

## Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp, *Monoctonus paulensis*

Amanda Chau & Manfred Mackauer

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada V5A 1S6

Accepted: February 4, 1997

**Key words:** *Acyrtosiphon pisum*, host choice, *Monoctonus paulensis*, offspring mortality risk, parasitoid, pea aphid

### Abstract

We examined the dropping behaviour of the pea aphid, *Acyrtosiphon pisum* (Harris), feeding on broad-bean plants in the laboratory. Aphid responses to foraging and oviposition by females of *Monoctonus paulensis* (Ashmead) were instar-specific and included kicking with the hind legs, walking away and dropping from the feeding site. Fourth nymphal instars were most likely to drop, followed by second, third, and first instars, in that order. Compared with first instars, the odds that a fourth-instar aphid will drop were 6-times higher independent of the stimulus, and 16-times higher after parasitoid attack. Dropping from the feeding site increases an aphid's mortality risk. If parasitoid offspring are more likely to survive in small pea aphids, it may be adaptive for *M. paulensis* to choose smaller hosts, regardless of possible fitness gains due to increased body size from development in larger aphids.

### Introduction

Host behaviour has a major influence on host choice by parasitoid wasps. Many insects actively defend themselves against attacks by parasitoids and predators, or excrete substances that act as deterrents, or otherwise try to escape (Dettner, 1987; Evans & Schmidt, 1990). Aphids show a broad range of defensive tactics including kicking with the hind legs, walking away, jumping, and dropping from the feeding site to the ground below (Klingauf, 1967; Roitberg & Myers, 1979; Heie, 1987). Some aphids escape by smearing a waxy secretion exuded from the siphunculi on an attacking natural enemy (Dixon, 1958). Defensive behaviours are often accompanied by the production of alarm pheromone (Nault et al., 1973; Pickett & Griffiths, 1980). As a consequence, even aphids that are not under direct attack may show altered behaviours in the presence of a parasitoid or predator (Dixon & Stewart, 1975; Roitberg & Myers, 1978; Dill et al., 1990).

*Monoctonus paulensis* (Ashmead) (Hymenoptera: Aphidiidae) is a solitary parasitoid of several species of aphids, including pea aphid, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae) (Calvert & van den

Bosch, 1972a). Females used the fore legs to capture and orientate a potential host for oviposition. The host was temporarily paralyzed, possibly by venom injection (Calvert & van den Bosch, 1972b). If the female accepted the host, she deposited an egg in the aphid's fused thoracic ganglia, a behaviour also observed in the related species, *Monoctonus crepidis* (Haliday) (Griffiths, 1960). Unusual among solitary parasitoids, females frequently deposited more than one egg during a single oviposition bout although only one offspring survived in superparasitized aphids (Michaud & Mackauer, 1995). In an extensive study of the oviposition behaviour of *M. paulensis*, A. Chau & M. Mackauer (unpubl.) found that females did not show a preference for a particular pea-aphid instar but generally selected the smaller aphids for oviposition when given a choice between hosts of different size that were equally available. Offspring successfully developed, and gained more in body size, in larger aphids, however.

'Optimal progeny allocation' theory (reviewed in Godfray, 1994) predicts that, other conditions being equal, parasitoids should select larger hosts over equally available smaller ones if host quality is a positive function of host size and if parasitoid fit-

ness increases with body size. Although host quality is difficult to quantify objectively (Mackauer & Sequeira, 1993), both assumptions are, apparently, satisfied in the interaction between pea aphid and *M. paulensis*. Therefore, a choice of the relatively smaller, as opposed to the relatively larger, available hosts requires a different explanation. Because aphid behaviour is instar-specific (Dill et al., 1990; Gerling et al., 1990), we tested the hypothesis that host choice in *M. paulensis* reflects aphid responses to parasitoid foraging and oviposition. If the probability of dropping after parasitism (and consequently the mortality risk of parasitoid offspring) varies between aphid instars, it may be adaptive for females to select aphids that are least likely to be disturbed and drop from the feeding site after handling, regardless of host size.

## Materials and methods

**Insect rearing.** We reared *M. paulensis* in the laboratory on early third-nymphal instars of pea aphid feeding on potted broad-bean plants, *Vicia faba* L. cv 'Broad Windsor'. Insect colonies were kept in growth chambers at  $20 \pm 1$  °C, 50–55% r.h., and continuous light. To obtain aphids of a particular age or instar, we caged reproductive virginoparae on fresh bean plants for 6 h. All offspring produced during this period were transferred to fresh plants and reared as a synchronous colony until they reached the desired age for experimentation (24 h, 48 h, 72 h, and 120 h); these four age classes corresponded approximately to the four nymphal instars, L1 to L4 (Sequeira & Mackauer, 1992).

**Behavioural interactions.** We compared the behavioural responses of the four aphid instars to encounter and attack by a female of *M. paulensis*. We set up four sets of replicated trials, one set for each aphid instar. The trials were designed so that aphids would settle naturally on the preferred feeding sites. On the day prior to testing, we released 15–20 nymphs on the base of a potted bean plant in a screened plexiglass cage (26 cm wide  $\times$  35 cm deep  $\times$  41 cm high). Each plant was 12 cm tall, with two fully developed leaves and two leaves that had just unfurled in addition to smaller leaves tightly curled around the growing tip. For ease of observing any dropped aphids, we placed a filter paper (Whatman #1; 15 cm in diameter) around the base of each bean stalk 1 h before a trial. Into each cage we released a single, 2-day-old female of

*M. paulensis* on the bean stalk, about 1 cm above the paper disk; all females were mated but had no prior experience with aphids. Behavioural interactions between the aphids and the wasp were observed continuously. A trial ( $n = 54$ ) ended when the female had encountered about 80% of the available aphids or after 30 min, whichever came first.

We distinguished between three events that could result in an aphid dropping from the feeding site: (1) An aphid could be disturbed by a foraging wasp and respond defensively by dropping from the feeding site. Disturbance is defined as kicking, backing off, walking and dropping in the absence of a direct physical attack by a wasp. Alternatively, an aphid could be attacked by a wasp and either (2) escape its attacker by dropping from the feeding site, or (3) drop after it was released by the wasp following oviposition. In the process of subduing a struggling aphid, a female parasitoid may drop off the plant, together with its host; these cases were noted. Not all encounters resulted in attack, and not all attacks were successful (see above). We were unable to differentiate between a wasp ignoring and rejecting an encountered aphid, however. Also, we could not determine if an attacked aphid released alarm pheromone, and if non-attacked aphids responded to pheromone or the presence of a wasp, or both.

Any aphid that dropped off a plant was removed immediately. At the end of each trial, we transferred 'droppers' and 'non-droppers' to bean plants in separate cages, distinguishing between aphids that had dropped due to disturbance (without being attacked) and those that had dropped following an attack. After 5 d of rearing, we dissected all aphids and counted the parasitoid larvae in each.

**Statistical analysis.** Aphid-parasitoid interactions may be influenced by crowding, which is a function of the number of aphids present in a constant plant-environment (Michaud & Mackauer, 1995). Therefore, we adjusted by ANCOVA the mean numbers of aphids disturbed and parasitized in each instar for differences in the numbers of available aphids in each cage; we used the approximate method of Gabriel (1978) to compare differences between the adjusted means. Pooling across females within treatments, we used the *G*-test of independence with Williams' correction (Sokal & Rohlf, 1995, p. 698) to test the statistical significance of differences between instars in the numbers of individuals parasitized and not parasitized. We assessed the relative risk of an aphid dropping from the feeding site by computing the log-odds ratio, corrected for

continuity, using L1 as a reference instar (Fleiss, 1981; Sokal & Rohlf, 1995, p. 764).

## Results

The mean numbers of aphids available and parasitized in each trial did not differ between instars (Table 1). Many dissected aphids contained more than one immature parasitoid (overall mean = 1.32 larvae), with superparasitism varying between an average of 1.22 larvae in L1 and 1.43 larvae in L3 hosts. Instars differed in the mean numbers of aphids disturbed by a foraging wasp (Table 1). First instars were the least likely, and L3 the most likely, to be disturbed and to display some form of anti-parasitoid behaviour including kicking, backing off, walking away and dropping in the absence of a direct attack. In contrast, after a successful attack and oviposition, L4 were the most likely, and L1 the least likely, to drop off a plant. We noticed, but could not quantify in our experiment, that L1, L2 and L3 aphids were all aggregated on the growing tips of bean plants, whereas L4 were more widely dispersed, feeding either alone or in loosely aggregated groups on the growing tip and the underside of the adjoining leaves.

Overall 34.5% ( $n = 911$ ) of pea aphids dropped from their feeding sites. In 67.5% of these cases, dropping occurred only after an ovipositional attack by *M. paulensis*. The proportions of 'droppers' varied between instars, in particular relatively more L4 than any younger instars dropped (Table 2). The relative risk of dropping, as measured by the odds ratio, was approximately 6-times higher for fourth- than first-instar pea aphids, independent of the stimulus, and approximately 16-times higher after successful parasitism. Dropping was not an effective tactic to prevent parasitism, however; once *M. paulensis* had grasped an aphid, none of the L1 and few L2 aphids succeeded in escaping (Figure 1). Also, rates of parasitism among 'droppers' and 'non-droppers' varied between instars. Whereas both groups were parasitized equally among L1 ( $G = 1.020$ ,  $P = 0.31$ ) and L3 ( $G = 1.994$ ,  $P = 0.16$ ), more 'droppers' than 'non-droppers' were parasitized among L2 ( $G = 11.881$ ,  $P = 0.0006$ ) and L4 ( $G = 28.667$ ,  $P < 0.0000$ ).

The percentage of females dropping from, or being knocked off, a plant due to an aphid's struggling against parasitism varied between 0% ( $n = 163$ ), 5.7% ( $n = 175$ ), 4.2% ( $n = 212$ ) and 23.6% ( $n = 123$ ) in encounters with L1, L2, L3 and L4, respectively; differences between L2 and L3 were not significant ( $G_3$

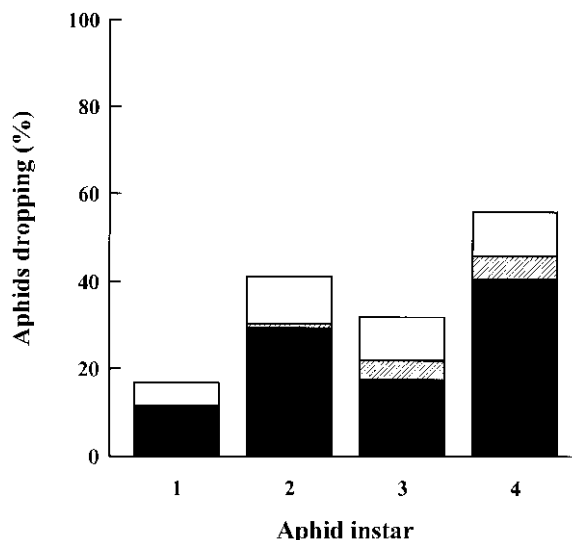


Figure 1. Percentages of individuals dropping among different nymphal instars of the pea aphid, *Acyrthosiphon pisum*, feeding on broad-bean plants in the laboratory. Dropping from the feeding site could be: 1 = a response to disturbance by a parasitoid wasp, *Monoctonus paulensis* (open bars); 2 = a consequence of an unsuccessful parasitoid attack, i.e., the aphid escaped (shaded bars); or 3 = a consequence of a successful parasitoid attack, i.e., the aphid dropped after handling by the parasitoid (solid bars). Percentages based on total numbers of aphids in each instar:  $n = 241$  (L1), 233 (L2), 272 (L3) and 165 (L4).

= 59.407,  $P = 0.0000$  for complete set;  $G = 0.441$ ,  $P = 0.932$  for non-significant subset by simultaneous test procedure).

## Discussion

Dropping from the feeding site is, perhaps, the greatest mortality risk in plant-feeding insects. Dropping in aphids is usually considered a defensive tactic against predators and parasitoids (Dixon, 1958; Montgomery & Nault, 1978; Roitberg & Myers, 1978, 1979; Clegg & Barlow, 1982; Brodsky & Barlow, 1986; Dill et al., 1990). Already parasitized pea aphids may drop from the feeding site when later attacked by a predator, a behaviour explained as 'adaptive suicide' (McAllister & Roitberg, 1987; McAllister et al., 1990). In contrast, in interactions between pea aphids and *M. paulensis*, dropping was mainly a side effect of successful parasitism. If dropping is a defense against attack, most 'droppers' should be unparasitized. Rates of parasitism were the same among 'droppers' and 'non-droppers' in L1 and L3, however, and relatively more, as opposed

Table 1. Interactions between *Monoctonus paulensis* and different nymphal instars of the pea aphid, *Acyrtosiphon pisum*; all aphids were feeding on broad-bean plants in the laboratory

	L1	L2	L3	L4	Significance
	<i>n</i> = 14	<i>n</i> = 14	<i>n</i> = 16	<i>n</i> = 10	
Available	17.21 ± 0.76	16.64 ± 0.55	17.00 ± 0.67	16.50 ± 0.40	$F_{3,50} = 0.238$ , $P = 0.869$
Disturbed	2.11 ± 0.99 <sup>a</sup>	5.54 ± 0.99 <sup>ab</sup>	7.02 ± 0.93 <sup>b</sup>	3.45 ± 1.17 <sup>a</sup>	$F_{3,49} = 4.976$ , $P = 0.004$
Parasitized	8.93 ± 0.87	7.74 ± 0.87	8.13 ± 0.81	7.05 ± 1.03	$F_{3,49} = 0.695$ , $P = 0.560$
Dropping	1.70 ± 0.51 <sup>a</sup>	4.58 ± 0.50 <sup>b</sup>	3.18 ± 0.47 <sup>ab</sup>	7.32 ± 0.60 <sup>c</sup>	$F_{3,49} = 18.51$ , $P = 0.000$

*n* = number of replicates (= females) in each treatment (= instar). Values are means ± SEM. Differences between means sharing the same letter(s) are not statistically significant ( $P \geq 0.05$ ). Mean numbers of aphids disturbed, parasitized, and dropping (after successful parasitoid attack) are adjusted by ANCOVA for numbers of aphids available in each trial; all differences between slopes are not significant.

Table 2. Differences in the proportions of individuals dropping between nymphal instars of the pea aphid, *Acyrtosiphon pisum*, in the presence of a parasitoid wasp, *Monoctonus paulensis*. All aphids were feeding on broad-bean plants in the laboratory

	L1	L2	L3	L4	Significance
	<i>n</i> = 241	<i>n</i> = 233	<i>n</i> = 272	<i>n</i> = 165	
Prop. dropping <sup>1)</sup>	0.166 <sup>a</sup>	0.412 <sup>b</sup>	0.316 <sup>b</sup>	0.558 <sup>c</sup>	$G_3 = 74.92$ , $P = 0.000$
Log-odds ratio	0.000	1.250 ± 0.217	0.836 ± 0.216	1.834 ± 0.233	
	<i>n</i> <sub>par</sub> = 127	<i>n</i> <sub>par</sub> = 107	<i>n</i> <sub>par</sub> = 131	<i>n</i> <sub>par</sub> = 69	
Prop. dropping <sup>2)</sup>	0.189 <sup>a</sup>	0.533 <sup>b</sup>	0.275 <sup>a</sup>	0.797 <sup>b</sup>	$G_3 = 87.62$ , $P = 0.000$
Log-odds ratio	0.000	1.571 ± 0.296	0.479 ± 0.297	2.783 ± 0.371	

*n* = total number of aphids in all trials; *n*<sub>par</sub> = total number of aphids parasitized in all trials. The proportions of aphids dropping, and the corresponding log-odds ratios, are computed in two ways: <sup>1)</sup> among all aphids (*n*), and <sup>2)</sup> among all aphids parasitized (*n*<sub>par</sub>); L1 is used as a reference instar. Differences between proportions sharing the same letter are not statistically significant ( $P \geq 0.05$ ).

to fewer, 'droppers' were parasitized in L2 and L4. Pea aphids attacked by *M. paulensis* were temporarily paralyzed (Calvert & van den Bosch, 1972b) and, therefore, were likely to fall off the plant when released by the parasitoid, especially large third and fourth instars. Similarly, winged sycamore aphids, *Drepanosiphum platanoidis* (Schrank), may be knocked off their feeding site when attacked by *Dyscritulus planiceps* Marshall; females of this parasitoid captured and subdued a host by grasping it with the fore legs after a short run (Völkl & Mackauer, 1996).

Dropping is potentially costly to an aphid in that it reduces feeding opportunity and, more important, increases mortality risk (Dill et al., 1990). Having dropped to the ground, an aphid may be exposed to ground-hunting predators or unable to return to a suitable host plant. When dropping is an active defense against attack, the probability of an aphid withdrawing its stylets and dropping should depend on the optimal balance between the value of the current feeding site and future mortality risk. First instars have low energy reserves and, therefore, should not favour drop-

ping as an escape tactic. In contrast, dropping is much less of a mortality risk in large L4 and adult aphids, which have higher energy reserves and are more mobile (Nault et al., 1973; Roitberg & Myers, 1978). Our results are consistent with this prediction with regard to first instars, but fourth instars did not drop from the feeding site as readily as expected to avoid an attack. Crowding increases the probability of an aphid dropping in response to alarm pheromone or physical contact. Because in our experiment fourth instars were least aggregated, they were less likely to be disturbed by a wasp's foraging activities.

In the absence of choice, females of *M. paulensis* equally accepted and oviposited in all four pea-aphid instars (Table 1), which suggests that large size did not prevent an attack. When given a choice, however, females selected the relatively smaller aphids among those available (A. Chau & M. Mackauer, unpubl.). Also, host choice was not correlated with parasitoid size in that small females preferred small and large females preferred large aphids. When host quality varies, parasitoids are expected to favour higher-quality,

large hosts over lower-quality, small hosts (Godfray, 1994). But a choice of small hosts may be adaptive if, for example, large hosts incur higher costs in opportunity time, are more difficult to capture and subdue, or put offspring at increased risk of mortality. A. Chau & M. Mackauer (unpubl.) showed that handling time and survival of *M. paulensis* varied between pea aphid instars, but differences were not of a magnitude to account for possible fitness costs in terms of reduced body size.

The escape tactics of aphids, presumably, have had a strong influence on the evolution of host choice and oviposition behaviour in the parasitoids of aphids. A parasitoid's strategy for host capture and oviposition may also act as a constraint on the range of available, as opposed to suitable, hosts. Theoretically, a female could attack a broad range of aphid sizes if she need not subdue the host for oviposition. In contrast, a female that must pursue and physically subdue a potential host is likely to be more successful attacking aphids that are small or not easily disturbed. For a female of *M. paulensis* to grasp an aphid with the fore legs, she must approach it within striking distance. Because first-nymphal instars of pea aphid were least likely to be disturbed (Dill et al., 1990; A. Chau & M. Mackauer, unpubl.), they were most susceptible to successive attacks by foraging wasps. Also, first-instar pea aphids were less at risk of dropping after successful parasitism than older instars. The probability of parasitoid offspring surviving in first, as opposed to later, instars consequently will be higher. Both hypotheses (increased availability and reduced offspring mortality risk), which are not mutually exclusive, can account for a flexible host choice, with the relatively smaller aphids being favoured by *M. paulensis* over larger and, in terms of resources for immature development, potentially more profitable instars.

## Acknowledgments

We thank Andrew Chow for useful discussion, and the Natural Sciences and Engineering Research Council of Canada for financial support.

## References

Brodsky, L. M. & C. A. Barlow, 1986. Escape responses of the pea aphid, *Acyrtosiphon pisum* (Harris) (Homoptera: Aphididae):

- influence of predator type and temperature. *Canadian Journal of Zoology* 64: 937–939.
- Calvert, D. J. & R. van den Bosch, 1972a. Host range and specificity of *Monoctonus paulensis* (Hymenoptera: Braconidae) a parasite of certain dactynotine aphids. *Annals of the Entomological Society of America* 65: 422–432.
- Calvert, D. J. & R. van den Bosch, 1972b. Behavior and biology of *Monoctonus paulensis* (Hymenoptera: Braconidae), a parasite of dactynotine aphids. *Annals of the Entomological Society of America* 65: 773–779.
- Clegg, J. M. & C. A. Barlow, 1982. Escape behaviour of the pea aphid *Acyrtosiphon pisum* (Harris) in response to alarm pheromone and vibration. *Canadian Journal of Zoology* 60: 2245–2252.
- Dettner, K., 1987. Chemosystematics and evolution of beetle chemical defenses. *Annual Review of Entomology* 32: 17–48.
- Dill, L. M., A. H. G. Fraser & B. D. Roitberg, 1990. The economics of escape behaviour in the pea aphid, *Acyrtosiphon pisum*. *Oecologia* 83: 473–478.
- Dixon, A. F. G., 1958. Escape responses shown by certain aphids to the presence of the coccinellid, *Adalia decempunctata* (L.). *Transactions of the Royal Entomological Society, London* 10: 319–334.
- Dixon, A. F. G. & W. A. Stewart, 1975. Function of the siphunculi in aphids with particular reference to the sycamore aphid, *Drepanosiphum platanoideis*. *Journal of Zoology, London* 175: 279–289.
- Evans, D. L. & J. O. Schmidt (eds), 1990. *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. State University of New York, Albany, NY.
- Fleiss, J. L., 1981. *Statistical Methods for Rates and Proportions*. 2nd ed. J. Wiley, NY.
- Gabriel, K. R., 1978. A simple method of multiple comparisons of means. *Journal of the American Statistical Association* 73: 724–729.
- Gerling, D., B. D. Roitberg & M. Mackauer, 1990. Instar-specific defense of the pea aphid, *Acyrtosiphon pisum*: influence on oviposition success of the parasite *Aphelinus asychis* (Hymenoptera: Aphelinidae). *Journal of Insect Behavior* 3: 501–514.
- Godfray, H. C. J., 1994. *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton, NJ.
- Griffiths, D. C., 1960. The behaviour and specificity of *Monoctonus paludum* Marshall (Hym., Braconidae), a parasite of *Nasonovia ribis-nigri* (Mosley) on lettuce. *Bulletin of Entomological Research* 51: 303–319.
- Heie, O. E., 1987. Morphological structures and adaptations. In: A. K. Minks & P. Harrewijn (eds), *Aphids: Their Biology, Natural Enemies and Control*. World Crop Pests, Vol. 2A. Elsevier, Amsterdam, pp. 393–400.
- Klingauf, F., 1967. Abwehr- und Meidereaktionen von Blattläusen (Aphididae) bei Bedrohung durch Räuber und Parasiten. *Zeitschrift für Angewandte Entomologie* 60: 269–317.
- Mackauer, M. & R. Sequeira, 1993. Patterns of development in insect parasites. In: N. E. Beckage, S. N. Thompson & B. A. Federici (eds), *Parasites and Pathogens of Insects*, Vol. 1: Parasites. Academic Press, San Diego, CA, pp. 1–23.
- McAllister, M. K. & B. D. Roitberg, 1987. Adaptive suicidal behaviour in pea aphids. *Nature* 328: 797–799.
- McAllister, M. K., B. D. Roitberg & K. L. Weldon, 1990. Adaptive suicide in pea aphids: decisions are cost sensitive. *Animal Behaviour* 40: 167–175.
- Michaud, J. P. & M. Mackauer, 1995. Oviposition behavior of *Monoctonus paulensis* (Hymenoptera: Aphididae): factors influencing reproductive allocation to hosts and host patches. *Annals of the Entomological Society of America* 88: 220–226.

- Montgomery, M. E. & L. R. Nault, 1978. Effects of age and wing polymorphism on the sensitivity of *Myzus persicae* to alarm pheromone. *Annals of the Entomological Society of America* 71: 788–790.
- Nault, L. R., L. J. Edwards & W. E. Styer, 1973. Aphid alarm pheromones: secretion and reception. *Environmental Entomology* 2: 101–105.
- Pickett, J. A. & D. C. Griffiths, 1980. Composition of aphid alarm pheromones. *Journal of Chemical Ecology* 6: 349–360.
- Roitberg, B. D. & J. H. Myers, 1978. Adaptation of alarm pheromone responses of the pea aphid *Acyrtosiphon pisum* (Harris). *Canadian Journal of Zoology* 56: 103–108.
- Roitberg, B. D. & J. H. Myers, 1979. Behavioural and physiological adaptations of pea aphids (Homoptera: Aphididae) to high ground temperature and predator disturbance. *The Canadian Entomologist* 111: 515–519.
- Sequeira, R. & M. Mackauer, 1992. Nutritional ecology of an insect host-parasitoid association: the pea aphid-*Aphidius ervi* system. *Ecology* 73: 183–189.
- Sokal, R. R. & F. J. Rohlf, 1995. *Biometry*. 3rd ed. W.H. Freeman, NY.
- Völkl, W. & M. Mackauer, 1996. ‘Sacking’ the host: oviposition behavior of a parasitoid wasp, *Dyscritulus planiceps* Marshall (Hymenoptera: Aphididae). *Journal of Insect Behavior* 9: 975–980.