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An experimental test of potential host range in the ant parasitoid *Apocephalus paraponerae*

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Abstract. 1. The work reported here tested experimentally whether specialisation in *Apocephalus paraponerae* was due to physiological interactions that limit the parasitoid to the host ant *Paraponera clavata*. The suitability of other ant species as hosts was tested, and behavioural traits that may promote a high degree of specificity within this host–parasitoid system are discussed.

2. Data for development time, number of puparia, and adult eclosion success for *A. paraponerae* ovipositing in the regular host *P. clavata* are provided. A new method for testing host suitability in parasitoids of ants is described. Eggs of *A. paraponerae* were transferred directly into potential ant hosts. The development time, number of puparia, and adults eclosed for the eggs transferred into other ant host species are compared with comparable data from *P. clavata*.

3. Seven ant species within the Ponerinae are suitable for the development of *A. paraponerae*. The potential host range is greater than the actual host range of *A. paraponerae*. Flies are not limited solely by host suitability of related ant species for the development of larvae. Host location and acceptance behaviours are proposed as the primary reasons for host specialisation. The large size of the primary host *P. clavata*, and the ability of multiple females to raise many offspring successfully from those hosts may influence the specialisation of *A. paraponerae* on *P. clavata*.

Key words. Ants, *Apocephalus*, Diptera, host range, host suitability, *Paraponera*, phorids, Ponerinae.

Introduction

Specialisation, the use of one or a few host species for development, is influenced by host location behaviours, the ecological availability of hosts, or the physiology of prospective hosts in herbivorous and parasitoid insects. Jaenike (1990) described several plant host traits that may encourage herbivore specialisation, such as host-specific adaptations to host physiology, interspecific competition for hosts, resistance to generalist predators, abundance and location of hosts, and mate finding. Several of these factors, such as mate finding, host-specific adaptations to host physiology, and resistance to generalist predators, also influence host range in parasitoids. Additionally, avoidance of competition and developmental strategy may limit host range to one or a few host species (Askew, 1994; Shaw, 1994), while trade-offs between detection of cues and the reliability of host presence in host location can constrain host selection (Vet *et al.*, 1991). Here, host-specific physiological adaptations by larvae to their hosts are assessed to determine whether they limit the range of hosts used by the specialised ant parasitoid *Apocephalus paraponerae* (Diptera: Phoridae).

Most recent research on specificity in insects has focused on phytophagous insects because many phytophagous groups have well-resolved host ranges and are especially diverse and abundant (Futuyma & Moreno, 1988; Farrell *et al.*, 1992). Traditionally, these studies rely on vast quantities of field collection records to assess whether herbivores are specialist or generalist in their feeding habits. Similar studies of parasitoid communities have described the relative host ranges of many hymenopteran parasitoids and, further, have identified historical influences, developmental strategy, and host feeding niche as factors that may influence host range (Askew & Shaw, 1986; Askew, 1994; Shaw, 1994). For example, in parasitoids, suitable hosts found within the same niche may be more likely to be found by searching parasitoids, and thereby accepted into the host range (Shaw, 1994).

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Although these nonexperimental studies rely heavily on published host records that may vary widely in quality and may not reflect actual host range accurately, the nonexperimental approach can provide a large quantity of data on different taxa from which generalities among groups can be drawn. There may, however, be collection biases based on whether the taxa are economically important or a group small enough to be sampled extensively (Shaw, 1994).

Experimental tests of the actual range of hosts for phytophagous insects are becoming more common. Host range of phytophagous insects can be tested through feeding trial experiments (Barone, 1996). In these experimental tests of host range, a number of ecological and physiological characteristics such as plant toughness, chemistry, light environment or growth rates have been identified as constraints on host range and reasons for herbivore specialisation (Coley & Barone, 1996; Bernays, 1998). A similar method has been used in hymenopteran parasitoids where females are manipulated to lay eggs on unusual host species, whereby the suitability of the host for parasitoid development can be assessed (Godfray, 1994).

The experimental strategy has a variety of advantages including the control of environmental conditions, specific criteria for selection of potential hosts, and definitive identification of hosts and emerging parasitoids. Additionally, the experimental approach can identify the potential host range rather than the actual range of hosts attacked in the field. By testing for additional suitable hosts within the habitat, it can be determined whether the organisms specialise because of physiological adaptations to their hosts or for other behavioural or ecological reasons.

In the work reported here, the actual host range of the specialist ant parasitoid *A. paraponerae* was compared with an experimentally determined potential host range to test the hypothesis that there are physiological limitations to host range.

Methods

Study system

Apocephalus paraponerae attacks primarily injured workers of the giant tropical ant *Paraponera clavata*. Two products from the mandibular glands of *P. clavata*, 4-methyl-3-heptanol and 4-methyl-3-heptanone, attract *A. paraponerae* males and females for feeding, mating, and ovipositing (Brown & Feener, 1991a; Feener *et al.*, 1996). These volatile chemicals are believed to be part of the alarm communication system of some ants but by themselves do not elicit an alarm response from *P. clavata* (Hermann *et al.*, 1984). Other studies have found *A. paraponerae* attracted to *Ectatomma* and *Pachycondyla* spp. in the field (Brown & Feener, 1991a; B. V. Brown, pers. comm.); however, behavioural, morphological, and genetic evidence indicates that *A. paraponerae* attacking *P. clavata* are highly specialised to this host only (Morehead, 1999).

Feener *et al.* (1996) hypothesised that the taxonomic distribution of olfactory cues determines *A. paraponerae* host range (i.e. only hosts with the same olfactory cues will be utilised by the parasitoid). The long-range host location chemicals used by *A. paraponerae* are found in several closely related ant species, including *Pachycondyla* spp. (Hölldobler & Wilson, 1990; Brown & Feener, 1991a). Feener *et al.* (1996) suggested that host range is not limited by host-specific physiological adaptations but that the distribution of the olfactory cues used by *A. paraponerae* and reinforcement in the use of these cues for mate location are more important. This idea is especially likely because *A. paraponerae* attacks injured and dying hosts that have reduced behavioural defences and compromised immune systems. Additionally, these parasitoid flies develop extremely rapidly, obviating the need for long-term evasion of the host's immune system and species-specific adaptations (Brown & Feener, 1991b). Physiological constraints were not therefore expected to limit host use and, further, it was predicted that *A. paraponerae* larvae could develop in a wide range of ant hosts.

Study sites

Experiments were performed at Barro Colorado Island, Panama (9°9′N, 79°51′W) and at La Selva Biological Research Station, Puerto Viejo de Sarapiqui, Heredia Province, Costa Rica (10°26′N, 83°59′W). Work at Barro Colorado Island was performed between June and September 1996, and August and September 1997, and at La Selva from June to August 1997, and January to March 1998. La Selva is a lowland tropical wet forest on the Atlantic slope of Costa Rica that receives about 4000 mm of rain per year (McDade *et al.*, 1994). Barro Colorado Island, part of the Smithsonian Tropical Research Institute, is an island within Lake Gatun in the centre of the Panama Canal. It is a lowland tropical moist forest, which experiences a more severe dry season than La Selva and receives about 2500 mm of rain per year (Leigh *et al.*, 1982).

Natural oviposition experiments

To establish a baseline for pupal success and adult eclosion rates in the natural host, *P. clavata*, natural egg-laying and larval development of *A. paraponerae* were quantified by measuring how many females were attracted to a single host, the total number of eggs laid per host, number of puparia, and the number of adults eclosing from these puparia. Two *P. clavata* workers collected from the same colony were crushed and set out 0.5 m apart on filter paper in small Petri plates within 1.0 m of the colony entrance. These ants were watched for 30 min, fly arrival and behaviour (whether feeding or ovipositing) being noted, although there were often too many flies to note all individuals. After 30 min, the flies present were collected, counted, and sexed. One ant from each pair was dissected and the number of fly eggs was counted. The other ant was placed in a plastic cup with a cotton ball and the number of larvae, puparia, and adults emerging was recorded. Adult flies that emerged were placed in vials containing 80% ethanol and preserved for further morpho-

logical analysis. By dissecting eggs from one ant, and allowing the other host's eggs to develop, estimates could be obtained of how many of the eggs laid by females survive to puparation without damaging the eggs by counting them in the host.

Forty-two of these trials were performed, 14 at Barro Colorado Island in 1997, 14 at La Selva in 1997, and 14 at La Selva in 1998. These data were pooled across sites and years for analysis because the number of trials was small for each site and year. The number of eggs laid in one set of workers was compared with the number of puparia from the other set using a paired *t*-test to assess whether the number of eggs laid was the same as the number of larvae that formed puparia. A paired *t*-test was also used to examine whether equal numbers of females were attracted to each ant host. If there were equal numbers of females attracted to each ant in the pair, it was assumed that approximately the same number of eggs was laid in each ant, the one dissected and the one from which larvae emerged. Pearson's correlation statistic was used to determine whether there was a significant correlation between the number of females attracted to an ant and the number of eggs or puparia from the ant. These data estimate the number of eggs laid per female and the number of eggs that survive to puparation. The number of adult flies eclosing from puparia was also assessed.

Table 1. Species tested for host suitability in the development of *Apocephalus paraponerae* and their mandibular gland chemicals.

Subfamily	Species	Mandibular gland product or alarm pheromone
Ponerinae	*P. clavata*	4-methyl-3-heptanone
	P. apicalis	4-methyl-3-heptanone*
	P. obscuricornis	4-methyl-3-heptanone*
	P. villosa	4-methyl-3-heptanone
	E. tuberculatum	2-hexanone†
	E. ruidum	2-hexanone†
	O. minutus	Alkyl pyrazines*
	O. opaciventris	Alkyl pyrazines*
	O. chelifer	Alkyl pyrazines†
	O. erythrocephalus	Alkly pyrazines *
Formicinae	*C. sericeiventris*	Formic acid*
Myrmicinae	*Atta colombica*	4/5-methyl-3-heptanone*

*Denotes probable alarm pheromone, based on that determined for congeneric species.
†As determined by A. Attygalle (pers. comm.).
Modified from Hölldobler and Wilson (1990).

Morphological measurements as indications of fly fitness and ant host suitability

In order to compare the body size of flies from various host species with the body size of adult flies from the regular host *P. clavata*, adult *A. paraponerae* flies emerging from *P. clavata* workers that had been parasitised under field conditions were measured. Two different characters were measured for morphological analysis of body size: hind femur length and length of the costal wing vein. Hind femur length was used as a measure of body size because it is a fixed and rigid body part that does not change with food consumption or number of eggs carried. Costal vein length was measured because wing length asymmetries have been shown to be important for survival and female choice in another dipteran, the domestic fly (Møller, 1996). Relative and absolute asymmetry in these morphological characters was tested using the same procedure as Møller (1996). All morphological measurements were obtained using an ocular micrometer attached to a Zeiss Stemi SV 6 dissecting scope (Carl Zeiss Inc., Thornwood, New York). Linear regression was used to determine whether pupal number had an effect on body size in order to assess whether crowded larval conditions reduce adult body size, as has been shown in a variety of hymenopteran parasitoids (Godfray, 1994). Pearson's correlation was used to test for correlation between hind femur length and costal vein length to determine whether these were consistent measures of adult body size.

Host suitability

Because *A. paraponerae* does not lay eggs reliably in host ants other than *Paraponera clavata* in the field, the potential range of hosts was assessed by artificially parasitising workers of other ant species to determine whether they were suitable for the development of *A. paraponerae*. Eggs for transfer into alternate host ant species were collected by leaving crushed *P. clavata* workers in the field for 1 h. These parasitised workers were brought into the laboratory and the parasitoid eggs were dissected out. The eggs are large and are generally laid in the abdomen between the sclerites or into the damaged parts of the ant's thorax (Brown & Feener, 1991a). The dissections were performed in Insect Dissection Ringers Solution (149 mM NaCl, 40 mM KCl, and 9 mM $MgCl_2$), and the eggs were kept in this solution until transfer (Miller, 1980).

Twelve ant species were collected from the field for artificial parasitisation. Species were chosen using three criteria: variation in taxonomic relatedness to *Paraponera clavata*, variation in size and colour relative to *P. clavata*, and chemistry of their mandibular glands (Table 1). At Barro Colorado Island, the species *Paraponera clavata*, *Ectatomma tuberculatum*, *E. ruidum*, *Camponotus sericeiventris*, *Atta colombica* (media worker caste), *Odontomachus chelifer*, *O. minutus*, and *Pachycondyla villosa* were used. At La Selva, the species that were parasitised artificially were *P. clavata*, *E. tuberculatum*, *E. ruidum*, *O. erythrocephalus*, *O. opaciventris*, *P. villosa*, *P. apicalis*, and *P. obscuricornis*.

Ant workers were chilled on ice for easier handling. Using a drawn-out glass pipette, an egg was inserted into the abdomen of a test worker, usually between the first and second dorsal tergite of the abdomen. Experimentally parasitised workers were then put in plastic cups or vials with a cotton ball, covered with cloth held in place with a rubber band, and left at ambient temperature. They were kept moist by daily misting.

Because many of the ants in this study were substantially smaller than *P. clavata*, and the interest was only in whether

the hosts were suitable for development of *A. paraponerae*, one egg was transferred into each test individual. Under natural conditions, an individual host of *P. clavata* may attract many females of *A. paraponerae* and receive up to 40 eggs. This method reduces larval competition within hosts, as might occur with more than one egg per host, and may maximise the probability of larval development within each ant species if there is a significant decrease in larval fitness as egg number increases. Alternatively, there may be a *group effect* whereby more eggs increase the success of the larvae by overwhelming the host's immune defences (Godfray, 1994). A test of the group effect in *A. paraponerae* larvae is described below.

Data were pooled by host species across sites and years because there were no significant differences between sites and years in the number of larvae emerging to form puparia. The numbers of fly puparia from the various species were compared using log-likelihood ratio (G) tests. Additionally, the data were pooled in two other ways: first by genus to test for phylogenetic effects, and second by body size to test for effects of host size. Small ants included *E. ruidum*, *E. tuberculatum*, *O. minutus*, and *P. obscuricornis* with average mass <0.02 g. Medium species with average mass 0.02–0.10 g were *O. chelifer*, *O. erythrocephalus*, *O. opaciventris*, *P. apicalis*, and *P. villosa*. *Paraponera clavata*, weighing >0.10 g, was the only large ant. These pooled data were also analysed using G-tests. The sequential Bonferroni procedure was used to adjust for multiple comparisons to a table-wide P-value of 0.05 (Rice, 1989).

The development time of *A. paraponerae* larvae in the different host species was also compared by recording the number of days to puparation. An ANOVA with days to puparation as the dependent variable and host species as the categorical independent variable was used to test whether host species affected development time.

Group transfer experiments

A large number of eggs laid naturally in *P. clavata* may increase the success of the parasitoids in developing from this host by a *group effect* (Godfray, 1994). Therefore, variable numbers of eggs were inserted into the abdomen of *Paraponera clavata* workers using the methods described above. In order to keep egg number in each class more consistent for each group, 10 eggs were inserted into one ant, five eggs into another, and one egg into five ants. A total of 12 groups was parasitised. The proportion of eggs developing into larvae within each ant from the three classes was compared. These data were arcsin transformed and analysed using one-way ANOVA. If there was a group effect, it was expected that a greater proportion of larvae would develop in the ants that had more eggs inserted.

Results

A direct measure of fitness of *A. paraponerae* emerging from various hosts is unavailable so several indirect fitness measures were used to assess host quality. First, larval development times, pupal success, and adult eclosion success for *A. paraponerae* with its regular host *P. clavata* are described. Larval development time, the number of pupae surviving, and the number of adult flies emerging from *P. clavata* hosts will give an estimate of the quality of *P. clavata* as a host for *A. paraponerae*. The morphometrics of flies emerging from *P. clavata* in natural ovipositing situations and in experimental egg transfers are also described. Adult fly costal vein length and hind femur length were used as indirect fitness measures, larger flies being more fit. Flies emerging from *P. clavata* are compared with other ant host species, using these indirect measures of fitness to compare the quality of these other ants as hosts for *A. paraponerae*.

Natural oviposition of Apocephalus paraponerae

Apocephalus paraponerae females laid a mean of 7.88 ($\pm$ 6.48) eggs per *P. clavata* worker. A mean of 8.64 ($\pm$ 8.02) puparia emerged from each worker, and a mean of 2.64 ($\pm$ 4.0) adults emerged from each ant worker. The number of females captured did not differ between the paired *P. clavata* workers (6.75 ± 5.3 vs. 7.94 ± 5.6, paired $t = 1.01$, d.f. = 35, P = NS). There was no significant difference between the number of eggs laid and puparia from the workers (paired t-test, $t = -0.52$, d.f. = 41, P = NS), however only 30% of the puparia produced adult flies. The number of females captured at the end of the trial (range 0–21 females) was correlated positively with the number of eggs laid ($r = 0.34$, $\chi^2 = 4.14$, $n = 36$, $P < 0.05$) and the number of puparia ($r = 0.65$, $\chi^2 = 18.7$, $n = 36$, $P < 0.001$). The number of eggs laid per ant ranged from zero to 25 and the number of puparia from each ant ranged from zero to 32. These results suggest that the number of females present may be a reliable indicator of the number of oviposition events.

Morphological analysis of body size

Adult body size in parasitoid insects is an indirect measure of fitness. Larger individuals generally produce more offspring than smaller individuals and may live longer (van den Assem *et al.*, 1988). Therefore the relative fitness of individuals from a variety of larval environments can be estimated by comparing adult body size. Analysis of morphological characters of eclosed *A. paraponerae* adults showed that males and females differed significantly for average costal vein length ($t = 11.44$, d.f. = 163, $P < 0.001$) and average hind femur length ($t = 6.98$, d.f. = 179, $P < 0.001$; Fig. 1). Males are smaller than females for both characters, so in all subsequent analyses, males and females were analysed separately. Average costal vein length and average hind femur length were correlated highly with one another in both females ($r = 0.87$, $n = 83$, $P < 0.001$) and males ($r = 0.77$, $n = 81$, $P < 0.001$), demonstrating that these are consistent measures of adult body size.

The larval environment of *A. paraponerae* had an impact on the body size of eclosing adults. There was no difference in the sex ratio of adult flies from *P. clavata* hosts (49% males,

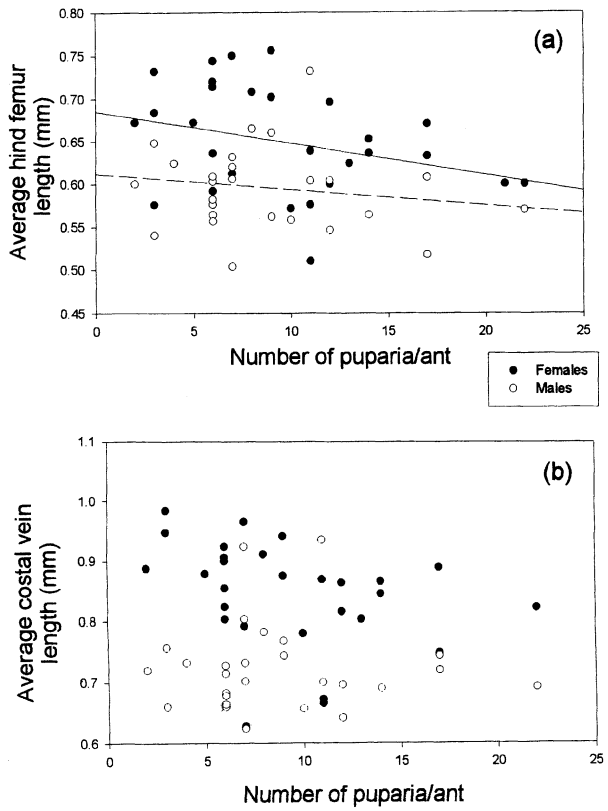

Fig. 1. Body size measurements of *Apocephalus paraponerae* vs. the number of puparia per ant from which the flies were emerging. The regression line for females is represented by a solid line, and for males by a dashed line. (a) Data for hind femur length. Females have a significantly negative regression of average hind femur length vs. number of puparia per ant ($y = 0.744–0.009x$, $r^2 = 0.24$, $n = 39$) as do males ($y = 0.657–0.005x$, $r^2 = 0.22$, $n = 38$). (b) Average costal vein length. Neither females nor males showed significant regression of costal vein length vs. puparia per ant host.

$\chi^2 = 0.085$, d.f. = 1, P = NS). There was a negative regression of average hind femur length on the number of puparia from an ant host for females ($r = –0.49$, $F_{1,37} = 11.93$, $P = 0.001$; Fig. 1a) but no regression of costal vein length of flies and puparia per ant ($F_{1,36} = 3.11$, P = NS; Fig. 1a).

Males showed a significant negative regression of average hind femur length on the number of puparia ($r = –0.47$, $F_{1,28} = 8.28$, $P < 0.001$; Fig. 1a), however there was no significant regression of costal vein length and puparia number for males ($F_{1,22} = 0.08$, P = NS, Fig. 1b). This result suggests that it may be important for adult flies to maintain absolute wing size but that hind femur length may be more variable because it may have little impact on fitness.

No relative or absolute asymmetry in the morphological characters, hind femur length and costal vein length, was detected in the measurements. Additionally, neither relative nor absolute asymmetry showed a significant regression of puparia number with either of the morphological characters measured. It is possible, however, that the measurements were not precise enough to detect any asymmetries (Møller, 1996), and further analysis of morphological characters may provide enough variation to assess host quality by this measure.

Host suitability

Apocephalus paraponerae adults developed successfully in seven species in four genera. An additional three species were suitable for larval development but none of these ant species produced puparia that eclosed as adults. Larvae did not develop in either *Atta colombica* (Myrmicinae) or *Camponotus sericeiventris* (Formicinae). Host quality differed among ponerine ant species as shown by the fraction of eggs that developed into puparia in different ant host species ($G = 67.12$, d.f. = 11, $P < 0.001$). The natural host *Paraponera clavata* had the second highest per cent success (30.7%) of puparia (Table 2). *Pachycondyla apicalis* had the highest per cent success (38.5%) but did not differ from *P. clavata* (Tukey's *post hoc* comparison). These species were followed in order by *P. villosa*, *P. obscuricornis*, *E. tuberculatum*, *O. chelifer*, *O. opaciventris*, *E. ruidum*, *O. erythrocephalus*, and *O. minutus* (all within the subfamily Ponerinae). Pooling these data across Ponerine genera, there is a significant difference among genera in the number of puparia from the transfer experiments ($G = 27.43$, d.f. = 3, $P < 0.01$). The genus *Pachycondyla* had 31.6% of the eggs developing into puparia, *Paraponera* 30.8%, followed by *Ectatomma* 19.2%, and *Odontomachus* 10.7%. Pooling species by body size, the number of puparia differed among size classes ($G = 4.77$, d.f. = 2, $P < 0.05$). Small ponerine ants had only 18.4% of puparia while the medium and large ants had 21.8 and 30.7% of puparia respectively.

Comparison of body size measurements of *A. paraponerae* emerging from *P. clavata* hosts in natural experiments and those from host transfer experiments showed that neither hind femur length nor costal vein length differed for males or females (Fig. 2). This result indicated that the experimental method did not have an impact on the adult body size of the flies developing in the primary host.

Morphological measurements of the adult *A. paraponerae* eclosing from hosts of the above genera showed that hind femur length of males was greater in the largest host size class ($F_{3,11} = 4.54$, $P < 0.05$), but not for females ($F_{3,8} = 0.35$, P = NS; Fig. 3). Average costal vein length did not differ among flies emerging in different genera for males ($F_{3,7} = 3.24$, P = NS) or females ($F_{3,5} = 0.81$, P = NS).

Group transfer of eggs

The proportion of eggs that resulted in puparia did not differ among ants that had 10 eggs (35%), five eggs (25%), or one egg (41%) artificially inserted ($F_{2,33} = 1.45$, P = NS). Average hind femur length did not differ between group egg transfers and single egg transfers into *P. clavata* ants for males ($t = 0.3$, d.f. = 5, P = NS) or females ($t = 0.4$, d.f. = 3, P = NS).

Table 2. Results of egg transfer experiments. Host ant species are ranked by average body size, followed by per cent adult success.

Subfamily	Size class	Species	Mass (mg)*	Number of trials	Number of puparia	% pupal success	Average days to puparation	Number of adults	% adult success
Ponerinae	Large	*Paraponera clavata*	128	130	40	30.7	6.73	5	12.5
	Medium	*Pachycondyla villosa*	34	32	9	28.0	5.22	2	22.2
		P. apicalis	24	52	20	38.5	6.40	5	25.0
		O. chelifer	29	51	7	13.7	6.14	0	0.0
		O. opaciventris	29	51	7	13.7	7.14	3	42.8
		O. erythrocephalus	29†	15	1	6.6	9.0	0	0.0
	Small	*E. tuberculatum*	19	131	33	25.1	6.48	7	21.2
		E. ruidum	11	87	9	10.3	6.56	1	11.0
		P. obscuricornis	17	52	14	26.9	7.21	4	28.5
		O. minutus	14	50	3	6.0	6.66	0	0.0
Myrmicinae	Medium	*A. colombica*‡	23	28	0	0.0	–	–	–
Formicinae	Medium	*C. sericeiventris*‡	29†	30	0	0.0	–	–	–

*Average mass of five or six individual workers collected in the field.
†Not measured directly. Average body mass of other species in the same size class.
‡Not included in size class analysis.

Discussion

This is the first study to determine potential host range experimentally for a dipteran parasitoid. It is also the first time that the method of direct egg transfer has been used to test host suitability. Most studies of hymenopteran parasitoids force females to oviposit on unusual hosts (Godfray, 1994); the method described here removes adult females from the process, thereby reducing the possibility of confounding effects from chemical, visual, and other signals that females may use in host choice. There is also no possibility that other chemicals laid with the egg would have an effect on larval success because this is not a significant factor in dipterans as it may be in hymenopteran parasitoids (Feener & Brown, 1997). This study highlights host characteristics that may encourage or influence the specialisation by this parasitoid and illustrates whether these specialists are limited by host location behaviours and chemical cues or by larval–host interactions at the physiological level.

These results show that several ant species within the subfamily Ponerinae are suitable for the development of *A. paraponerae* larvae. They also suggest that larvae are not highly adapted to *Paraponera clavata* hosts and that physiological interactions between *A. paraponerae* larvae and their hosts are not the only factors that limit host range. Ant species in the subfamilies Myrmicinae and Formicinae, however, while not tested extensively, do not appear to provide suitable larval environments for parasitoid development, indicating a taxonomic limit to host suitability; *A. paraponerae* cannot use every ant species available in the environment. This result suggests that there are some physiological or immunological constraints that correspond with ant species relatedness whereby *A. paraponerae* can develop in ant species closely related to their regular hosts, but not in all ant species. In the experimental tests, none of the eggs transplanted into *Atta* and *Camponotus* developed into larvae, suggesting that the eggs died before emerging as larvae. There may be some structural, chemical, or nutritional aspect of host quality that constrains *A. paraponerae* to ponerine ant species.

These results also showed that *Pachycondyla* species, which are in the tribe Ponerini, are excellent hosts for *A. paraponerae* larvae. This genus ranked higher in pupal success than *Paraponera*, the regular host. The similarities in mandibular gland contents between *Paraponera* and *Pachycondyla*, despite being in different taxonomic tribes, may indicate other chemical or immunological similarities (see Table 1) that contribute to *Pachycondyla* being highly suitable hosts.

Another possible explanation for high larval success in *Pachycondyla* is that smaller ants like *Pachycondyla* spp. may have softer exoskeletons/cuticles, allowing easier larval exit and therefore higher larval success in these experiments. The large number of eggs normally laid in *P. clavata* hosts may help emerging larvae to breach the exoskeleton of the ant, however the results from multiple egg transfer experiments showed that more eggs do not result in higher adult eclosion from *Paraponera clavata* hosts and do not support that hypothesis.

There may be other traits that influence *A. paraponerae* to lay eggs on *Paraponera* over the equally suitable *Pachycondyla*. The most obvious host characteristic difference between the two genera at La Selva and Barro Colorado Island is that *Paraponera clavata* are substantially larger than all the *Pachycondyla* species, and therefore can probably support a larger number of offspring. The analysis of host body size, discussed below, supports this suggestion.

Ectatomma, from the tribe Ectatommini along with *Paraponera clavata*, is also a good host, as demonstrated by the number of puparia. The genus *Odontomachus*, from the tribe Odontomachini, had the next highest number of puparia. These genera have different mandibular gland chemistry than

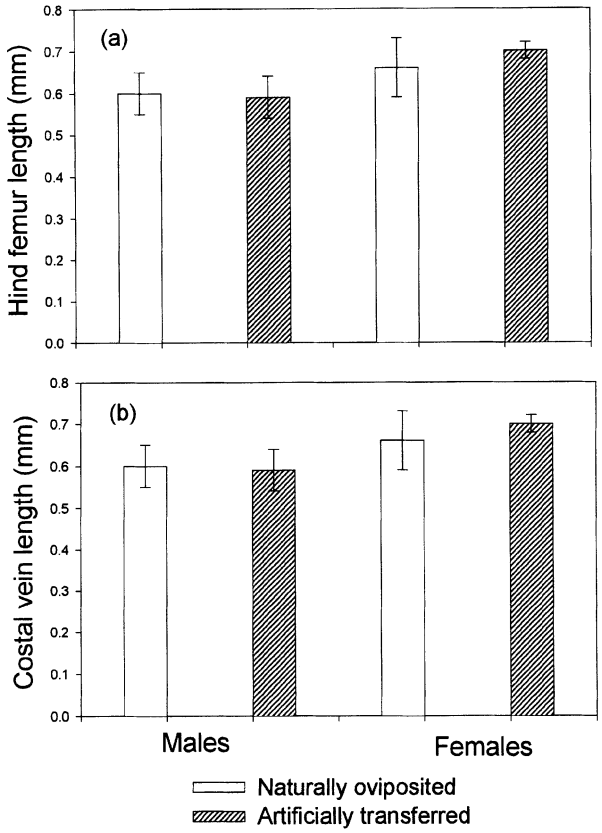

Fig. 2. Comparison of (a) average hind femur length and (b) average costal vein length in *Paraponera clavata* from natural oviposition events and egg transfer experiments. Error bars represent 1 SD.

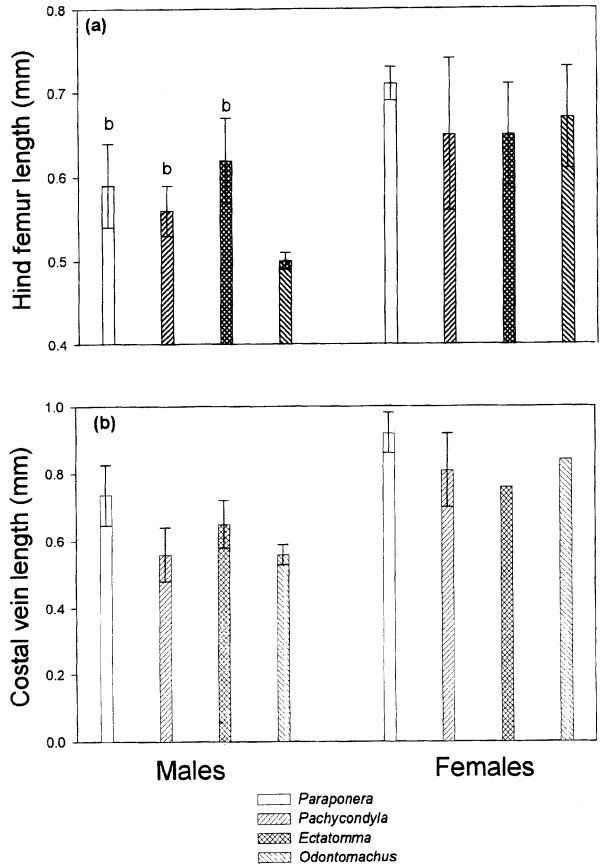

Fig. 3. (a) Average hind femur length and (b) costal vein length of *Apocephalus paraponerae* emerging from different host genera. Error bars represent 1 SD. Where there are no error bars, $n = 1$. Letters above bars indicate significant differences among genera (Tukey's HSD, $P < 0.05$).

Paraponera (Hölldobler & Wilson, 1990; A. Attygalle, pers. comm.), and there may also be differences in nutritional quality of these ants that affect *A. paraponerae* development.

Host ant body size contributed to an ant species' suitability as a potential host. Larger ants were generally better hosts, and flies were more likely to develop in a large host. It is unknown, however, whether larger body size inherently makes those species better hosts or whether larger species are easier to parasitise artificially. Further study on the impact of host body size effects on larval development of *A. paraponerae* is necessary in order to eliminate the possibility of experimental artefact of transferring fly eggs into smaller hosts.

Body size of adult parasitoid insects is often correlated positively with fecundity (Godfray, 1994). Measurements of adult body size of flies emerging from *P. clavata* that were parasitised naturally in the field versus those parasitised artificially in the laboratory suggest that the experimental procedure did not have any measurable effect on *A. paraponerae* adult body size. This result is important to establish a baseline expectation of adult body size within the transfer experiment and for comparisons of different genera as potential hosts. The measurements of adult body size of flies eclosing from the experimental genera indicate that, while there were no differences in the sex ratio of adult flies emerging throughout the experiments, males with smaller hind femur length emerged from the smaller ant genera. Males from *Odontomachus* species were smaller than all the others, suggesting that host size and quality may affect host choice and the realised host range. The results for females are inconclusive. Two genera, *Ectatomma* and *Odontomachus*, had only one female adult eclose, which greatly affected statistical power. These observations suggest that adult size may be more important for females than for males, and males can be relatively successful, despite smaller body size. Male body size may be less important than female body size because males perform courtship displays in flight (S. A. Morehead, pers. obs.; D. Feener, pers. comm.) and may need to be good fliers, not large individuals, in order to obtain mates. In females, however, fitness is proportional to the number of eggs a female can carry, which is related directly to overall body size.

The results presented here suggest that there are plenty of suitable hosts within the environment so it is unlikely that

specificity is being driven by host-specific adaptations of larvae to hosts. Additionally, the result that host body size affects adult fly body size suggests that it is more likely that body size of hosts is an important factor in host choice. Previous study has shown that the flies use body size as a visual signal to distinguish among host species (S. A. Morehead and D. H. Feener, unpublished). Host acceptance behaviours can limit host range more effectively because they depend on cues that are host species-specific, rather than the long-range olfactory cues that are shared by a variety of possible ant hosts (Godfray, 1994).

Additionally, host choice of *P. clavata* may be based on its body size and ability to support a large number of larvae. In this study, *Pachycondyla* species are equally suitable hosts for *A. paraponerae*. They have the same long-range olfactory signals as *P. clavata* but are not consistently attacked in the field and probably do not have the necessary short-range visual and chemical host acceptance cues that *A. paraponerae* use to find their hosts (S. A. Morehead and D. H. Feener, unpublished). They are also smaller in body size and different in colour from *P. clavata*. *Apocephalus paraponerae* may be adapted to efficiently locate high quality, large hosts such as *P. clavata* for mating and ovipositing because the flies lay gregariously and hosts are probably rare and ephemeral. Alternatively, Fox (1993) has suggested that females may avoid laying eggs on closely related suitable host species in order to avoid similar host species that may be unsuitable for larval development. This behaviour is not important in this system because there are other hosts that have similar chemical cues and are also suitable hosts for larval development.

Long- and short-range host location cues, host size, and physiology influence host range and, in general, may cause parasitoid ranges to be relatively narrow (Shaw, 1994), however insects that have a broad potential host range and a narrow actual host range may have the opportunity to expand their host range or switch hosts, perhaps leading to sympatric speciation.

Speciation occurring via the incorporation of new hosts into the host range is not without precedent. Jaenike and Papaj (1992) suggested that learning and motivation of searching female herbivores may allow phenotypic plasticity in host choice and may promote subsequent speciation. Another study of dipteran herbivores suggested that proximity of appropriate host odours to other possible hosts can promote host switching and possibly lead to sympatric speciation (Prokopy, 1996). Learning host cues to reinforce host use has not been ruled out as a factor in host choice in the *A. paraponerae* system. These results indicate that a host switch based on similarities in host chemistry, especially between the related genera *Paraponera* and *Pachycondyla*, is possible because these flies are not physiologically limited to one host species. Additionally, a host switch onto another distantly related ant species with similar glandular chemistry and geographic distribution is possible. The behaviours influencing host specificity by *A. paraponerae* require further investigation in a variety of locales and will provide valuable insight into the possible mechanisms that maintain and modify host range in parasitoids.

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