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Convergence of chemical mimicry in a guild of aphid predators

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Abstract. 1. A variety of insects prey on honeydew-producing Homoptera and many do so even in the presence of ants that tend, and endeavour to protect, these trophobionts from natural enemies. Few studies have explored the semiochemical mechanisms by which these predators circumvent attack by otherwise aggressive ants.

2. Ants use specific mixtures of cuticular hydrocarbons (CHCs) as recognition labels, but this simple mechanism is frequently circumvented by nest parasites that engage in 'chemical mimicry' of their host ants by producing or acquiring a critical suite of these CHCs.

3. Analysis of the CHCs from the North American woolly alder aphid, *Prociphilus tessellatus* (Homoptera: Aphididae), their tending ants, and aphid predators from three insect orders, *Feniseca tarquinius* (Lepidoptera: Lycaenidae), *Chrysopa slossonae* (Neuroptera: Chrysopidae), and *Syrphus ribesii* (Diptera: Syrphidae), showed that while the CHC profile of each predatory species was distinct, each was chemically more similar to the aphids than to either tending ant species. Further, the CHCs of each predator species were a subset of the compounds found in the aphids' profile.

4. These results implicate CHCs as a recognition cue used by ants to discriminate trophobionts from potential prey and a probable mechanism by which trophobiont predators circumvent detection by aphids and their tending ants.

5. Although several features of the aphids' CHC profile are shared among the chemically mimetic taxa, variation in the precision of mimicry among the members of this predatory guild demonstrates that a chemical mimic need not replicate every feature of its model.

Key words. Chemical camouflage, chemical mimicry, cuticular hydrocarbons, life history, myrmecophily, pheromone, predation, semiochemical, tritrophic interaction, trophobiont.

Introduction

Most ants that harvest honeydew secretions from aphids, coccids, jaccids, and other Hemiptera/Homoptera (but hereafter referred to as Homoptera) also defend these mutualists from predators and parasitoids (Pontin, 1959; Way, 1963; Yao *et al.*, 2000). However, the ants' protection is ineffective against a number of specialised predatory taxa, including certain coccinellid beetles, syrphid fly larvae, neuropteran larvae, lycaenid butterfly caterpillars, and aphidiid wasps. These insects have evolved mechanisms to evade ant attack while they feed on or oviposit among aphids and other Homoptera (Dodd, 1912; Eisner *et al.*, 1978; Liepert & Dettner, 1993; Pierce, 1995; Völkl, 1995; Way, 1963). However, few investigations have quantified pheromonal mechanisms used by predators of Homoptera to circumvent attack by trophobiont-tending ants.

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While the hardened elytra of adult ladybird beetles (Coccinellidae) are usually sufficient to protect them from attacking ants, most soft-bodied insects must resort to avoidance strategies and camouflage (Völkl, 1995). For example, the aphid parasitoid *Trioxys angelicae* Haliday (Hymenoptera: Aphidiidae) appears 'nervous' in the presence of ants and generally avoids them by fleeing the aphid colony before (often lethal) contact with ants (Liepert & Dettner, 1993; Völkl & Mackauer, 1993). Larvae of the green lacewing *Chrysopa slossonae* Banks (Neuroptera: Chrysopidae) camouflage themselves by harvesting the long-chain ketoester wax, or 'wool', produced by their *Prociphilus tessellatus* (Fitch) (Homoptera: Aphididae) aphid prey and embedding it within hooked bristles on their dorsum (Fig. 1; Meinwald *et al*., 1975). Removal of this waxen cloak causes the lacewing to be attacked by ants, suggesting that the covering serves a dual purpose as both physical and chemical camouflage (Eisner *et al*., 1978).

Ants, like many other social insects, use the suite of hydrocarbon compounds in their cuticular wax as a label for recognising nestmates (Lahav *et al*., 1999; Thomas *et al*., 1999; Wagner *et al*., 2000) and distinguishing among castes (Bonavita-Cougourdan *et al*., 1993; Wagner *et al*., 1998). Many arthropod predators of ants, including some beetles, syrphid fly larvae, crickets, and lycaenid butterfly larvae, have evolved the ability to produce or acquire the characteristic mixture of hydrocarbons found in the cuticular wax of ant workers or larvae (Vander Meer & Wojcik, 1982; Hölldobler & Wilson, 1990; Howard *et al*., 1990a, b; Akino *et al*., 1996, 1999). Chemical mimicry

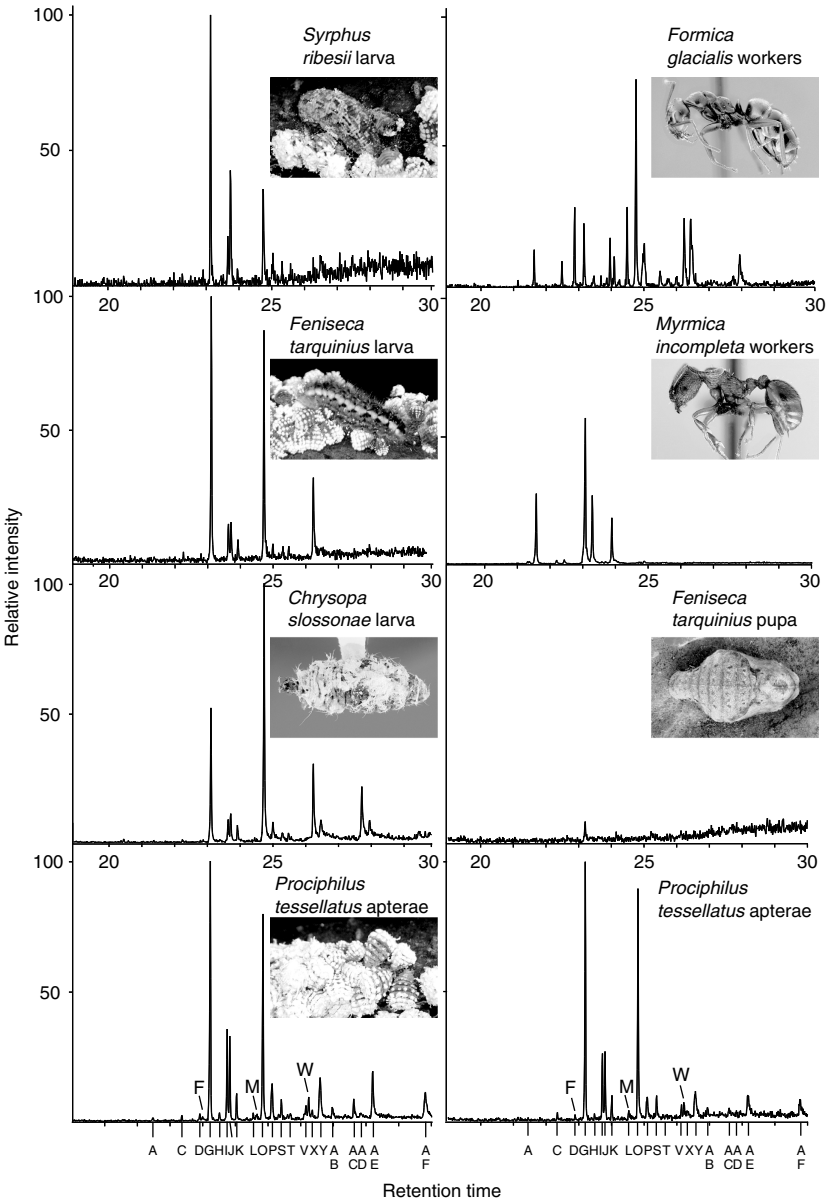

Fig. 1. Gas chromatograms of cuticular hydrocarbons from the woolly aphid *Prociphilus tessellatus*, its guild of insect predators, and workers of its tending ants. Chromatograms from two different samples of *P. tessellatus* are shown, one in each column, for comparison. Select peaks from these chromatograms are coded with letters and identified in Appendix 2.

seems to be necessary and sufficient for these invaders to enter well-guarded ant nests with impunity, often to feed on ant regurgitations or ant brood (Dettner & Liepert, 1994).

The North American woolly alder aphid, *P. tessellatus*, is tended by a variety of ants in at least three subfamilies (Mathew, 2003), yet falls prey to a small menagerie of predators and parasitoids that apparently go undetected by the ants (Edwards, 1886; Pergande, 1912). Although each predator differs in its life history and behaviour, three prominent members of this carnivorous guild appear to use chemical mimicry to avoid being detected by their prey and/or to avoid being attacked by the various ant species that tend *P. tessellatus*. The cuticular hydrocarbon (CHC) profiles of these predators, the aphids, and their tending ants were examined using capillary gas chromatography–mass spectrometry (GC–MS) to test the hypothesis that any or all of these predatory taxa chemically mimic their aphid prey or the aphids' tending ants, and to assess similarities among the CHC profiles of participants in this tritrophic interaction.

Materials and methods

Natural history and collection of experimental taxa

Fundatrices and alates of *P. tessellatus* develop on the undersides of sugar maple leaves, *Acer saccharinum*, from May to July. Alates then migrate to the branches and leaves of alders, *Alnus* spp., where they reproduce parthenogenetically until alate sexuparae return to *A. saccharinum* in September (Blackman & Eastop, 1994). Pergande (1912) listed six insect predators and five tending ants of *P. tessellatus*. Mathew (2003) adds eight more species to the list of ants known to tend *P. tessellatus*[*]. All aphids in the present study were collected from speckled alder, *Alnus rugosa*, at several localities in the north-eastern United States (Appendix 1).

Feniseca tarquinius (Abbot) (Lepidoptera: Lycaenidae) is the sole North American member of the wholly aphytophagous lycaenid subfamily Miletinae, and represents the first documented carnivorous species in the Lepidoptera (Riley, 1886). Eggs are laid among the host aphids and newly hatched larvae spin a silken tunnel in which they shelter from marauding ants (Edwards, 1886). Within this tunnel they seize aphids from below, and hollow aphid mummies are subsequently used to decorate the tunnel. Later instars venture outside the tunnel to feed with impunity on aphids in the presence of ants. Perhaps because of their carnivorous habit, the larval stage lasts an unusually short 8–11 days (Clark, 1926; Scudder, 1899).

Chrysopa slossonae is a specialist lacewing predator on the aphid *P. tessellatus*, and appears to have evolved from a generalist predatory ancestor. For this reason, *C. slossonae* and its sister species, *C. quadripunctata* Burmeister have become a model system for studying the evolution of specialised insect predators (e.g. Tauber *et al.*, 1993; Albuquerque *et al.*, 1996, 1997).

The aphid-eating larvae of the common, Holarctic flower fly species *Syrphus ribesii* (Linnaeus) (Diptera: Syrphidae) are known to feed on several species of aphid, but the present study is possibly the first record of a pemphigine aphid host. This syrphid species is obligately aphidophagous as a larva, and ovipositing females have become a model for the study of host searching and oviposition behaviour (e.g. Sadeghi & Gilbert, 2000a, b, c).

Semiochemical extraction and analysis

All animals were collected in the field and transported to the laboratory in ventilated plastic containers within 1–2 days of capture. Collecting locality and voucher information for all samples in this analysis are provided in Appendix 1. Each animal was killed by freezing in a −80 °C freezer for 5–10 min, thawed, and air-dried for 10–20 min. Insects were then immersed in a measured amount of HPLC grade hexanes (50–300 μl; EMI Science, Gibbstown, New Jersey) for 5 min, and the resulting solution transferred to sample vials via syringe. All *F. tarquinius* caterpillars were placed on wire mesh and blown with pressurized air to remove extraneous aphid wool prior to solvent extraction. Extracts from *P. tessellatus* aphids were also centrifuged for 3 min to remove aphid wool from suspension in the solvent; injections from these centrifuged samples used only the resulting supernatant. In a preliminary experiment, wax filaments incinerated in the injection port, resulting in a large smear of unidentifiable compounds eluting from 30 to 90 min at 310 °C. Chromatograms from these samples and those of centrifuged samples differed only in the presence of this smear. All samples were stored at −4 °C before analysis with a Hewlett-Packard 5890 Series II gas chromatograph with a J & W Scientific DB-1 Capillary column (30 m, 0.25 mm ID, 0.25 μm film thickness) in conjunction with a JEOL SX-102 A magnetic sector mass spectrometer as the detector. GC program was as follows: He carrier gas, splitless injection at 250 °C, oven isothermal at 60 °C for 1 min, then increased to 310 °C at 10 °C min^{-1}, and held at the maximum temperature for 7 min. MS mode: electron impact at 70 eV.

Statistical analyses

Multivariate statistical analyses – including the techniques used here – treat each variable (chemical compound, in this case) with equal weight, when in fact some variables are likely to be less important than others (e.g. unbranched alkanes, see Discussion). Multivariate statistics are thus a blunt tool for assessing biologically important similarities among complex CHC profiles because they do not take into account what may be important differences in the

[*]Our spelling of *Prociphilus tessellatus* follows Remaudière (1997). Alternate names include *Prociphilus tesselatus* (Eastop & Hille Ris Lambers, 1997) and *Paraprociphilus tessellatus* (Blackman & Eastop, 1994).

perception of different compounds. For this reason, all statistical analyses in this study were performed on two sets of data, the 'full' data set, and a 'reduced' data matrix from which all unbranched alkanes had been removed.

To assess overall similarity among hydrocarbon profiles, the area of each detectable peak in every chromatogram was converted to its proportional contribution to total peak area in that sample and transformed by taking the arcsine of the square root (Sokal & Rohlf, 1995; Wagner *et al*., 1998). Because some peaks contained more than one compound (Appendix 2), peaks, rather than individual chemicals, were the units on which statistical analyses were performed. Cluster analyses were performed on a matrix of the relative amounts of each of the 32 peaks found in the CHC profile of each of the 22 samples using unweighted pair-group average (UPGMA), weighted pair-group average (WPGMA), unweighted pair-group centroid (UPGMC), and weighted pair-group centroid (WPGMC) algorithms using Euclidean and squared Euclidean distances.

A variety of multivariate statistical methods have been used to assess similarities among CHC chromatograms, but most studies investigate variation among colonies or castes of a single ant species (e.g. Dahbi & Lenoir, 1998; Lahav *et al*., 2001; Boulay *et al*., 2003). Most conspecific ants have the same suite of CHCs, and the proportions of these CHC compounds differ in a colony- or caste-specific fashion. However, some of the species in the present analysis have compounds not found on other species, resulting in many 'missing data' values and significant non-normality of variables. For this reason, non-metric multidimensional scaling (NMDS) was used to assess overall similarities among CHC profiles. This technique uses the rank order of variates rather than their Euclidean distances, making it robust to non-normal data (James & McCulloch, 1990; Krzanowski, 1988; Statsoft, 2001). NMDS has been fruitfully applied to interspecific comparisons of *Myrmica* ant CHC profiles (Elmes *et al*., 2002). Using the results from a scree test of principal components analyses of the data, we chose to implement three dimensional NMDS analyses using both correlation and dissimilarity matrices. All statistical analyses were performed using Statistica 6.0 (Statsoft, 2001). The rarity and highly patchy distributions of some of the experimental taxa in this study prevented collection of large numbers of samples, but low within-species variation relative to between-species variation adds strength to statistical inferences drawn from these data.

Results

Hydrocarbon constituents from 32 chromatographic peaks with sufficiently strong signal:noise ratios were tentatively identified using electron impact (EI) mass spectra. No insect possessed every peak (Fig. 1; Appendix 2), and several peaks contained mixtures of constitutional isomers. Branched alkanes were the most numerous type of hydrocarbon, but the unbranched alkanes were proportionally more abundant. Relative proportions of different hydrocarbon peaks showed little variation among individuals of *P. tessellatus* and *F. tarquinius* larvae (the two most heavily sampled species in this study; Fig. 2). The aphid *P. tessellatus* and its associated insects had similar hydrocarbon profiles, and – with the exception of a single trace compound (Appendix 2, peak E) – every compound on the aphidophagous predators was also found on their prey (Appendix 2). This exception, pentacosene (x-$C_{25:1}$), was found on a single *S. ribesii* larva that was tended by *Myrmica incompleta* Weber ants (Hymenoptera: Formicidae). This compound is a major component of the *M. incompleta* profile, and may have been acquired by the syrphid larva through external contact with the ants. The profiles of ants tending aphids differed greatly from the profiles of the other insects in this analysis and from each other (Fig. 1).

Phenograms constructed with the UPGMA clustering algorithm using squared Euclidean distances were chosen *a priori* for presentation (Fig. 3). This algorithm efficiently recovers 'natural clusters' in which the number of cases per cluster is uneven, and use of *squared* Euclidean distances places progressively greater weight on objects that are farther apart (Statsoft, 2001) and thus emphasises the presence vs. absence of a compound. Other clustering algorithms produced similar topologies. While each method grouped species/life stages together, the clusters occasionally differed in their relationship to one another, and some methods placed the neuropteran, *C. slossonae*, within the aphids. This result is not surprising, as the neuropterans steal their woollen coat from their aphid prey (Eisner *et al*., 1978). The exclusion of unbranched alkanes had only minor effects on the topology of the cluster diagram; aphids and their predators clustered more closely together. The CHC profiles of *F. tarquinius*'s larval and pupal stages differed markedly; the possible significance of this change is discussed below.

Output from the NMDS analyses mirrored the results of the cluster analysis: although each species tends to have a characteristic profile of CHCs, aphids and their predators had similar hydrocarbon profiles that differed markedly from those of the ants tending these aphids and from pupae of the lycaenid predator *F. tarquinius*. In addition, collection locality appeared not to affect the similarity of interspecific CHC profiles. NMDS analyses using correlation and dissimilarity matrices were qualitatively identical; results from the former are presented (Fig. 4). Exclusion of unbranched alkanes from the full data set did not noticeably affect the arrangement of samples in the NMDS analyses; results from these supernumerary analyses are not shown.

Discussion

Chemical mimicry and the role of CHCs in trophobiont recognition

This study documents striking convergence in CHC profiles between an ant-tended aphid and three of its principle predators, each from a different insect order. CHC profiles

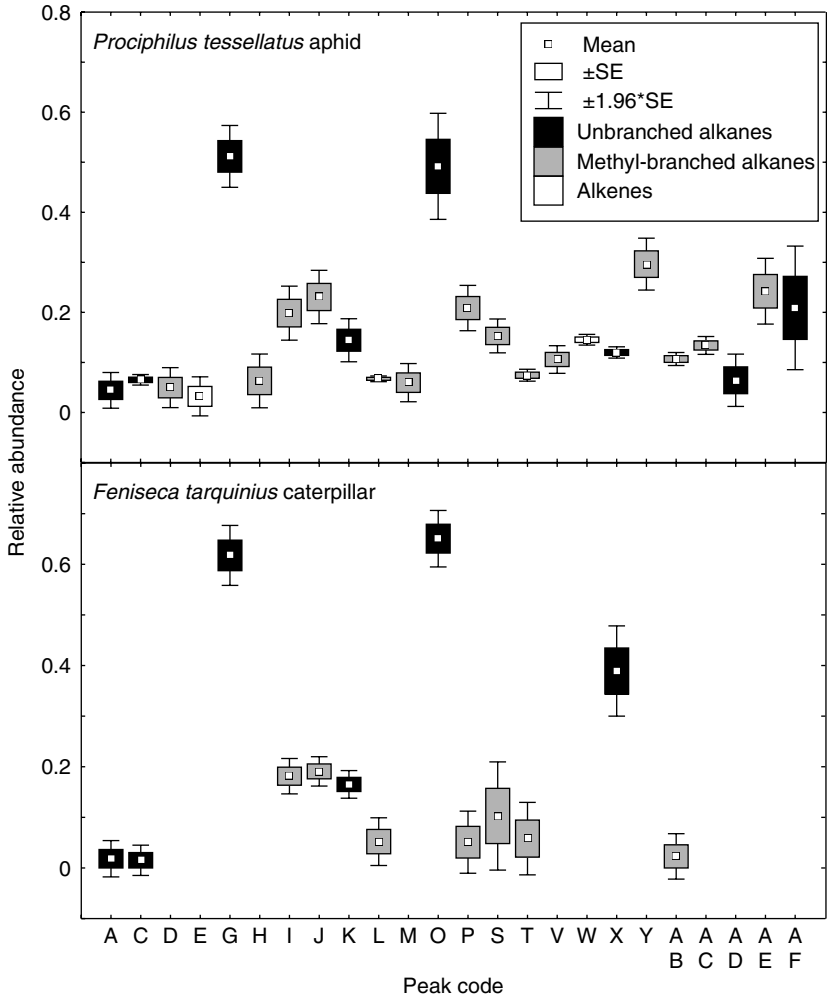

Fig. 2. Box plots showing variation of individual compounds within the cuticular hydrocarbon profiles of the aphid *Prociphilus tessellatus* and larvae of the butterfly *Feniseca tarquinius*. Values are based on the arcsine-square root transformed proportional contribution of each compound to the total hydrocarbons within the sample. Codes along the abscissa refer to peaks and their chemical constituents listed in Appendix 2.

of neither model nor mimic appear to resemble those of the tending ants. Although only one sample of *M. incompleta* was included in these analyses, the CHCs of the ants in this sample, collected in New Hampshire, share the same major hydrocarbon components as populations from Idaho (Howard *et al.*, 1990b) and Quebec (Lenoir *et al.*, 1997): *n*-C23, $C_{25:1}$, *n*-C_{25}, and 3-MeC_{25}.

Hydrocarbons appear to be an essential ingredient in the cuticular wax of all insects because they play an important role in water retention (Gibbs, 1998). Recognising variation in the amount and type of hydrocarbons within the cuticular wax and using these chemical signatures as recognition labels to communicate group identity seems to have evolved independently in ants, termites, and many other social insects. In the ant species so far examined, CHCs appear to be the primary nestmate recognition cue (Lahav *et al.*, 1999; Thomas *et al.*, 1999; Wagner *et al.*, 2000). The chemoreceptive and cognitive ability of ants to use CHCs as recognition cues may therefore predispose them to utilise the same suite of compounds for recognising their trophobiotic insect associates. Ants may have evolved the ability to recognise their trophobionts on the basis of CHC signature, or to associate a trophobiont's CHC profile with another feature, such as gustatory cues from honeydew. However, the reliance by ants on this mixture of common chemicals to discriminate friend from foe appears to be an Achilles' Heel. Because all insects have the biosynthetic machinery to produce CHCs, fine-tuning the mixture to match that of a social insect can enable entry into those insects' normally closed society. It appears that this has evolved many times among socially parasitic taxa (Howard *et al.*, 1990b; Dettner & Liepert, 1994).

Many ant inquilines possess a blend of CHCs similar to that of their hosts, and this 'chemical mimicry' is believed to allow integration of these foreign species into an otherwise well-guarded ant nest (Hölldobler & Wilson, 1990; Dettner & Liepert, 1994). Few studies have investigated the role of chemical mimicry in relationships outside the ant nest (but see Akino & Yamaoka, 1998), and even fewer studies have studied the role of chemical mimicry in tritrophic interactions involving ant-tended trophobionts and their natural enemies (but see Liepert & Dettner, 1993, 1996).

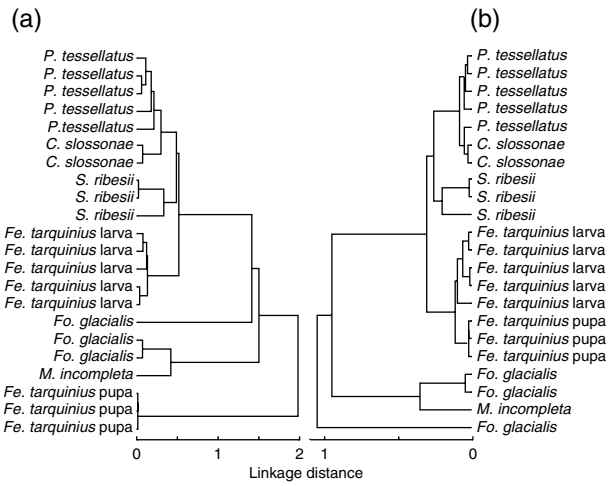

Fig. 3. Unweighted pair-group average (UPGMA) cluster analysis of squared Euclidean distances derived from arcsine square root transformed proportions of cuticular hydrocarbons showing hierarchical similarity of hydrocarbon profiles among the woolly aphid *P. tessellatus*, its guild of insect predators, and workers of its tending ants. A: Analysis of all cuticular hydrocarbons; B: analysis in which normal alkanes have been excluded.

The present study, as well as those of Liepert and Dettner (1993, 1996), indicate that chemically mimicking the CHCs of their aphid prey allows insect predators to avoid detection by the aphids and by their tending ants. The benefits of chemical mimicry are thus two-fold. Predators of aphids may circumvent elicitation of an alarm or escape response by their prey, allowing them to get closer than might otherwise be possible. These predators simultaneously prevent the aphids' normally aggressive tending ants from detecting and attacking them.

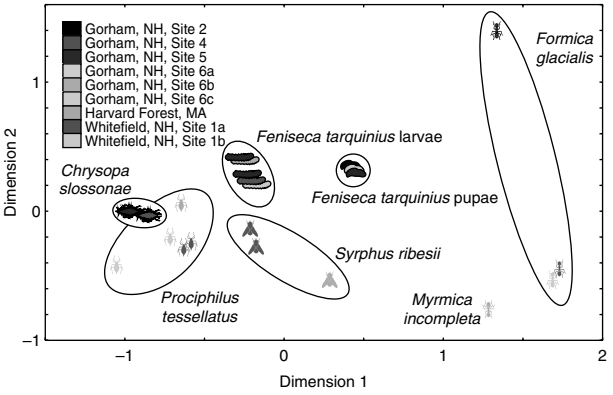

Fig. 4. Two-dimensional projection of a three-dimensional, nonmetric scaling ordination plot, derived from analysis of a correlation matrix of arcsine square root transformed proportions of cuticular hydrocarbons of the insects listed in Appendix 1. The cuticular hydrocarbon profiles of three aphid predators (*C. slossonae*, *S. ribesii*, and *Fe. tarquinius* larvae) are more similar to the hydrocarbon profiles of their aphid prey (*P. tessellatus*) than to the aphids' tending ants (*Fo. glacialis* and *M. incompleta*).

Prociphilus tessellatus is tended by a wide variety of ants in at least three subfamilies, and the tending ant species of individual free-living, plant-feeding trophobionts can change over time (Fraser *et al*., 2001). Mimicry of one ant species may be ineffective against another, and a strategy of mimicking aphids rather than ants avoids this problem associated with turnover of tending ants. Predatory invertebrates inside ant nests (inquilines) – where the species of ant is not likely to change – chemically mimic the CHCs of their ant hosts (Hölldobler & Wilson, 1990; Howard *et al*., 1990a, b). These two tactics may be viewed as two adaptive strategies or as the by-product of passive hydrocarbon acquisition via ingestion or cuticular adsorption (see below).

While many chemically mimetic insects duplicate the CHC profiles of ant workers or brood, chemical mimicry of ant trophobionts appears to be uncommon. As all three species in this study prey on the same aphid host, a novel interspecific pattern can be observed: each predator has a species-typical mimetic CHC pattern that matches the host's 'model' pattern to differing degrees. While the CHC profile of the larval neuropteran *C. slossonae* falls within the range of variation shown by the aphid *P. tessellatus*, samples from *F. tarquinius* and *S. ribesii* form distinct clusters in the NMDS and cluster analyses (Figs 3 and 4). That none of the observed tending ant species or aphids seemed to recognised these 'imperfect' mimics as predators – presumably because they were not detected or perceived as enemies – suggests that every compound on the model need not be present on the mimic to dupe the ants. An acceptable level of pheromonal noise seems to exist which does not exceed the discrimination threshold of the ants in the relevant sensory modality (Hölldobler & Carlin, 1987).

Acquisition of a mimetic CHC profile

De novo production of another species' hydrocarbons is only one strategy used by chemical mimics. Acquisition of at least some of an ant colony's hydrocarbon label seems to be another tactic, and CHCs may be acquired through direct contact (e.g. the wasp *Paralipsis eikoae*; Akino & Yamaoka, 1998), by eating ants (e.g. the salticid spider *Cosmophasis bitaeniata* Keyserling; Elgar & Allan 2004), or by grooming and accepting regurgitations from ants (e.g. the xenobiotic 'shampoo ant' *Formicoxenus provancheri* Emery; Lenoir *et al*. 1997). CHCs derived from insect prey may be incorporated into an insect's CHC profile (Blomquist & Jackson, 1973; Liang & Silverman, 2000; Elgar & Allan, 2004), and ingestion of *P. tessellatus* aphids by various other insects may enable those predators to obtain the necessary blend of CHCs needed to evade ant detection and attack. Orally acquired hydrocarbons appear to be involved in the homogenisation of colony odour of ants. Hydrocarbons are acquired through trophallaxis and allogrooming, and are subsequently incorporated into a

worker's cuticular blend, thus creating the colony's uniform Gestalt odour (Dahbi *et al*., 1999; Boulay *et al*., 2000).

Previous investigations of lycaenid caterpillars that live within ant nests have found a consistent pattern: larval lycaenids chemically mimic the larvae of their host ants (Henning, 1983, 1997; Akino *et al*., 1999; Schönrogge *et al*., 2004). The socially parasitic caterpillars of *Maculinea rebeli* (Hirschke) produce a mixture of hydrocarbon compounds resembling the CHC profile of their host ant's larvae, *Myrmica schencki* Emery. This mimetic chemical cocktail induces *M. schencki* workers to carry *M. rebeli* caterpillars into their nests, where they are fed trophallactically by ants (Akino *et al*., 1999; Schönrogge *et al*., 2004). These researchers found that the caterpillars acquire several more, presumably colony-specific, hydrocarbons after a week in their host ant nests, and become even better chemical mimics of the larval ants' hydrocarbons. To verify that the putative brood pheromones were successfully extracted, small glass rods treated with extracts from either ant larvae or caterpillars were offered to ants alongside suitable controls, and both sets of extract-coated rods were placed in brood chambers of laboratory nests, while control rods were ignored (Akino *et al*., 1999; Schönrogge *et al*., 2004). Unfortunately, experimental manipulation of soft-bodied caterpillars, maggots, and neuropteran larvae is not feasible because the solvents needed to remove or apply hydrocarbons easily penetrate the insect's soft cuticle and kill it (Akino *et al*., 1999). In the absence of significant advances in bioassay methodology, dependence on previous bioassays with chemically manipulated inanimate objects in concert with the correlative patterns among CHC profiles remain the best means of examining cases of putative chemical mimicry.

CHC chemical structure and ant chemoreception

The common CHCs of insects may be classified into three groups based on their chemical structure: normal (unbranched, saturated) alkanes, branched alkanes, and alkenes. Normal alkanes might be viewed as 'strings of carbon' with no branches or double bonds, whereas branched alkanes possess one or more methyl side chains and alkenes possess one or more double bonds. Of these, normal alkanes comprise the bulk of most insect CHC profiles (Lockey, 1988; Nelson & Blomquist, 1995). However, recent investigations have shown that, among some social insects, normal alkanes seem to have little or no utility in nestmate recognition, perhaps because they are not perceived (Dani *et al*., 2001). Whereas the three-dimensional structures of branched and unsaturated hydrocarbons are folded in a variety of shape conformations, linear hydrocarbons remain consistently linear in shape. This difference appears to play a role in olfaction/gustation. Based on the current paradigm of insect chemoreception, it seems likely that an insect olfactory receptor can discriminate between the many and varied shapes of branched and unsaturated hydrocarbons, but not between long, linear molecules that are very similar in length (Jones *et al*., 2002).

To discern friend from foe, social insects seem to detect and compare the CHC profile of an encountered insect with the hydrocarbon 'template' that the insect has learned to associate with its own colony. A close fit does not trigger an aggressive response, whereas conspicuous differences may lead to an attack (Vander Meer & Morel, 1998). If unbranched alkane hydrocarbons are not used in recognition, then their inclusion in analyses of similarity among CHCs may not be appropriate. It is still unknown – for any social insect – how closely an insect's hydrocarbon profile must match the learned template of colony odour, whether particular hydrocarbon compounds can act as 'flags' labelling particular individuals or groups of individuals, or whether ants are capable of perceiving all possible structural variations in a hydrocarbon's chemical structure.

Early instar caterpillars of *F. tarquinius* are occasionally attacked by ants and consequently spend much of their time within their silk tunnels consuming aphids from below. However, as the caterpillar matures, the ants' hostility towards them seems to lessen, and they frequently roam among the aphids and ants in their final instar, with no particular notice paid them by the ants (Edwards, 1886; Scudder, 1899). This may be because the caterpillars are covered with protective hairs that provide mechanical defence against ants. However, the change is also consistent with a scenario in which the caterpillars' cuticular profile becomes more similar to that of their aphid prey as they consume more and more aphids. Adaptation to placating ant aggression may therefore initially be behavioural (hiding in a silk shelter) and revert to pheromonal subterfuge as the caterpillar acquires the recognition pheromones of its prey and becomes indistinguishable from them in the sensory *umvelt* of the ants. The caterpillars examined in this study were all in their final instar; variability among instars was therefore not observed.

This ontogenetic change in CHCs seems to change drastically at pupation, when the total mass of CHCs decreases by an order of magnitude (quantitation data not shown), and decreases in hydrocarbon diversity from approximately 10 compounds to one: *n*-pentacosane (Appendix 2, Fig. 3). *Feniseca tarquinius* caterpillars leave the aphid colony and travel a considerable distance before pupating (Edwards, 1886); the pupae are thus physically isolated from the ants with which they associated as larvae.

The variation in CHC patterns among species in this study highlights the acceptable noise within ant recognition of their trophobionts, and indicates that multiple paths to chemical mimicry may lead to a common result. The neuropteran *C. slossonae* steals its woollen coat directly from the chemical 'model', while the other two 'wolf' species, *F. tarquinius* and *S. ribesii*, appear to produce or acquire their 'sheep's clothing' by other means. It remains to be determined whether these predatory insects produce or acquire their mimetic semiochemicals, or how these profiles change during the insects' development.

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Appendix 1

Collection locality, species identification, and voucher information for each cuticular hydrocarbon sample. Sample ID refers to the GC–MS sample number; Voucher no. refers to the accession number of the specimen lodged in the DNA and Tissues Collection of the Museum of Comparative Zoology, Harvard University. Different site numbers within the same collecting locality refer to sites more than 100 m apart (e.g. Gorham, NH, Site 1 and Site 2); sites differentiated by letters (e.g. Sites 1a and 1b) refer to specimens collected from different trees at the same site.

Common name	Species	Life stage	Chemical sample ID	Voucher no.	Locality
Woolly aphids	*Prociphilus tessellatus*	Apterae	ANTS191	DL-01-F002	Whitefield, NH, Site 1a
	P. tessellatus	Apterae	ANTS189	–	Harvard Forest, MA
	P. tessellatus	Apterae	ANTS190	DL-01-F016	Gorham, NH, Site 4
	P. tessellatus	Apterae	ANTS184	DL-01-F033	Gorham, NH, Site 6a
	P. tessellatus	Apterae	ANTS141	DL-01-F027	Gorham, NH, Site 6c
Harvester butterflies	*Feniseca tarquinius*	Larva	ANTS178	DL-01-F006b	Whitefield, NH, Site 1b
	Fe. tarquinius	Larva	ANTS165	DL-01-F017	Gorham, NH, Site 4
	Fe. tarquinius	Larva	ANTS168	DL-01-F019	Gorham, NH, Site 5
	Fe. tarquinius	Larva	ANTS172	DL-01-F025	Gorham, NH, Site 5
	Fe. tarquinius	Larva	ANTS144	DL-01-F030	Gorham, NH, Site 6b
	Fe. tarquinius	Pupa	ANTS173	DL-01-F011	Gorham, NH, Site 2
	Fe. tarquinius	Pupa	ANTS163	DL-01-F022	Gorham, NH, Site 5
	Fe. tarquinius	Pupa	ANTS166	DL-01-F032	Gorham, NH, Site 6a
Green lacewings	*Chrysopa slossonae*	Larva	ANTS160	–	Gorham, NH, Site 4
	C. slossonae	Larva	ANTS179	DL-01-F024	Gorham, NH, Site 5
Syrphid flies	*Syrphus ribesii*	Larva	ANTS176	–	Whitefield, NH, Site 1a
	S. ribesii	Larva	ANTS153	–	Whitefield, NH, Site 1a
	S. ribesii	Larva	ANTS175	DL-01-F029	Gorham, NH, Site 6b
Ants	*Formica glacialis*	Workers	ANTS149	DL-01-F003	Whitefield, NH, Site 1a
	Fo. glacialis	Workers	ANTS156	DL-01-F004	Whitefield, NH, Site 1b
	Fo. glacialis	Workers	ANTS151	–	Gorham, NH, Site 5
	Myrmica incompleta	Workers	ANTS158	DL-01-F010	Gorham, NH, Site 6b

Appendix 2

Cuticular hydrocarbons from the woolly aphid *Prociphilus tessellatus*, its insect predators, and tending ants. Chromatograms from each species and life stage are presented in Fig. 1. ECL, equivalent chain length of *n*-alkane (Nelson & Sukkestad, 1975); CN, carbon number; MI, molecular ion (*m*/*z*); **X**, major component in all samples from a given species; ×, minor component of all samples; X, major component of some samples; ×, minor component of some samples; peaks were designated as 'minor' if they were approximately less than 10% as tall as the highest peak in the chromatogram.

			Diagnostic EI ions			Aphids	Ants		Predators			
Peak code	Compound(s)	ECL	CN	MI	Ion peaks (*m*/*z*)	PT	FG	MI	CS	SR	FTL	FTP
A	*n*-C_{23}	23	23	324		x	X	**X**	x	x	x	
B	*x*-$C_{24:1}$	23.75	24	336			**x**	**x**				
C	*n*-C_{24}	24	24	338		**x**	x	**x**	x	x	x	
D	4-MeC_{24}	24.64	25	352	71, 308/9	x			x			
E	*x*-$C_{25:1}$	24.75	25	350			**X**	**X**		x		
F	*y*-$C_{25:1}$	24.85	25	350		x						
G	n-C_{25}	25	25	352		**X**	**X**	**X**	**X**	**X**	**X**	**X**
H	11-; 13-MeC_{25}	25.33	26	366	168/9, 224/5; 196/7	x	**x**					
I	4-MeC_{25}	25.64	26	366	71, 322/3	**X**			**x**	**x**	**x**	
J	3-MeC_{25}	25.75	26	366	57, 336/7	**X**	**x**	**X**	**x**	**X**	**x**	
K	*n*-C_{26}	26	26	366		**X**			**x**	**x**	**x**	
L	4-MeC_{26}	26.64	27	380	71, 336/7	**x**			x		x	
M	3-MeC_{26}	26.75	27	380	57, 350/1	x						
N	*x*-$C_{27:1}$	26.75	27	378			**X**					
O	*n*-C_{27}	27	27	380		**X**	**x**	**x**	**X**	**X**	**X**	
P	9-; 11-; 13-; 14-MeC_{27}	27.33	28	394	140/1, 280/1; 168/9, 252/3; 196/7, 224/5; 210/1	**X**			**x**	**x**	x	
Q	11-; 13-MeC_{27}	27.33	28	394	168/9, 252/3; 196/7, 224/5		X					
R	*v*,*w*-diMeC_{27}	27.73	28	394			x				X	
S	3-MeC_{27}	27.75	28	394	57, 364/5	**x**			**x**	**x**	x	
T	*x*,*y*-diMeC_{27}	27.85	28	394		**x**			**x**		x	
U	*x*-$C_{29:1}$	28.75	29	406			x					
V	4-MeC_{28}	28.75	29	408	71, 364/5	**x**			**x**			
W	*x*-$C_{29:1}$	28.84	29	406		**x**			**x**			
X	*n*-C_{29}	29	29	408		**x**			**X**		**X**	
Y	8-; 11-; 12-; 13-; 14-MeC_{29}	29.33	30	422	126/7, 322/3; 280/1, 168/9; 182/3, 266/7; 196/7, 252/3; 224/5	**X**			**x**	**x**		
Z	11-; 13-; 15-MeC_{29}	29.33	30	422	280/1, 168/9; 196/7, 252/3; 224/5		x					
AA	11,15-diMeC_{29}	29.64	30	422	168/9, 224/5, 238/9, 294/5		X					
AB	5,16-diMeC_{29}	29.83	31	436	85, 210/1, 252/3, 378/9	**x**			x		x	
AC	8,12,16-triMeC_{30}	30.70	33	464	126/7, 196/7, 224/5, 266/7, 294/5, 364/5	**x**			**x**			
AD	*n*-C_{31}	31	31	436		x			**X**			
AE	13-; 15-MeC_{31}	31.33	32	450	196/7, 280/1; 224/5, 252/3	**X**			**x**			
AF	*n*-C_{33}	33	33	464		x			**x**			

PT, *Prociphilus tessellatus*; FG, *Formica glacialis*; MI, *Myrmica incompleta*; CS, *Chrysopa slossonae*; SR, *Syrphus ribesii*; FTL, *Feniseca tarquinius* larva; FTP, *Feniseca tarquinius* pupa.