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Host stage preference and sex allocation in *Aenasius vexans*, an encyrtid parasitoid of the cassava mealybug

Catherine Bertschy, Ted C. J. Turlings[1,*], Anthony Bellotti[2] & Silvia Dorn

Institute of Plant Sciences, Applied Entomology, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland; [1]*Present address: Institut de Zoologie, Université de Neuchâtel, CH-2007 Neuchâtel, Switzerland;* [2]*Centro International de Agricultura Tropical, CIAT, Cali, Colombia;* [*]*Author for correspondence (E-mail: ted.turlings@zool.unine.ch)*

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Abstract

The solitary endoparasitoid *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae) is used for augmentative releases against the cassava mealybug, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae), an important pest on cassava in South America. In light of the need for large numbers of high quality females, experiments were conducted on host stage suitability and sex allocation. In choice and no-choice experiments, individual female wasps were offered second and third instar, as well as adult, hosts. During the first five days after emergence, the wasps showed a steady increase in the number of hosts they successfully parasitised per day, but the respective secondary sex ratio for each instar remained constant. Parasitism was highest for third instar hosts in no-choice tests, while in choice tests parasitism was highest in both third instars and adults. The later the developmental stage of the host at oviposition, the faster the parasitoids developed and emerged, and for each host stage, the development time of males was shorter than for females. The sex ratio of the wasps emerging from hosts that were parasitised as second instars was strongly male-biased, while the apparently preferred later stages yielded significantly more females than males. Female and male *A. vexans* emerging from hosts parasitised at the third instar were significantly larger than for the other stages. This may explain the preference for the third instar as well as the female-biased sex ratio, as size is usually positively correlated with higher fitness, especially in females. The results suggest that third instar hosts are the most suitable for rearing high numbers of large females.

Introduction

The cassava mealybug, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae), has rather suddenly become a pest in South America. It was first found in Northeast Brazil and was later reported in Colombia, Venezuela, and Guyana (Bellotti et al., 1994, 1999; CIAT, 1984, 1987, 1988, 1990). The encyrtid parasitoid *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae) is a potential biological control agent against this pest. *Aenasius vexans* was released in 1994 and 1995 in Brazil together with two other encyrtids, *Acerophagus coccois* Smith and *Apoanagyrus (Epidinocarsis) diversicornis* Howard (Bellotti et al., 1994, 1999). Additional augmentative releases of parasitoids are currently being considered.

We found differences between the three wasp species in their attraction to odours of healthy and mealybug-infested cassava plants, with *Aenasius vexans* showing the strongest attraction to infested plants and a better ability to distinguish infested from healthy plants (Bertschy et al., 1997). *A. vexans* appears to be the most specialised of the three parasitoid species and may offer advantages especially when released in pure cassava stands (Dorn, 1996). Such releases require large numbers of healthy females, but the rearing colony of *A. vexans* at CIAT (Centro Internacional de Agricultura Tropical) in Cali, Colombia, presented on

several occasions an extremely male-biased sex ratio. To resolve this problem, current sex allocation theory was considered in the design of a series of experiments to optimise the sex ratio and quality of the colony.

Most Hymenoptera exhibit arrhenotokous parthenogenesis in which males develop from unfertilised haploid eggs and females from fertilised diploid eggs. This haplodiploid sex determination mechanism allows the insects to precisely control the sex of their offspring. In parasitic Hymenoptera, one observed sex allocation strategy is that female eggs are preferentially laid in larger hosts (King, 1993). This may be explained by differential fitness consequences; parasitoid size is positively correlated with host size, and female fitness may increase more strongly with size than for males (Charnov, 1982; King, 1993). Waage (1982) suggested that size-dependent sex allocation is unlikely to occur in koinobiont parasitoids, which lay eggs in still growing hosts, because at oviposition a female wasp is unable to determine the eventual size of a host. However, several studies demonstrate size-dependent sex ratios for koinobiotic parasitoids (reviewed by King, 1989, 1993). This is also expected to be the case for *A. vexans*. After parasitism, hosts continue to grow for about 10 days and can moult during that period. Hosts that are parasitised at different stages may thus represent resources of different quality during parasitoid development and the wasp may have adapted its sex allocation accordingly.

Local mate competition is another aspect of the biology of some parasitoids that may shape sex allocation. It is the consequence of males staying close to their site of emergence in order to mate with females that emerge at the same site (Hamilton, 1967; Taylor & Bulmer, 1980; Godfray, 1994; Hardy, 1994). In such situations, the likelihood of mating among siblings is high and sex ratios are female-biased, which reduces competition among brothers. This is commonly used to explain extreme female-biased sex ratios in gregarious parasitoids where more than one parasitoid develops per host (Godfray, 1994). However, it may also apply to solitary parasitoids that attack spatially grouped hosts, such as aphids, mealybugs, and egg masses (Waage, 1982). *Phenacoccus herreni* usually occurs in groups of various instars on individual cassava leaves and an *A. vexans* female parasitises several individuals once it has found such a cluster of hosts. Therefore, a female-biased sex ratio might be expected as an adaptation to local mate competition if parasitoid dispersal is low. These hypotheses were considered in the design of a series of no-choice and choice experiments that were conducted to optimise mass-rearing methods of *A. vexans*.

Materials and methods

Plants. Cassava plants were grown at CIAT in Cali, Colombia. Twenty cm long stakes of the variety CMC40 were planted every week in pots and kept in a screened compartment subjected to natural weather conditions but protected from rain. Approximately six weeks after planting, the plants were used in experiments, when they had 10–30 leaves.

Hosts. The cassava mealybug, *P. herreni*, was reared at CIAT on 30–40 cm potted cassava plants (var. CMC40) as described by Van Driesche et al. (1987). First instar mealybugs are much smaller than *A. vexans* adults, are rarely parasitised, and, if they are, no parasitoid offspring emerges from them (CIAT, 1987–1991). Therefore, only subsequent instars were used for the experiments.

Hosts were collected from the rearing colony, using individuals of approximately the same size for each instar. Second instar nymphs of the mealybug were chosen when they were big enough to be recognised as females, which remain white, while males turn rosy before they start spinning a cocoon. Third instar mealybugs were chosen at a mid-development average size. Adult females were used after they had moulted, but before they started producing ovisacs.

Wasps. The parasitoid, *A. vexans*, has been continuously reared at CIAT on mealybug-infested cassava plants since this parasitoid was first collected in Venezuela in 1990. Parasitoids were maintained in a greenhouse at 35 °C under natural light conditions. Five days before each experiment, mummies (parasitised mealybugs), which can be recognised by their dark grey colour and hard consistency, were collected from the colony. They were kept individually in gelatine capsules and stored at about 28 °C.

Preparation of the experimental plants. Leaves on a living plant were enclosed in Petri dishes (15 cm diam.). The dishes were fixed to a thick iron wire planted into the soil so that the leaves would remain in their natural position. In order to reduce the condensation due to plant respiration, two holes of 10 cm each were cut in the upper and lower sides of the Petri dishes and covered with nylon gauze. Two holes of

1 cm diameter were drilled in the opposite sides of the vertical rims. One hole was used to introduce the plant petiole. Cotton wool was wrapped around the petiole to protect it and to plug the hole. The second hole was used for the introduction and capture of parasitoids. After introduction of the insects, the dishes were wrapped with Parafilm® to seal the holes and the space between the two dish halves. Three Petri dishes were placed on each plant.

For the no-choice experiment, 50 mealybugs of the same instar were transferred from an infested cassava plant onto a leaf enclosed in a Petri dish, using a small paintbrush. In each of three dishes on one plant we placed mealybugs of a different instar. Fresh plants were prepared like this for five days in a row. Care was taken to prevent the stylet, which was usually inserted in the plant, from breaking. The insects were placed on the upper side of the leaf, from where most of them then moved to the lower side. A few wasps escaped so that the experiment was replicated 19 times for the second instars and adults and 17 times for third instars.

For the choice experiment, 20 mealybugs of each instar were transferred to a dish attached to a leaf as described above. This experiment was replicated with 21 *A. vexans* females. The following day, after they had been exposed to a parasitoid, the mealybugs from each dish were separated by instar and transferred into three new Petri dishes on a fresh plant.

Parasitism. On the first morning of an experiment, newly emerged *A. vexans* females (1–6 h old) were individually introduced into a Petri dish containing a mealybug-infested leaf. A few females were introduced alone and unmated to confirm that virgin females can reproduce, but are constrained to only produce males. The other females were accompanied by a male to allow them to copulate. At about 10 AM on each morning of the next four days, the parasitoid couple was transferred into a new dish on a new plant with fresh mealybugs of the same instar or instar combination.

Plants with parasitised mealybugs were kept for two weeks in a greenhouse at an average temperature of 32 °C (max. 40 °C) during the day and 23 °C (min. 19 °C) at night and an average daytime humidity of 40% and 74% at night. After this period, mummies were removed and kept individually in gelatine capsules. The sex and the time between oviposition and emergence was recorded for each emerging parasitoid. Wasps that emerged over the weekend were not included in the final analysis of development time. In the choice experiment, we also measured the length of the hind tibia of each wasp, as a measure of parasitoid size.

Statistics. Percentage data for sex ratio and preference experiments were subjected to a one-way analysis of variance after arcsine transformation. The lengths of the tibia and the duration of development were not normal distributed, even after a log transformation. For these data, Kruskal–Wallis one-way ANOVA on ranks was used.

Results

Sex ratios. Mealybugs of the second nymphal instar yielded a considerably higher proportion of *A. vexans* males than did third instar or adult hosts, in both no-choice and choice experiments (Figures 1a and b). The sex ratio was only measured at emergence (secondary sex ratio). It should be noted that differential mortality of the sexes before emergence may have contributed to the observed differences in secondary sex ratio among the different host instars. During the mummy stage, however, *A. vexans* mortality was low and similar for all host instars (about 5% and 8% for no-choice and choice experiments, respectively).

Over the five-day oviposition period, the sex ratios of wasps emerging from the different instars remained relatively constant in both experiments (Figure 2). However, male ratios in the choice experiments showed a slight, but not statistically significant, trend to decrease for third and fourth instars and increase for second instar over time (Figure 2b).

Host stage preference. In the no-choice experiment, significantly more wasps emerged from third instar hosts and fewest from the adult stage (Figure 3a). The apparent preference for the third instar was also reflected in the results for the choice experiment, but here, the third instar emergence was not significantly different from the emergence from adults (Figure 3b).

The curves for daily parasitism all show a significant increase over the five-day period (Figure 4). The increase was most pronounced for the second instar hosts in a no-choice situation. In contrast to the parasitism of second and adult stages, the parasitism rate for the third instar was already high on the first two days when the wasps had no choice.

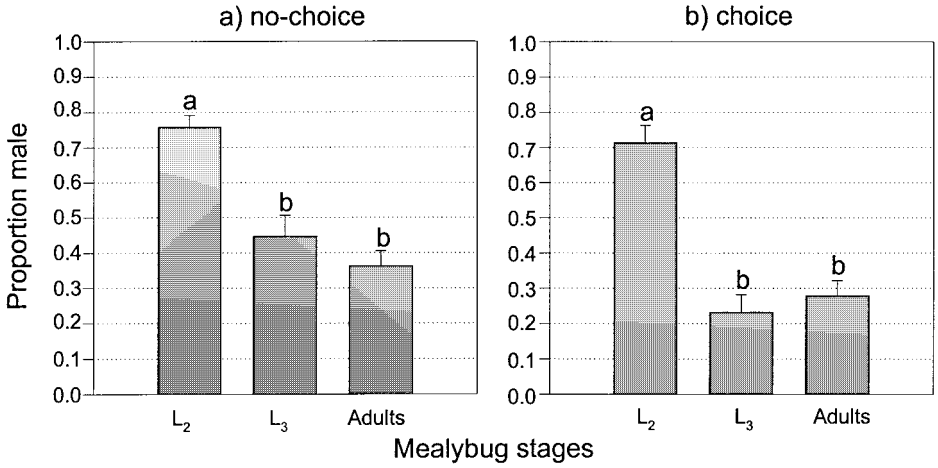

Figure 1. Proportion (± S.E.) of *A. vexans* males that emerged from second and third instar nymphs and adult mealybugs. (a) no-choice: individual *A. vexans* females were offered 50 hosts of one stage only (n=19, 17, 19 for second, third, and fourth stages, respectively). (b) choice: individual *A. vexans* females were offered 20 mealybugs of each stage (n=21). Different letters within each graph represent data that are statistically different ($P<0.05$).

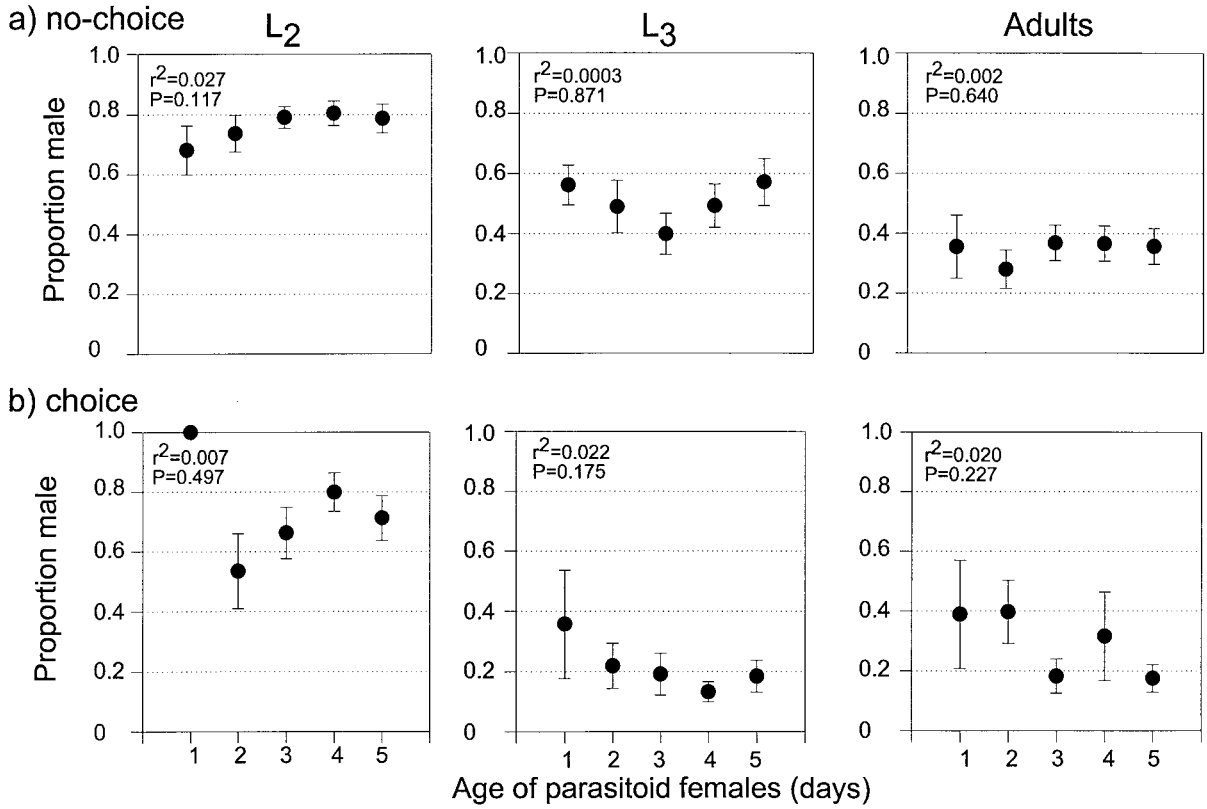

Figure 2. Proportion (± S.E.) of males produced per day and per host stage by *A. vexans* females over five days. Same experiments as described for Figure 1. The regression values indicate that the sex ratios did not significantly change over time.

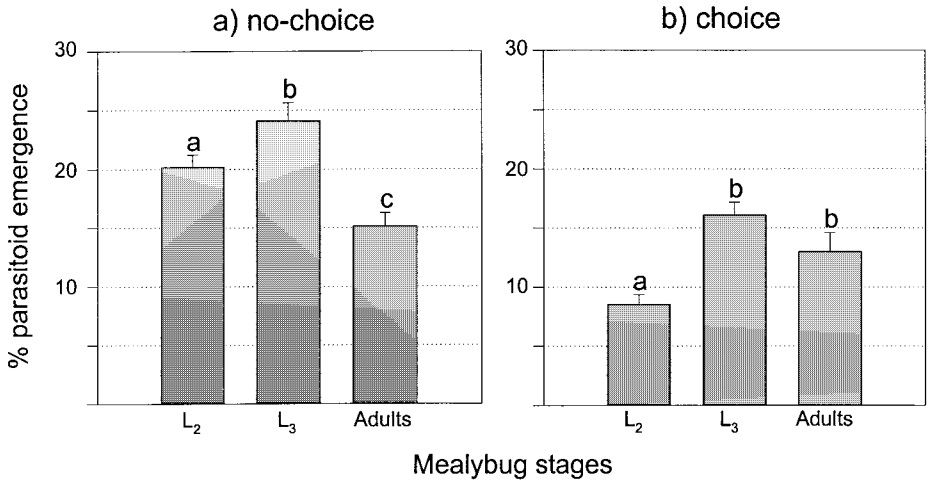

Figure 3. Average percentage of hosts from which a parasitoid emerged (± S.E.) for each host stage. Same experiments as described for Figure 1. Different letters within each graph represent data that are statistically different ($P<0.05$).

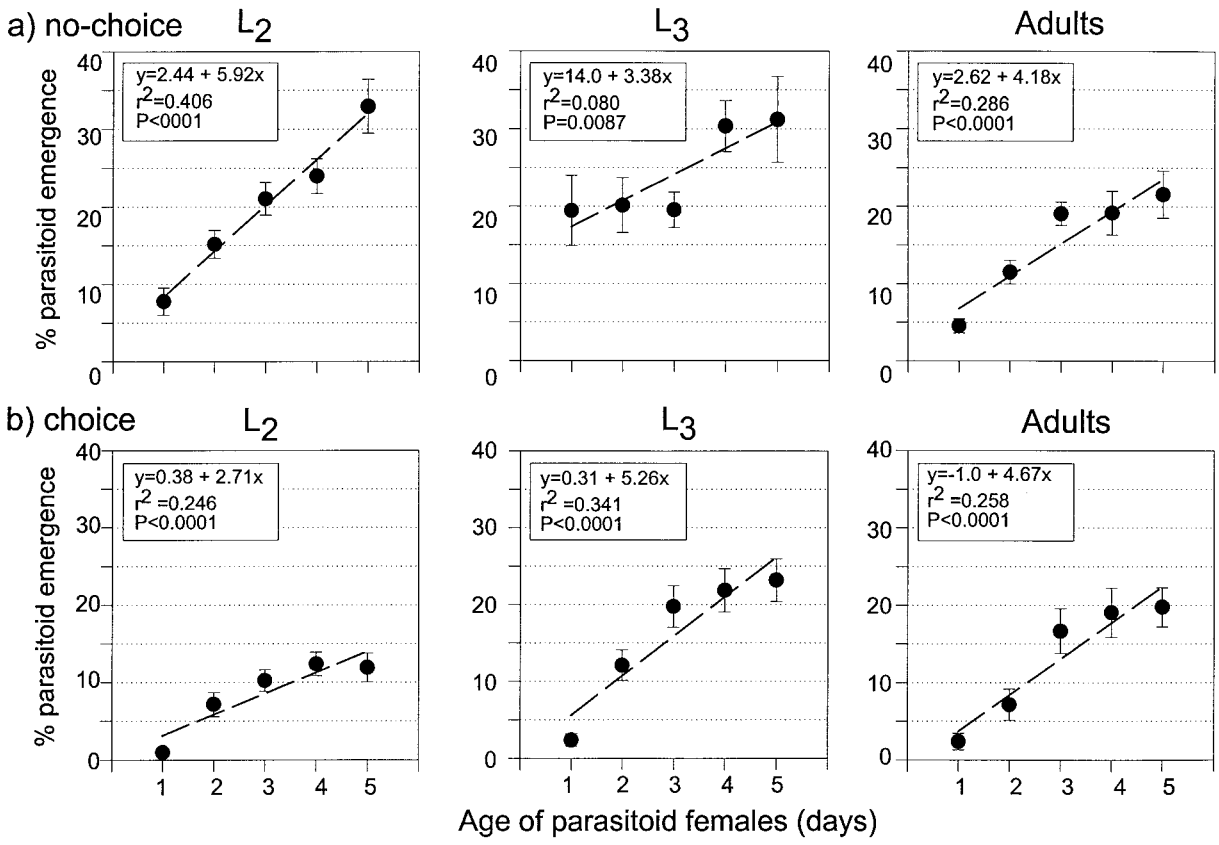

Figure 4. Average percentage of hosts from which a parasitoid emerged (± S.E.) for each host stage per oviposition day. Same experiments as described for Figure 1. The regression values indicate that for both experiments and all three host stages the parasitisation rate increased over time.

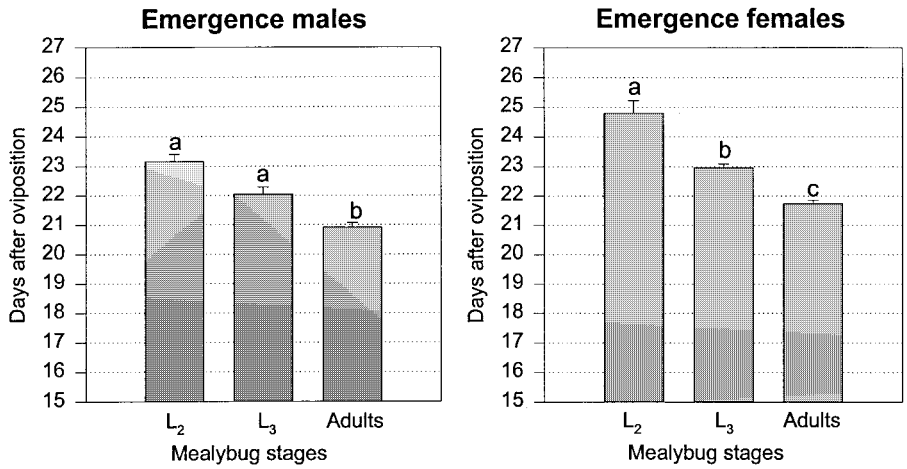

Figure 5. Average emergence time (days), after oviposition, of *A. vexans* for the different host stages. Same experiments as described for Figure 1. Different letters within each graph represent data that are statistically different ($P<0.05$).

Wasp development time and wasp size. The older the host that was attacked, the faster a wasp would develop (Figure 5). Moreover, in each host instar males developed significantly faster than females. *Aenasius vexans* females were significantly larger than males ($P<0.0001$). Female as well as male parasitoids emerging from hosts of third instars were significantly larger (longer hind tibia) than parasitoids emerging from second instars and adults (Figure 6). The two last mentioned groups were not significantly different from each other.

Discussion

The size of wasps emerging from *P. herreni* was strongly correlated with host stage (size) at the time of parasitism. Third instar hosts of the mealybug produced larger parasitoids than adults and second instars, which is similar to what Cadée & van Alphen (1997) found for the citrus mealybug, *Planococcus citri* (Risso) and its encyrtid parasitoid, *Leptomastidea abnormis* (Girault). Cadée & van Alphen (1997) suggest that parasitoids that emerge from adult mealybugs are lighter because resources that the adults use for egg production are not available to the parasitoid larvae. Late nymphal stages do indeed appear to provide the parasitoids with superior resources, resulting in larger size wasps. The size of adult parasitoids is usually correlated with fitness, especially in females; larger females lay more eggs over their lifetime (Sandlan, 1979; Charnov et al., 1981; van Dijken & van Alphen, 1991; Srivastava & Singh, 1995). Third instar larvae of mealybugs are therefore likely to be the most suitable for *A. vexans.* However, development time is most favourable in adult hosts; males and females both developed fastest in adult hosts (Figure 5).

The sex ratio we found for the three different host stages is in accordance with the apparent suitability of the third instar host larvae. Third instar and adult mealybugs produced more female parasitoids, while second instar produced more males. This host-stage dependent sex ratio corroborates Charnov's model (Charnov et al., 1981), which postulates that parasitoids preferentially lay female eggs in the most suitable hosts. The model also predicts that sex ratio will depend on relative host size distribution, thus sex allocation decisions when parasitising a host of a particular size are influenced by the availability of hosts of different sizes. However, the results for *A. vexans* suggest an innate ability to recognise second instar hosts as less suitable for females, because the strongly male-biased sex ratio for second instar hosts was very similar in the no-choice and choice situation. On the other hand, in the no-choice test, wasps appeared to lay more male eggs in the third instar and adult hosts than in the choice test. Interestingly, the secondary sex ratios for these two stages in the no-choice test was almost identical (about 0.40) to the overall sex ratio in the choice test. De Jong & van Alphen (1989) found a very similar host stage preference and sex allocation for *Leptomastix dactylopii* (Howard) parasitising the citrus mealybug, *P. citri*, which attacks the last three of five developmental stages. They too found a clearly female-biased overall sex ratio.

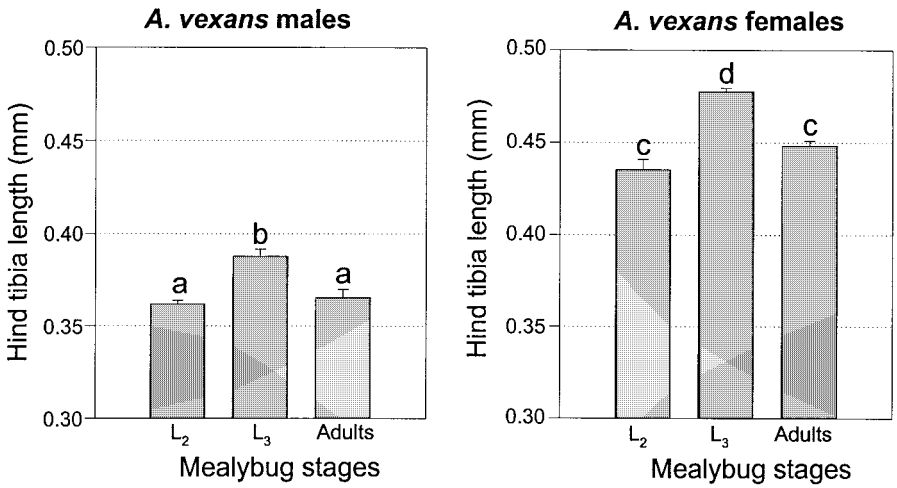

Figure 6. Average hind tibia length (mm) of *A. vexans* insects that emerged from different host stages. Same experiments as described for Figure 1. (a) tibia length of the emerging males, (b) tibia length of the emerging females. Different letters within both graphs represent data that are statistically different ($P<0.05$).

Local mate competition theory (Hamilton, 1967) predicts that female-biased sex ratios will evolve in cases where related males compete for mates, given that one male can inseminate more than one female. *Aenasius vexans* is a solitary parasitoid, but one female attacks several hosts within an aggregated batch. Therefore, frequent mating between brothers and sisters is possible and sex allocation could be adapted accordingly. *Aenasius vexans* shows an average secondary sex ratio that is moderately, but clearly female-biased. Such ratios could be adaptations to partial local mating (Hardy, 1994; Hardy & Mayhew, 1998; West & Herre, 1998). To determine if local mating affects sex allocation in *A. vexans* requires additional studies on the wasp's mating behaviour and the effects of parasitoid density.

One confusing result was that in the no-choice experiment, adult hosts yielded the fewest parasitoids, while in the choice experiment, the numbers were lowest for the second instar host and statistically not different between third instar and adult hosts (Figure 3). However, overall parasitism rates in both experiments were similar. Also, the increase in parasitisation over time was very similar in both experiments (Figure 4). Adult mealybugs show aggressive defensive behaviour, which may result in increased handling time. Long handling times in the no-choice test with only adult hosts may have limited the number of hosts *A. vexans* could parasitise in the allotted time.

Our results are based on the sex ratio at emergence, which may not correspond to the sex allocation at oviposition. Differential mortality during larval development may have influenced emergence, giving a misleading impression of preferences and sex allocation strategies. Mortality in mummified hosts, however, was low and did not significantly differ among the stages, nor between the experiments (about 6% and 8.5% for no-choice and choice experiments, respectively). Still, the sex ratio may have been influenced by a differential mortality of the sexes at an earlier preimaginal stage. One cause of mortality is encapsulation of the parasitoid inside the host. It has been frequently reported that hosts have a better encapsulation ability at a later stage in their development (Brodeur & Vet, 1995; Benrey & Denno, 1997; Blumberg, 1997). This seems to be different for *P. herreni*; adults are less successful at encapsulating the parasitoid *A. diversicornis* than the second instar mealybugs (Van Driesche *et al.*, 1986). However, this difference in the encapsulation rate for *A. diversicornis* was not very high (15% vs. 10%) (van Driesche et al., 1986) and is unlikely to have a significant effect on host preference.

Differential mortality for parasitoid males and females also occurs in cases of superparasitism (King, 1987). In our experiments, superparasitism is unlikely to have been important. In previous studies at CIAT (1992), five *A. vexans* couples were offered different mealybug nymph densities for 24 h. At the same density that was used for the no-choice experiment (250 mealybugs for five *A. vexans* couples), superparasitism was on average only 1.3% (CIAT, 1992).

Following Sandlan's example (1979), we compared the mortality of the progeny of virgin and mated females. Male offspring of virgin females showed a mortality for third instars (11.4%) and adults (14.6%) that was four times as high as for second instars (3%) (the difference between second instars and adults was significant). Although these results should be regarded with caution because few virgin females were tested (n=5 for second instars and adults, n=7 for third instars), they do suggest that males suffer higher mortality in larger hosts, which may have influenced the emergent sex ratio.

Aenasius vexans is one of several candidates being considered for further augmentative releases against the cassava mealybug. As third instar hosts yielded more and larger female wasps than the other two stages of hosts, we recommend that the former be used for mass rearing. If, however, fast development of parasitoids is an important consideration, adult hosts may be more suitable. Other studies that aim to optimise rearing of large numbers of high quality biological control agents may also benefit from considering sex allocation theory in the design of experiments.

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