. First, we tested the hypothesis that host choice varies with parasitoid size. Because females of *M. paulensis* deposit their eggs in a specific location, the physical size of a female, especially the length of her ovipositor, may constrain the size of the aphids that she can parasitize (Brandl & Vidal, 1987). Although large females had nearly twice the body mass of small females, and presumably had a correspondingly longer ovipositor, both kinds of female showed the same degree of preference for the smaller L2 over the larger L4 instars (Fig. 1).

Second, we asked if host choice is affected by female age. Older females are expected to be less choosy than younger counterparts (Roitberg et al., 1993). Contrary to this prediction, 7-day-old, as opposed to 2-day-old, females attacked fewer aphids per unit of time and parasitized proportionally more L1 than L3 instars (Fig. 2). Two explanations that are not mutually exclusive could account for these results. One explanation is that egg maturation was affected by host deprivation. If this is true, older females that were deprived of hosts had probably fewer eggs available for oviposition than younger counterparts. For example, Mackauer (1983b) found that, in *Aphidius smithi* Sharma & Subba Rao, another parasitoid of the pea aphid, total fecundity declined when females were limited to less than 20 aphids per day throughout life. However, Aphidiinae are not known to resorb eggs (Le Ralec, 1991) and cannot therefore repartition energy resources to prolong longevity in the absence of suitable hosts. An alternative explanation is that females of *M. paulensis* with age became less effective in subduing and handling pea aphids, in particular any larger instars. Weisser (1994) showed that older females of *Lysiphlebus cardui* Marshall, a parasitoid of *Aphis fabae cirsiiacanthoides* Scopoli, needed more time than younger females for host handling and oviposition.

Third, we tested the hypothesis that parasitoid preference reflects differences in handling costs and oviposition success between host types (Iwasa et al., 1984; Gerling et al., 1990; Chau & Mackauer, 1997; Losey & Denno, 1998). Anti-parasitoid behaviour including dropping from the feeding site and kicking with the hind legs are generally better developed in older instars and adult aphids than in first instars. Using anaesthetized aphids, we evaluated the influence of host mobility and defensive behaviour on parasitoid choice. Contrary to expectations, we found that parasitoids accepted anaesthetized aphids and their normal counterparts equally and, furthermore, preferred L2 over L4 instars under both conditions (Fig. 3). Because we could not exclude the possibility that host choice was influenced by other, and perhaps more subtle behavioural differences between the four pea-aphid instars, we used the total time needed to capture, position, and parasitize an aphid as a proxy of host-parasitoid behavioural interactions. Females needed on average twice as much time for a large L4 instar as compared with any of the three younger instars. Moreover, L4 aphids were most likely to escape or otherwise avoid parasitism. Similarly, Chau & Mackauer (1999) reported that handling time (i.e., the time needed to capture and subdue a potential host) increased with pea-aphid instar, but oviposition time varied with the number of eggs laid. Because females often laid more than one egg during a single ovipositor insertion, especially in L4 aphids, time costs increased with aphid instar.

In this study, we have used aphid size and age synonymously. As size increases with age, the results could equally well support the hypothesis that *M. paulensis* prefers relatively younger rather than smaller pea aphids. As shown by Brough et al. (1990) in *Megoura viciae* Buckton, the allocation pattern of nutritional resources to somatic and gonadal tissues changes as aphids approach reproductive age. Such physiological changes in the host, in turn, may alter host quality and ultimately influence host choice. However, females of *M. paulensis* seem to be indiscriminate and oviposit even in aphids that are unsuitable for offspring development (Calvert, 1973), a behaviour also observed in *M. crepidis* (Griffiths, 1960). Moreover, in a direct test of the relative importance of aphid age versus size, Kouamé & Mackauer (1991) demonstrated that the ranking order of different host types was dependent on their size and not age. Comparing starved pea aphids and aphids feeding normally on broad beans, they found that females of *Ephedrus californicus* Baker attacked and parasitized more starved (ie., small) than non-starved (ie., large) aphids of the same age; however, differences in host size had no influence on parasitoid attack rates when aphids of the same age were anaesthetized so that they could not escape or defend themselves.

In conclusion, our results show that females of *M. paulensis* do not selectively parasitize first- and second-instar pea aphids but attack the relatively smaller aphids among those available independent of instar: L1 > L2 > L3 > L4. Choice was not influenced by parasitoid size in that large females showed the same preference pattern as small females. Moreover, the observed ranking order was not reversed in females given a choice between large and small anaesthetized aphids. In spite of their lower growth potential (Mackauer et al., 1997), early-instar pea aphids may be perceived by a female as having a higher value than late-instar and adult aphids. First- and second-instar pea aphids are more abundant, can be captured more easily, and present a lower mortality risk for parasitoid offspring than older aphids, which are less abundant, can vigorously defend themselves against an attack, and present a high mortality risk for offspring (Chau & Mackauer, 1997; Losey & Denno, 1998).

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Received November 5, 1999; accepted February 24, 2000