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Evidence for a cryptic species complex in the ant parasitoid *Apocephalus paraponerae* (Diptera: Phoridae)

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ABSTRACT

Cryptic species complexes occur in many taxa, in particular in the insect order Diptera. Here we describe a possible new cryptic species complex in the family Phoridae. Three lines of evidence suggest that *Apocephalus paraponerae*, an ant parasitoid, is actually a complex of at least four genetically distinct but morphologically almost indistinguishable populations attacking at least three different ant hosts. First, the host-location cues used by *A. paraponerae* to locate two of the host species differ. Second, *A. paraponerae* attracted to these two ant host species differ consistently in average hind femur length and costal vein length, two measures of body size. Finally, mtDNA sequence comparisons of individuals from a variety of locations and host ant species indicate high sequence divergence between populations and low sequence divergence within populations. We discuss aspects of host location behaviour that may be important in cryptic species formation, and we speculate that many such cryptic complexes may exist in this family and others with similar mechanisms of host location and exploitation.

Keywords: Ants, *Apocephalus*, cryptic species, *Ectatomma*, host location, parasitoids, phorids, *Paraponera*.

INTRODUCTION

Cryptic species, morphologically indistinguishable but genetically distinct populations, have been found in a wide variety of taxa (Mayr and Ashlock, 1991). Many cryptic species complexes can be distinguished only by studying genomic characters (e.g. McLea and Lambert, 1985; Beebe and Saul, 1995) or cuticular hydrocarbons (Sutton and Carlson, 1997). However, some populations of herbivores and parasites have distinct ecological or behavioural characteristics, such as the use of different host species, that may also allow

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identification of the separate species (Waring *et al.*, 1990; Clopton and Gold, 1996). In parasites and herbivores, host- and mate-finding behaviours may induce the separation of populations (Bush, 1975; Prokopy *et al.*, 1988). Here we describe a complex of cryptic species in which populations exhibit highly specialized mating and oviposition behaviours that are specific to particular host ant species.

Diptera is one of the largest insect orders and includes about 60,000 described species of parasitic flies (Feener and Brown, 1997). Cryptic species complexes have been identified in at least a dozen dipteran families, including Culicidae, Psychodidae, Neriidae, Tephritidae, Simuliidae, Calliphoridae and Tabanidae. Although these groups are not parasitic, many of the cryptic species show highly specialized behaviours and resource use. Many were discovered after the failure of eradication projects for pests and disease vectors (Richardson *et al.*, 1982) and the subsequent identification of subtle genetic and behavioural differences among morphologically indistinguishable populations.

Here we show that the specialized ant parasitoid *Apocephalus paraponerae* (Diptera: Phoridae) includes several sympatric and allopatric cryptic species that vary in host-finding, mating and oviposition behaviour.

METHODS

Study system

Apocephalus paraponerae males and females mate, feed and oviposit on injured workers of the ant *Paraponera clavata* (Formicidae: Ponerinae). Many flies are attracted to an ant host, and several females can lay multiple eggs within a host. Flies lay eggs between sutures in the ant's exoskeleton or in wounds in the exoskeleton (Brown and Feener, 1991a). Eggs develop rapidly inside the ant, emerging from the host as larvae after about 3 days. The larvae then crawl into the leaf litter for puparation (Brown and Feener, 1991b).

The compounds 4-methyl-3-heptanone and 4-methyl-3-heptanol, which are found in the mandibular glands of *P clavata*, are used by adult *A. paraponerae* as long-range host-location cues (Brown and Feener, 1991a; Feener *et al.*, 1996). These compounds, which are released when the ants are injured or alarmed, are believed to be part of the ant's alarm communication system, even though either compound alone does not elicit the alarm response in *P clavata* (Hermann *et al.*, 1984).

Apocephalus paraponerae is occasionally attracted to other ant species in the subfamily Ponerinae, including *Ectatomma tuberculatum* and *Pachycondyla* species. *Ectatomma* is thought to be closely related to *Paraponera*, although *Paraponera* has recently been placed in its own tribe (Lattke, 1994).

Study sites

We performed field experiments at La Selva Biological Research Station in Heredia Province, Costa Rica, and at Barro Colorado Island (BCI), Panama. La Selva is a well-studied lowland tropical wet forest on the Atlantic slope of Costa Rica, which receives about 4000 mm of rain per year (McDade *et al.*, 1994). BCI is located in Lake Gatun of the Panama Canal; it receives approximately 2500 mm of rainfall annually and experiences a more severe dry season than La Selva (Windsor, 1990).

Chemical attraction experiments were performed within the successional plots and in the Holdridge Arboretum at La Selva in June of 1995 and 1997 and during January to April 1998. Egg transfer experiments were performed at La Selva and BCI during 1997 and 1998. Specimens for DNA sequencing were collected at both field sites in 1997 and 1998 and were stored in 80% ethanol.

Chemical attraction experiments

To document the cues used by *A. paraponerae* to locate alternative ant hosts, we conducted two experiments to address whether 4-methyl-3-heptanone and 4-methyl-3-heptanol were used to find the host *E. tuberculatum*. In the first experiment, we assessed whether the long-range host-location cues were in the heads of *Ectatomma*, as in *P. clavata*, and whether head removal would reduce the attractiveness of the ant to *A. paraponerae*. We immobilized two ant workers of either *E. tuberculatum* or *P. clavata* by crushing the thorax. We removed the head of one worker (headless), whereas the other ant was left intact. The ants were placed on filter paper in small petri dishes approximately 1.0 m apart near a colony entrance. All flies attracted to the ant workers during a 15-min interval were collected with an aspirator. The flies were later brought to the laboratory, counted and sexed under a dissecting microscope.

In the second experiment, we wished to determine whether we could rescue the attractiveness of headless ant workers of *E. tuberculatum* and *P. clavata* by direct application of the long-range olfactory cues used by *A. paraponerae* in host location. In these trials, headless workers of both species were placed with a cotton ball soaked in 4-methyl-3-heptanone and 4-methyl-3-heptanol in a 9 : 1 ratio. This amount and mixture is equivalent to that contained in a single worker of *P. clavata* (Hermann *et al.*, 1984). A total of 300 μg of these compounds was added to olive oil in a 1 : 10 ratio (Feener *et al.*, 1996). Again, all flies attracted to the two ants were collected, counted and their sex determined.

We performed one trial at each of six colonies of *P. clavata* and six colonies of *E. tuberculatum*. The number of flies attracted to each species was pooled across trials within experiments. The results were analysed using binomial probabilities with the expectation that each worker would attract an equal number of flies ($p = q = 0.5$). Additionally, we analysed our results in two other ways; first, within an experiment across ant host species and, second, within host species across experiments using log-likelihood ratios (G-tests).

Body size measurements

In observing *A. paraponerae* attracted to *P. clavata* and *E. tuberculatum* hosts in the field, we noted that the flies attracted to *P. clavata* appeared to be larger than those attracted to *E. tuberculatum*. Overall body size, however, is not generally used as a morphological character for taxonomic purposes in insects because body size within insect parasitoid species frequently varies with host size (Waage, 1986; Godfray, 1994). Larger individuals tend to emerge from larger hosts. We quantified body-size differences in the flies at the two hosts and then looked for consistent size differences among flies that were attracted to and emerged from two hosts of different sizes.

To compare the body size of *A. paraponerae* in the two host ant species, we measured adult *A. paraponerae* flies eclosing from *P. clavata* and *E. tuberculatum* workers. We also measured adult flies that were attracted to these two hosts in the field. We measured two characters for 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 body part that does not change with food consumption or number of eggs carried. We measured costal vein length because wing length asymmetries have been shown to be important for survival and for female choice in another dipteran, the domestic fly (Møller, 1996). Morphological measurements were obtained with an ocular micrometer attached to a dissecting microscope. We measured 18 males and 23 females from *P. clavata* hosts and 8 males and 18 females from *E. tuberculatum* hosts. We used two-way analysis of variance to compare the morphological characters among sexes and species. We also used analysis of variance to test for differences in body size in flies either emerged from, or attracted to, the two hosts.

DNA extraction, sequencing and phylogeny reconstruction

Fly populations were designated by the host to which they were attracted and by their collection location. Thus an *A. paraponerae* individual collected in Costa Rica at *Paraponera clavata* represents the 'Paraponera Costa Rica' population. Two other phorid species were used as outgroups, *Apocephalus* sp. 181 and *Megaselia scalaris*. *Apocephalus* sp. 181, which is closely related to *A. paraponerae*, is within the *Apocephalus miricauda*-group together with *A. paraponerae*, but attacks a different subfamily of ants. *M. scalaris* is a distant saprophagous phorid. Flies collected in Costa Rica and Panama were preserved in 80% ethanol; flies collected in California, Ecuador and Colombia were dried in hexamethyldisilazane (HDMS) and mounted on pins (Brown, 1993).

All flies were dried overnight at 50°C before DNA extraction. Individual specimens were ground over dry ice in 0.65 ml microcentrifuge tubes with disposable pestles made from melted pipette tips. Conventional SDS/pronase extraction (Evans, 1993) was followed by DNA precipitation on diatoms (Carter and Milton, 1993). A 0.9 kb segment of the mitochondrial cytochrome oxidase I gene was amplified with primers C1-J-2183 and TL2-N-3014 (Simon *et al.*, 1994); typical reaction conditions involved 36 cycles of (94°C, 20 s; 39°C, 30 s; 72°C, 45 s). PCR products were purified (QIAquick) before direct sequencing with dye-terminator chemistry on ABI 377 automated fluorescent sequencers. The sequences described here are available from GenBank under accession numbers AF217464-AF217483.

Sequences were easily aligned to each other and to the *Drosophila yakuba* COI sequence (Clary and Wolstenholme, 1985). Gene trees were inferred by distance, parsimony and maximum likelihood methods, and evaluated by bootstrap analysis, using programs in PHYLIP 3.57 (Felsenstein, 1995). Pairwise synonymous (K_S) and non-synonymous (K_A) substitutions per synonymous and non-synonymous site, respectively, were estimated by the method of Li (1993) and Pamilo and Bianchi (1993); neighbour-joining and UPGMA trees were inferred from the resulting matrix of K_S values. The maximum likelihood model used empirical base frequencies, a transition–transversion ratio of 10, and three rate categories (10, 1, 40) for first, second and third codon positions, respectively.

RESULTS

Attraction of A. paraponerae *to long-range olfactory cues*

Head removal of *P. clavata* ant workers reduced the number of *A. paraponerae* parasitoids attracted by > 50% (binomial probability $P < 0.0001$; Table 1). However, compared to intact workers, the application of attractant chemicals to headless *P. clavata* workers doubled the

number of *A. paraponerae* attracted ($P < 0.0001$; Table 2). Headless *P. clavata* workers with attractant compounds attracted more *A. paraponerae* than intact workers (Table 2) and headless workers attracted fewer flies than intact *P. clavata* (Table 1). The pattern in fly attractiveness to *P. clavata* differed between the two experiments ($G = 36.9$, $P < 0.0001$). These results demonstrate that *A. paraponerae* can effectively find their hosts by detecting 4-methyl-3-heptanone and 4-methyl-3-heptanol, even when the hosts lack mandibular glands. Furthermore, the attractiveness of headless workers with added long-range attraction cues exceeds that of intact workers alone.

Headless *E. tuberculatum* workers near *E. tuberculatum* colonies did not attract *A. paraponerae* flies (Table 1; $P < 0.0001$). Application of 4-methyl-3-heptanone and 4-methyl-3-heptanol did not increase the number of *A. paraponerae* attracted to *E. tuberculatum* workers, whether the workers were intact or headless ($P < 0.0001$). These results show that *A. paraponerae* are attracted to compounds found in the heads of *E. tuberculatum*; however, these compounds are apparently not 4-methyl-3-heptanone and 4-methyl-3-heptanol as in *P. clavata*.

P. clavata attracted many more flies than *E. tuberculatum* both as intact and as headless workers. Headless workers of *P. clavata* were also relatively more attractive than those of *E. tuberculatum* (Table 1; $G = 29.5$, $P < 0.0001$) even following application of exogenous host-location attractants ($G = 67.3$, $P < 0.0001$). These results further support the idea that *A. paraponerae* flies attracted to *E. tuberculatum* are not using the chemicals 4-methyl-3-heptanone and 4-methyl-3-heptanol to locate their hosts.

Table 1. Number of *Apocephalus paraponerae* attracted to *Ectatomma tuberculatum* and *Paraponera clavata* ant hosts at La Selva Biological Research Station: Intact versus headless ants

Host species	Intact ants[a]	Headless ants	*P*
P. clavata	109	43	<0.001
E. tuberculatum	53	0	<0.001

Note: Overall $G = 29.5$, $P < 0.0001$.
[a] The results are pooled for experiments at six colonies. *P* is the binomial probability with $p = q = 0.5$.

Table 2. Number of *Apocephalus paraponerae* attracted to *Ectatomma tuberculatum* and *Paraponera clavata* ant hosts at La Selva Biological Research Station: Intact versus test ants

Host species	Intact ants[a]	Test ants[b]	*P*
P. clavata	44	80	<0.001
E. tuberculatum	41	0	<0.001

Note: Overall $G = 67.3$, $P < 0.0001$.
[a] The results are pooled for experiments at six colonies. *P* is the binomial probability with $p = q = 0.5$.
[b] Test ants are headless workers with 300 μg of a mixture of the two mandibular gland components.

Body size differences

P clavata workers are substantially larger than workers of *E. tuberculatum*, suggesting that host body size may determine the relative sizes of adult flies as in other parasitoids. To distinguish environmental from genetic influences on parasitoid body size, we analysed the size of flies both attracted to and emerging from the two ant host species. We reasoned that if there were separate non-interbreeding populations, then the flies attracted to and emerging from the two hosts would be consistent within ant host species but would differ between host species. If, however, adult fly body size were solely the result of host size, then flies attracted to the two ant species would be approximately the same size, but flies emerging from *P clavata* would be larger.

Average hind femur length and average costal vein length of *A. paraponerae* varied significantly with sex and with host ant species both for flies attracted to and flies emerging from the two hosts (Fig. 1; Table 3). None of the two- or three-way interactions among these variables were significant. Males were significantly smaller than females for both morphological traits in both ant species. The collection source, whether flies attracted to or emerging from the ant hosts, did not influence average hind femur length ($F_{1,51} = 0.1$, $P = 0.7$) or costal vein length ($F_{1,51} = 0.0$, $P = 0.9$) of adult flies. *A. paraponerae* attracted to and emerging from *E. tuberculatum* hosts were significantly smaller than those attracted to and emerging from *Paraponera clavata* workers ($t = -3.85$, d.f. = 14.5, $P < 0.01$). Figure 1 shows the results for all flies emerging from the two ant host species. Small flies are consistently attracted to the small host *E. tuberculatum* and large flies are attracted to the large *P clavata*, supporting the hypothesis that there are separate populations attracted to the two ant hosts.

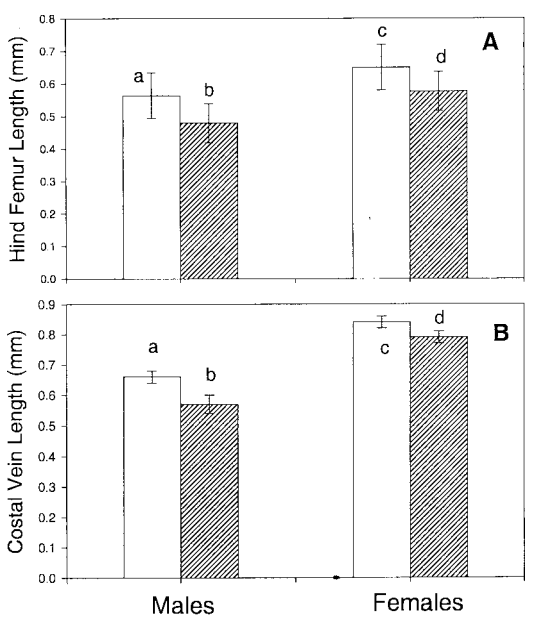

Fig. 1. Hind femur length (A) and costal vein length (B) for adult *A. paraponerae* emerging from the host ant species *P. clavata* (open bars) and *E. tuberculatum* (hatched bars). Error bars represent standard deviations. Different letters represent significant differences ($P > 0.05$).

Table 3. Average hind femur length and average costal vein length compared among sexes, host species and collection sources (attracted to or emerging from host ant species)

Source	d.f.	MS	*F*	*P*
Average hind femur length				
Host species	1	0.0437	10.5	0.002
Fly sex	1	0.0707	17.1	0.0001
Collection source	1	0.0005	0.1	0.72
Species × sex	1	0.0006	0.1	0.70
Species × collection	1	0.0026	0.1	0.80
Sex × collection	1	0.0097	2.4	0.12
Species × sex × collection	1	0.0050	1.2	0.27
Error	51	0.0041		
Average costal vein length				
Host species	1	0.0359	5.1	0.028
Fly sex	1	0.2959	41.9	0.0001
Collection source	1	0.0001	0.0	0.90
Species × sex	1	0.0002	0.0	0.85
Species × collection	1	0.0033	0.5	0.49
Sex × collection	1	0.0173	2.4	0.12
Species × sex × collection	1	0.0031	0.4	0.51
Error	51	0.0071		

Phylogenetic analysis of populations

A. paraponerae attracted to the two ant hosts *Paraponera* and *Ectatomma* apparently use different host-location cues and also differ consistently in size. To determine whether *A. paraponerae* is one generalized panmictic population or several host-specific populations, we analysed DNA sequences from the COI gene.

Intra-population variation. We sequenced 420 base pairs of the COI gene for one to four individuals from each population sampled. A population consisted of flies attracted to one species of ant at one location. Intra-population sequence divergence was always less than 1.9% and typically on the order of 0.5% (Table 4). We extended the sequences of one representative individual from each population to a total length of 804 bp and estimated the phylogenetic relationships of these longer sequences.

Topology. Trees generated from maximum likelihood, parsimony, neighbour-joining and UPGMA methods all have the same topology (Fig. 2). Parsimony analysis produced one most-parsimonious tree with 217 steps. Bootstrap analysis of parsimony trees showed moderate to strong support for most internal nodes (64–100% of 200 replicates).

A. paraponerae collected from *Paraponera* hosts in both Panama and Costa Rica showed little sequence divergence, suggesting that these 'Central American Paraponera' flies belong to a single population, which is the sister taxon to flies collected from *Paraponera* hosts in Colombia and Ecuador (South America). However, South American and Central American

Table 4. Sequence variation for multiple individuals from the same collection location

Host ant genus	Collection location	Number of individuals	Mean sequence divergence (π)	Max. sequence divergence (π)
Paraponera	Costa Rica	3	0.0047	0.0047
Paraponera	Panama	3	0.0047	0.0070
Paraponera	Ecuador	2	0.0	0.0
Paraponera	Colombia	3	0.0035	0.0040
Ectatomma	Panama	3	0.0170	0.0190
Ectatomma	Costa Rica	4	0.0047	0.0047

Note: Only one sequence was obtained for *A. paraponerae* attracted to *Pachycondyla* and for the outgroups *Apocephalus* sp. 181 and *Megaselia scalaris*.

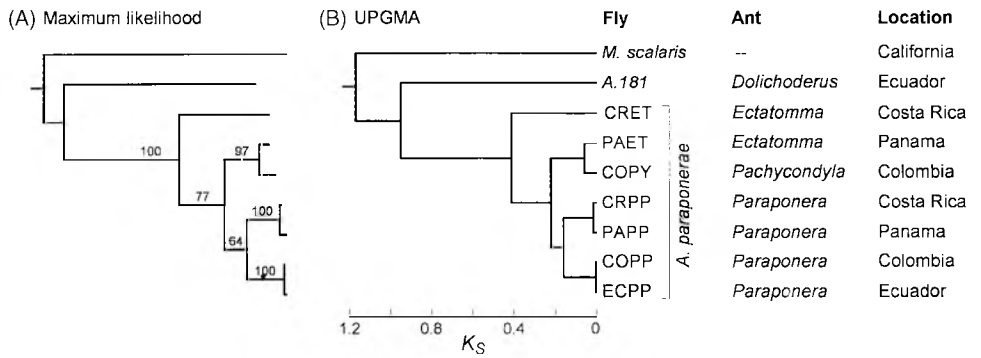

Fig. 2. COI gene trees for *A. paraponerae* populations. Each population is identified by the species of fly, followed by the ant host species and the collection location. (A) Maximum likelihood tree with bootstrap values from a parsimony analysis, which gives the same topology. (B) UPGMA tree constructed from synonymous divergences (K_S). Neighbour-joining analysis gives the same topology.

flies collected from *P clavata* showed considerably greater divergence (5%). Dipterans described as separate species sometimes differ by only 1.5% or less at COI or other, less well-conserved mitochondrial loci (Xiong and Kocher, 1991). A calibration of the COI molecular clock for insects implies that a 5% divergence corresponds to a separation of three Myr (Brower, 1994).

Pairwise total (π) and synonymous (K_S) divergence values are shown in Table 5. Together, the *A. paraponerae* attracted to *Paraponera* ant hosts form a clade with respect to the flies attracted to *Ectatomma* in Panama ($K_S = 0.2$). The 'Ectatomma Panama' flies are most similar to 'Pachycondyla Colombia', differing at 13 base pairs (1.6%, $K_S = 0.06$), but they have identical amino acid sequences. The 'Ectatomma Costa Rica' population is basal to all other *A. paraponerae* populations in our sample. The outgroups, *Apocephalus* sp. 181 and *Megaselia scalaris*, are well separated from all of the *A. paraponerae* ($K_S = 0.95$ and 1.17, respectively).

A. paraponerae that use *Paraponera* hosts are most similar to each other and they vary in relatedness to flies using *Ectatomma* hosts. *Paraponera*-using populations form a distinct

Table 5. Pairwise raw divergence (π) and synonymous divergence (K_S) estimates for COI sequences from *A. paraponerae* populations and the outgroups *Apocephalus* sp. 181 and *Megaselia scalaris*

	K_S								
π	Mscal	*A.* 181	CRET	PAET	COPY	CRPP	PAPP	COPP	ECPP
Mscal	—	1.16	1.07	1.49	1.51	1.00	0.97	1.10	1.10
A. 181	0.158	—	0.99	0.91	0.96	1.05	1.03	0.87	0.87
CRET	0.167	0.153	—	0.39	0.42	0.40	0.41	0.43	0.43
PAET	0.157	0.128	0.086	—	0.06	0.21	0.22	0.22	0.22
COPY	0.160	0.137	0.092	0.016	—	0.21	0.22	0.23	0.23
CRPP	0.144	0.143	0.087	0.053	0.053	—	0.02	0.15	0.16
PAPP	0.144	0.143	0.091	0.056	0.056	0.005	—	0.16	0.17
COPP	0.146	0.131	0.093	0.053	0.056	0.042	0.045	—	0.01
ECPP	0.144	0.131	0.093	0.055	0.057	0.044	0.046	0.001	—

Abbreviations: Mscal = *Megaselia scalaris*, *A.* 181 = *Apocephalus* sp. 181, CRET = 'Costa Rica Ectatomma', PAET = 'Panama Ectatomma', COPY = 'Colombia Pachycondyla', CRPP = 'Costa Rica Paraponera', PAPP = 'Panama Paraponera', COPP = 'Colombia Paraponera', ECPP = 'Ecuador Paraponera'.

clade nested inside populations using other hosts. This implies that the use of *Paraponera* as a host for *A. paraponerae* is a relatively recent innovation. *A. paraponerae* appears to have originally attacked *Ectatomma*; later, various populations moved onto *Pachycondyla* and *Paraponera*. The diverging populations appear to have been separated by host use primarily, but the Central and South American *Paraponera* populations also appear to represent distinct geographically separated groups.

Sympatric populations at different hosts. While the intra-population divergence among flies collected at *Ectatomma* hosts in Costa Rica averages 0.5%, these flies differ by about 10% from sympatric *A. paraponerae* attracted to *Paraponera*. Similarly, the Panamanian flies attracted by *Ectatomma* and *Paraponera* hosts differ by 5.6%, suggesting that these are cryptic sympatric groups utilizing different hosts. The 'Pachycondyla Colombia' individual also differed by about 5.6% from sympatric 'Paraponera Colombia', indicating that it, too, represents a distinct non-interbreeding population or species.

DISCUSSION

Three lines of evidence suggest that there are four or more distinct populations of *A. paraponerae*. First, the flies attracted to *Ectatomma* and *Paraponera* hosts consistently differ in size; *A. paraponerae* at *Paraponera* hosts are always larger. The larger body size of *Paraponera* has some effect on adult fly size; *A. paraponerae* emerging from *Paraponera clavata* hosts are larger, and more flies emerge from each individual *P clavata* ant host than from *Ectatomma tuberculatum* (S. Morehead, personal observation). Additionally, other behavioural studies have shown that *A. paraponerae* uses the large body size of *P clavata* as a short-range visual cue in host location and acceptance (Morehead and Feener, 2000). These results suggest an explanation for a host switch from *Ectatomma* hosts to *Paraponera*.

Second, the results of the chemical attraction experiments show that *A. paraponerae* use different long-range host-location cues to find the host ants *E. tuberculatum* and *P clavata*.

Whereas *A. paraponerae* flies are attracted to *P. clavata* hosts by 4-methyl-3-heptanone and 4-methyl-3-heptanol, these compounds were not sufficient to attract *A. paraponerae* to *E. tuberculatum* ants. These behavioural differences indicate separate groups that are specialized to each host species. Because *Ectatomma* have been shown to have a different major chemical component in their mandibular glands, 2-hexanone (A. Attygalle, personal communication), and we have shown that the attractive compounds for *A. paraponerae* are found in the head capsule of both *E. tuberculatum* and *P. clavata*, it is probable that *A. paraponerae* populations use different attractants to locate their hosts.

Finally, there are significant genetic differences among individual flies attracted to the different host ants, even at the same location. Sequence divergence among groups varies between 2.5 and 10%. In many instances, differences of this magnitude are sufficient to warrant separation of populations into species. In combination with the behavioural information, this genetic evidence implies that populations of *A. paraponerae* have long been separated by their attraction to and exploitation of different host ants.

Mitochondrial COI sequences diverge by about 1.7% per million years in various arthropod groups (Brower, 1994). On the basis of this calibration, our estimates of sequence divergence among these populations suggest that the initial split between the 'Ectatomma Costa Rica' group and the other populations occurred about 7 million years ago; the Central and South American split would have occurred about 2.5 million years ago. These divergence times for the fly populations do not agree with the hypothesized ages of the ant host genera. The Ectatommini, to which *Ectatomma* belongs, is believed to have originated in the late Oligocene, about 23 million years ago (Lattke, 1994). *Ectatomma* and *Paraponera* are believed to be closely related, however, and are probably somewhat younger than the estimate of 23 million years. There is a distinct incongruity between the fly divergence times and the proposed ant speciation events, which suggests that there was a host switch rather than co-speciation. We suggest that an ancestral population of *A. paraponerae* switched from the smaller *Ectatomma* onto larger *Paraponera* hosts. As mentioned above, more flies emerge from *P. clavata* hosts than from *E. tuberculatum* hosts, so an increase in reproductive success might have favoured this initial host switch.

Cryptic species that are highly specialized to a single host species could arise through sympatric speciation (Bush, 1975). It was initially believed that *A. paraponerae* was attracted to at least three species, *P. clavata*, *E. tuberculatum* and *Pachycondyla* spp. (Brown and Feener, 1991b); however, the genetic and behavioural differences among populations we describe here suggest that any given group of *A. paraponerae* attacks only one ant host species. Specialization on different host species may cause populations to diverge genetically. Additionally, these flies mate and lay eggs at the host (Brown and Feener, 1991a,b), providing an ideal situation for host-race formation and sympatric speciation. Morphological divergence may be slowed by very similar environmental conditions and by the absence of any need to use morphological cues in mate recognition. Geographic differences between some closely related taxa suggest that allopatric speciation has also occurred.

Cryptic species complexes appear to be very common within the Diptera. The *Apocephalus miricauda* group, to which *A. paraponerae* belongs, currently includes over 80 species, many of which are as yet undescribed (B.V. Brown, personal observation). Many of these species are highly species-specific on a variety of ant hosts. However, some species have been collected at several ant host species, suggesting that these, too, may represent cryptic species complexes.

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