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THE EFFECTS OF ANTS ON THE ENTOMOPHAGOUS BUTTERFLY CATERPILLAR *Feniseca tarquinius*, AND THE PUTATIVE ROLE OF CHEMICAL CAMOUFLAGE IN THE *Feniseca*–ANT INTERACTION

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Abstract—Butterfly caterpillars in the lycaenid subfamily Miletinae are predators of ant-tended Homoptera, yet they lack specialized secretory and call-production organs crucial to ant association in other lycaenids. Here, we address the question of how miletine caterpillars have invaded the ant–Homoptera symbiosis through a study of the only New World miletine, *Feniseca tarquinius*, a predator of the wooly aphid *Prociphilus tesselatus*. Previous interpretations have suggested that *F. tarquinius* and other miletine caterpillars avoid ant aggression by concealing themselves under silken webs. In contrast, our field data indicate that *F. tarquinius* caterpillars are less likely to be concealed in the presence of the ants *Camponotus pennsylvanicus* and *Formica obscuriventris* than in the absence of ants, although caterpillar and ant behaviors vary between years. Chemical analysis and behavioral assays suggest that chemical camouflage, not physical concealment, is responsible for the ants' failure to detect and remove *F. tarquinius* caterpillars from aphid colonies. Analyses by gas chromatography indicate that the cuticular lipid composition of caterpillars are similar to that of their aphid prey, although it varies with prey species. Behavioral assays confirm that solvent extracts of *F. tarquinius* caterpillars and *P. tesselatus* aphids evoke similar behavioral responses in *C. pennsylvanicus* ants. Chemical camouflage is well known in social parasites

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of ants, but the present study represents one of a few documented cases where chemical deceit is important to interactions with ants outside the nest.

Key Words—Lycaenidae, Miletinae, *Feniseca tarquinius*, chemical camouflage, cuticular hydrocarbons, lycaenid–ant interactions, carnivorous caterpillars, *Camponotus pennsylvanicus*, Eriosomatidae, *Prociphilus tesselatus*.

INTRODUCTION

Caterpillars in the butterfly family Lycaenidae are unique in their propensity to form symbioses with ants. An estimated 6000 species of lycaenids account for nearly 50% of all butterflies, and about 75% of documented lycaenid caterpillars are associated with ants (DeVries, 2001; Pierce et al., 2002). Many lycaenid–ant associations are deemed mutualistic; here, ants protect caterpillars from predators and parasitoids in exchange for food secretions, and caterpillars mediate these symbioses through a suite of secretory and call production organs (Malicky, 1970; Atstatt, 1981; Cottrell, 1984; DeVries, 1988, 1990; Cushman et al., 1994; Pierce et al., 2002). Other lycaenid caterpillars are social parasites that infiltrate ant nests and consume ant brood or food regurgitations (Cottrell, 1984; Pierce, 1995). These caterpillars appear to employ one of two strategies. Some are recognized as intruders but are heavily armored to survive attack; others mimic the cuticular hydrocarbons by which ants recognize their brood and are accepted into the ant nest where they are tended and sometimes fed (Cottrell, 1984; Akino et al., 1999).

In marked contrast to other lycaenid groups, the predominantly Old World subfamily Miletinae has a unique relationship with Homoptera and ants. Miletine adults feed on the honeydew of homopterans, and the caterpillars are predators of the same homopterans, which are also tended by ants for their honeydew excretions (Clark, 1926; Cottrell, 1984). Thus, unlike other lycaenids, miletines compete with ants for homopteran resources (Cottrell, 1984; Maschwitz et al., 1988). All miletine caterpillars lack the secretory organs considered crucial to myrmecophily, and they are not exceptionally well armored (Cottrell, 1984). This raises the question of how miletines have invaded the ant–homopteran symbiosis, and how ants affect miletine fitness.

Our understanding of miletine–ant interactions is drawn from largely anecdotal evidence. Miletine caterpillars are often concealed under silken webs thought to protect them from aggressive ants (Edwards, 1886; Scudder, 1889; Atstatt, 1981; Cottrell, 1984). It appears that some miletine caterpillars are attractive or neutral to ants or are bitten only in moments of stress; others are known to survive within ant nests, perhaps as social parasites (Cottrell, 1984; Kitching, 1987; Maschwitz et al., 1988; Pierce, 1995). Further evidence is needed to clarify the nature of miletine–ant interactions. Here, we address the

behavioral and chemical ecology of the miletine caterpillar *Feniseca tarquinius* (Fabricius) and the ants that tend its aphid prey.

F. tarquinius is the only known miletine in the New World and is the sole representative of the genus. It is widespread in eastern North America and feeds predominantly on wooly alder aphids, *Prociphilus tesselatus* (Fitch), that are facultatively tended by various formicine, dolichoderine, and myrmicine ants (Scudder, 1889; Clark, 1926; Holldobler and Wilson, 1990; Youngsteadt, personal observations). Like other miletine caterpillars, *F. tarquinius* often conceals itself under a silken web covered with carcasses and "wool" of its aphid prey. A series of field and garden observations in the late 1800s gave rise to the prevailing hypothesis that physical concealment is crucial to survival of *F. tarquinius* caterpillars among ants (Edwards, 1886; Scudder, 1889).

Observations presented here suggest that physical concealment is not a defense against ants, but support the alternative hypothesis that *F. tarquinius* caterpillars are chemically concealed among their prey. Chemical mimicry and camouflage have been documented among taxonomically diverse nest parasites of social insects. Such parasites infiltrate their host's nestmate recognition system by bearing cuticular hydrocarbons similar to those of the host (reviewed in Holldobler and Wilson, 1990; see also Howard et al., 1990; Akino et al., 1996, 1999; Allan et al., 2002). Chemical similarity is deemed mimicry if a parasite synthesizes host-like hydrocarbons or camouflage if the parasite incorporates host-synthesized hydrocarbons into its own cuticle (Howard et al., 1990; but see alternative definitions in Dettner and Liepert, 1994). The mechanism by which camouflaged insects incorporate host hydrocarbons into their cuticles probably varies among species. One mechanism, implied or demonstrated in various insect–insect interactions, is passive transfer of hydrocarbons by physical contact (e.g., Vander Meer and Wojcik, 1982; Akino et al., 1996; Liang and Silverman, 2000).

Ants may also defend resources outside the nest, such as homopterans and extrafloral nectaries (Way, 1963; Holldobler and Wilson, 1990; Huxley and Cutler, 1991). Other insects invade these symbioses as competitors and circumvent ant defenses by various means (e.g., Eisner et al., 1978; DeVries and Baker, 1989; Liepert and Dettner, 1993, 1996). However, with the exception of one aphid parasitoid that evades ant aggression by chemical resemblance of its host aphids (Liepert and Dettner, 1993, 1996), the role of cuticular hydrocarbons in competitive interactions with ants outside the nest has received little attention. The present study of *Feniseca*–ant interactions offers further evidence for the role of chemical deceit in the exploitation of ant-tended resources outside the nest.

Here, we examine aspects of the *Feniseca*–ant interaction through field observations, chemical analyses, and behavioral assays that address two questions. First, is caterpillar physical concealment a defense against ants?

Second, is chemical camouflage responsible for the ants' apparent inability to perceive *Feniseca* caterpillars?

METHODS AND MATERIALS

Field Observations. To characterize the *Feniseca*–ant interaction, field observations were made on *F. tarquinius* caterpillars during August and September 2001, and July through September 2002, at two study areas in southeast Wisconsin (USA). At one site, the carpenter ant *Camponotus pennsylvanicus* (DeGeer) was the predominant species tending *P. tesselatus* aphids; at the other, *Formica montana* Emery and *F. obscuriventris* Mayr dominated. Ants were identified by using Creighton (1950), and voucher specimens are housed in Youngsteadt's collection.

In 2001, caterpillars were censused daily on all known aphid colonies and the following data were recorded: species and number of ants present on the host aphid colony, dimensions of the aphid colony, caterpillar instar, and presence or absence of a silken web over the caterpillar. Direct caterpillar–ant interactions and caterpillar disappearance were also recorded. Caterpillars develop over four instars, each 2–5 days in duration, before dropping from the aphid colony and wandering to a pupation site on other vegetation (Scudder, 1889; Youngsteadt, personal observations). Therefore, disappearance of a caterpillar prior to the second day of its fourth instar was attributed to death; later disappearance was attributed to pupation.

In 2002, censuses were conducted weekly, so fates of individual caterpillars were not followed; all other variables were recorded as in 2001. Survey intervals made it unlikely that an individual caterpillar was recorded repeatedly and certainly never more than once in a given instar.

Dependence of concealment behavior on presence and species of ants was evaluated by using a series of Fisher's exact tests (Sokal and Rohlf, 1981), in which, for each caterpillar instar, behavior in the presence of each ant species was compared with behavior in the absence of ants. Hence, up to three comparisons were performed per instar. Resultant P values were subjected to Bonferroni corrections to reflect family-wise type II error rates within each instar. Site effects were not considered in the analysis of concealment behavior because the entire study area was composed of similar habitat, caterpillar behavior varied between ant species within a site, and caterpillars at nontended colonies behaved the same way at both sites. Pupation rate was also tested for ant dependence with a Fisher's exact test (Sokal and Rohlf, 1981). A Mann–Whitney U-test compared ant density on aphid colonies between 2001 and 2002 (Sokal and Rohlf, 1981).

Chemical Analysis. Cuticular lipid composition of *F. tarquinius* caterpillars was compared to that of ants and aphids to evaluate whether chemical similarity to another species played a role in ants accepting caterpillars on aphid colonies. When results indicated that caterpillar surface lipid composition was similar to that of *P. tesselatus*, caterpillars were also reared on a novel aphid host to test whether caterpillars were true *P. tesselatus* mimics or whether they were camouflaged with surface lipids acquired from the host. Live specimens of *P. tesselatus*, *F. tarquinius*, *F. montana*, and *F. obscuriventris* were collected, frozen, and immediately stored at −80°C until analysis.

For the mimicry vs. camouflage experiment, five *F. tarquinius* caterpillars were reared from the second instar on an unidentified dogwood aphid species (Aphididae). Well into the fourth instar, caterpillars and their dogwood aphid hosts were killed and stored as above.

To extract cuticular lipids, insects (1 caterpillar, 5 ants, 7 *P. tesselatus*, or 60 dogwood aphids per extract) were placed in a 10 ml borosilicate conical bottomed screw-cap centrifuge tube and immersed in two sequential 3 ml hexane washes for 2.5 min each. The two washes were combined and spiked with 7.5 μg each of *n*-tricosane ($C_{23}H_{48}$) and *n*-dotriacontane ($C_{32}H_{66}$) as internal standards. The extract was concentrated to dryness under a gentle stream of N_2 and resuspended in 30 μl hexane. Periodically, a hexane blank including internal standards was prepared by the same method to confirm absence of contamination.

Extracts were analyzed by gas–liquid chromatography (GC). The gas chromatograph was a Hewlett-Packard 5890-II equipped with flame ionization detector and HP 3396-II integrator. Injection was split, with a split ratio of 129:1; injections were manual, 1.5 μl. The column was a fused silica capillary column (30 m × 0.32 mm) with a 1-μm DB-1 stationary phase. Helium was the carrier gas at a flow of 0.5 ml/min. Programmed conditions were as follows: injection port 280°C, FID 320°C, oven at 80°C 1 min, ramp at 10°C/min, 310°C for 30 min.

Components were distinguished by retention times. Bray–Curtis similarities, computed with fourth root transformed relative abundances of 42 components, were used to compare lipid profiles. The similarity matrix of pairwise comparisons among all samples was represented in a nonmetric multidimensional scaling ordination plot and analyzed in the statistical package Primer Version 5. Clarke (1993) and Elmes et al. (2002) discussed these methods. Included in the analysis were 5 extracts each of *P. tesselatus* and *F. tarquinius*, 3 extracts each of *F. montana*, dogwood aphids, and *F. tarquinius* reared on dogwood aphids, and 2 extracts of *F. obscuriventris*. Total cuticular lipids were considered, and these may include, in addition to hydrocarbons, other lipid classes not specifically implicated in ant recognition systems (Singer, 1998; Lahav et al., 1999).

To describe robust patterns of peak presence and absence underlying the mathematical similarities between caterpillars and aphids, we prepared a reduced dataset that included only those components that were present in all individuals of at least two species, or that were present in all individuals of at least one species but absent in all individuals of at least one other species. Excluded from this dataset were components that were variable in all species, present in one species but variable in all others, or absent in one or more species but variable in others. The remaining "indicator peaks" form the basis for descriptive statements about patterns of peak presence and absence.

Behavioral Assays. To test for behavioral relevance of the hexane extracts analyzed by GC, comparable extracts were offered to workers from three different *C. pennsylvanicus* colonies in the context of *P. tesselatus* colonies during September 2002. Extracts and blanks were prepared as described above, excluding the internal standards. Except for ants and aphids (as above), each extract was of a single insect. Solvent blanks ($N = 5$) and extracts of the following insects were offered: *Formica ulkei* Emery ($N = 7$ extracts); *P. tesselatus* ($N = 6$); adults of the aphid predator *Harmonia axyridis* (Pallas) (Coleoptera: Coccinelidae) ($N = 4$); and fourth instar *F. tarquinius* caterpillars ($N = 6$).

In the field, filter paper disks (5-mm diam) were treated with 30-μl insect extract (i.e., equivalent of 1 caterpillar, 1 beetle, 5 ants, or 7 aphids) or pure hexane, allowed to dry, then pinned onto the alder adjacent to the tended aphid colony. A single experimental set consisted of a blank and a series of the 4 extracts, or a subset thereof, in variable haphazard order. If conditions allowed for a second set to be presented immediately to the same ants, it was presented in reverse order to the previous series. Ant response to each extract was recorded for 20 min. Total number of ants present was recorded, along with the number of each of the following behaviors: lunge bite, bite, open-jawed lunge, open jawed inspection, closed-jawed inspection, ignore. Repeated instances of the same behavior were recorded when an ant discontinued, then reestablished physical contact with the disk. An encounter was scored as "ignore" if an ant made antennal contact with the disk in passing, but did not maintain contact or show observable change in behavior or orientation.

For analysis, behaviors were pooled as either aggressive or nonaggressive, and ant response to each extract or blank was quantified in two ways: proportion of aggressive acts out of total number of encounters and number of aggressive acts per ant per minute. Ant response to blanks and extracts was compared using two Kruskal–Wallis tests—one for proportional aggression and one for absolute aggression (Sokal and Rohlf, 1981). Transformation of proportion data was not necessary in this case (Zar, 1999). To test for qualitative differences in ant aggression toward different extracts and blanks, aggression was subdivided into its four component categories. Using a logistic regression model in the SAS

system proc genmod, extracts and blanks were tested for differences in the likelihood of eliciting each category of aggressive behavior.

Caterpillar Morphology. To determine whether the setae of *F. tarquinius* caterpillars are modified in such a way as to enhance collection or dissemination of semiochemicals, we preserved field-collected fourth instar *F. tarquinius* in 80% EtOH and examined them under a scanning electron microscope.

RESULTS

Field Observations. Table 1 presents frequencies of caterpillar concealment in 2001 and 2002. In 2001, behavior of second through fourth instar *F. tarquinius* caterpillars appeared to depend on the presence and species of ant: caterpillars were concealed less often in the presence of *C. pennsylvanicus* and *F. obscuriventris* than *F. montana* or no ants. First instar caterpillars showed a similar trend. In 2002, this effect was marginally significant only for third instars. Other caterpillars were predominantly concealed regardless of ants. However, density of attendant *C. pennsylvanicus* differed significantly among years (Figure 1). Ants tended *P. tesselatus* colonies at a consistently higher density in 2001, but this was lower and more sporadic in 2002.

Caterpillar survival data were available only for one site in 2001. Here, 54% of caterpillars on nontended aphid colonies disappeared prior to pupation, whereas only 35% of those on *C. pennsylvanicus*-tended colonies disappeared. Pupation among treatments did not differ significantly ($P = 0.472$, $N = 13$ for nontended colonies, 20 for tended colonies).

Few direct caterpillar–ant interactions were observed: ants generally ignored caterpillars or tapped them with their antennae before moving away. On one occasion, when a tended aphid colony was disrupted by the observer bumping a branch, a *C. pennsylvanicus* bit a third instar caterpillar and dropped it from the colony. However, the caterpillar remained suspended on a thread and returned to the colony unharmed and without further incident. Once a *C. pennsylvanicus* ant was observed to kill and consume a freshly pupated *F. tarquinius* that had anchored itself on a vine entwined with a tended aphid-infested alder.

Chemical Analysis. Representative chromatograms are shown in Figure 2. Mean Bray–Curtis pairwise similarities (Table 2) indicate that cuticular lipid similarity was higher within species (77–90%) than between any pair of species (41–76%). Of the between-species comparisons, two similarities were unusually large: that between *P. tesselatus* and "normal" *F. tarquinius* that had eaten *P. tesselatus* (76%) and that between "normal" *F. tarquinius* and *F. tarquinius* that ate dogwood aphids (71%). All other between-species comparisons,

TABLE 1. FREQUENCY OF CATERPILLAR CONCEALMENT ON APHID COLONIES IN THE ABSENCE OR PRESENCE OF EACH OF THREE ANT SPECIES

Ant species	*N*	Percentage of caterpillars concealed (%)	*P* (two-tailed)
Year: 2001 (caterpillars censused on 29 aphid colonies)			
Fourth instar			
C. pennsylvanicus	12	0	<0.001
F. obscuriventris	12	0	<0.001
F. montana	11	73	ns
No ants	13	85	
Third instar			
C. pennsylvanicus	12	17	<0.001
F. obscuriventris	4	0	<0.005
F. montana	11	91	ns
No ants	10	100	
Second instar			
C. pennsylvanicus	15	60	<0.05
F. obscuriventris	3	67	ns
F. montana	11	91	ns
No ants	19	100	
First instar	15		
C. pennsylvanicus	4	75	ns
F. montana	3	100	ns
No ants	8	100	
Year: 2002 (caterpillars censused on 34 aphid colonies)			
Fourth instar			
C. pennsylvanicus	19	79	ns
F. montana	6	67	ns
No ants	23	87	
Third instar			
C. pennsylvanicus	17	76	0.07
F. montana	7	100	ns
No ants	20	100	
Second instar	33		
C. pennsylvanicus	14	100	ns
No ants	19	100	
First instar	6		
C. pennsylvanicus	3	100	ns
No ants	3	100	

Probabilities are shown for pairwise comparison of each ant species to "no ants" (Fisher's exact test with Bonferroni correction). No data are presented for *F. obscuriventris* in 2002 because no aphids established near the one known *F. obscuriventris* nest.
ns = Not significant.

including those between caterpillars and ants, yielded similarities ≤57%. The nonmetric MDS ordination plot (Figure 3) discriminates among species. Analysis of similarities (ANOSIM) is typically used to test for significance of MDS groupings (Clarke, 1993). The global ANOSIM test was significant for this

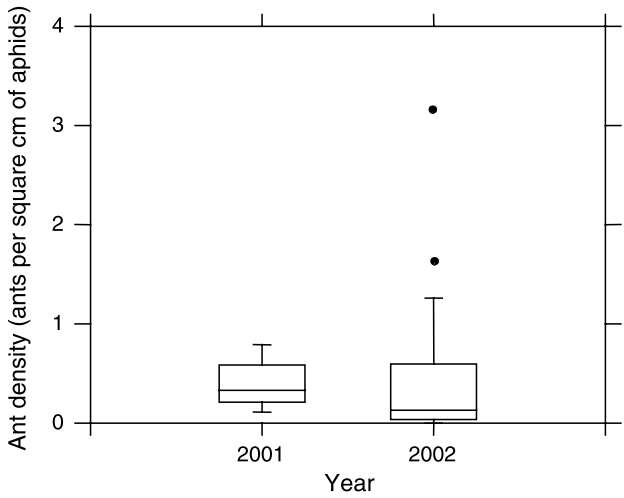

FIG. 1. Density of attendant *C. pennsylvanicus* ants on *P. tesselatus* aphid colonies in 2001 and 2002 based on 32 and 40 measurements, respectively. Mann–Whitney U = 859.0, P = 0.013. Each box encompasses the midrange; central horizontal line is the median; whiskers extend to furthest data points within 1.5 midranges of q1 and q3. Data points further dispersed are indicated by dots.

dataset (R = 0.97, P = 0.001), but sample sizes were too small for valid pairwise comparisons between species.

The "indicator peaks" found in *P. tesselatus*, "normal" *F. tarquinius*, dogwood aphids, and *F. tarquinius* that ate dogwood aphids are presented in Table 3. *P. tesselatus* and "normal" *F. tarquinius* have 15 indicator peaks in common, five of which are unique to this pair; they differ by only three. Of these three, two are present in *F. tarquinius* but not in *P. tesselatus*, whereas one is present in *P. tesselatus* but not in *F. tarquinius*.

All peaks present on *F. tarquinius* caterpillars that ate dogwood aphids were a subset of those found on "normal" *F. tarquinius*—including the two peaks absent in *P. tesselatus* aphids. On the other hand, "normal" *F. tarquinius* had five additional peaks not found in *F. tarquinius* that ate dogwood aphids, and all of these were shared with *P. tesselatus*. Peak 15 was notably more abundant in both caterpillar groups than in either aphid species.

Dogwood aphids had a simple lipid profile that included 10 indicator peaks. Two of these were absent from *F. tarquinius* that ate dogwood aphids. Six peaks were common to dogwood aphids and caterpillars that ate them, but were not unique to this pair, and three of these differed markedly and consistently in abundance. Finally, *Feniseca* that ate dogwood aphids had three

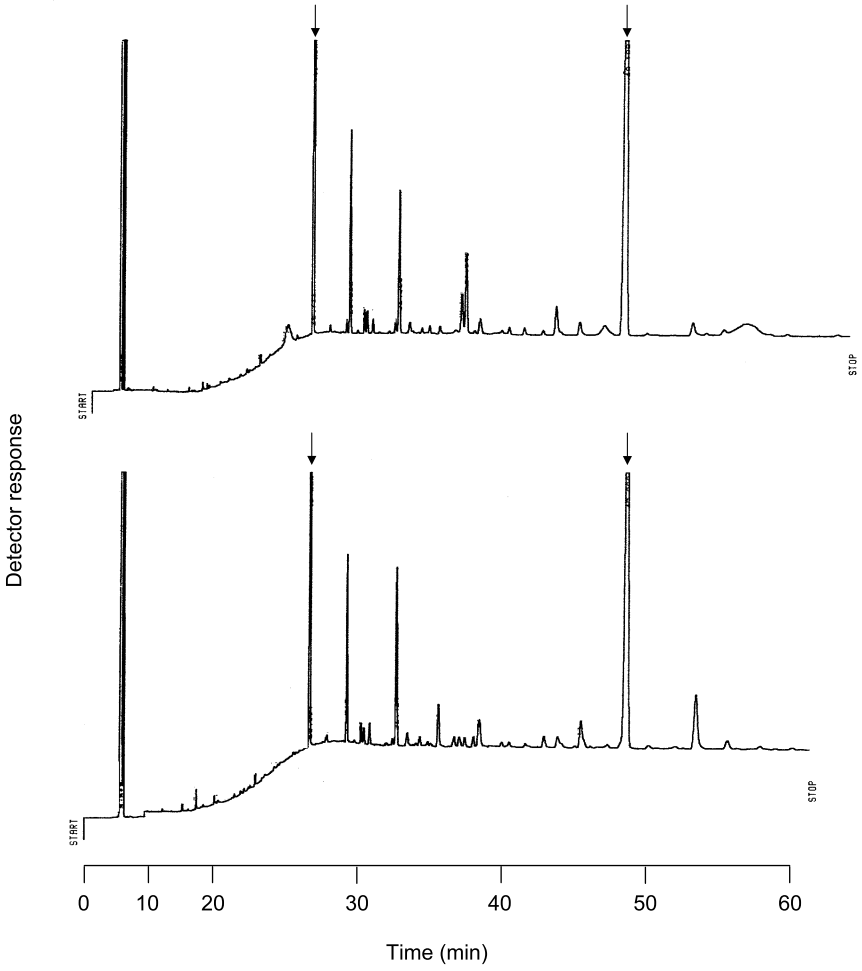

FIG. 2. Gas chromatograms of hexane extract of cuticles of fourth instar *F. tarquinius* caterpillar (top) and adult *P. tesselatus* aphids (bottom). Internal standards n-C_{23} and n-C_{32} are indicated by arrows.

peaks that were absent from those aphids, but which also occurred in normal *F. tarquinius*.

Although the *F. tarquinius* lipid profile varied with diet, the amount of lipids extracted did not differ between the two groups of caterpillars. Total cuticular lipids recovered per caterpillar were 4.73 ± 1.058 μg (mean ± SD) for normal caterpillars and 5.45 ± 0.433 μg for caterpillars reared on dogwood aphids (N = 8 and 5, respectively).

TABLE 2. MEAN BRAY–CURTIS PERCENT SIMILARITIES ± SDS WITHIN AND BETWEEN SPECIES IN THE *Feniseca* SYSTEM BASED ON 42 CUTICULAR LIPID COMPONENTS

	Pt	Ft	FD	DA	Fm	Fo
Pt	89.5 ± 4.9 (10)					
Ft	76.1 ± 4.3 (25)	86.9 ± 4.8 (10)				
FD	53.4 ± 4.3 (15)	70.5 ± 3.2 (15)	76.9 ± 9.5 (3)			
DA	52.8 ± 3.6 (15)	54.6 ± 6.9 (15)	57.2 ± 6.2 (9)	85.0 ± 2.5 (3)		
Fm	40.8 ± 3.0 (15)	53.3 ± 2.6 (15)	54.5 ± 4.4 (9)	43.1 ± 5.8 (9)	85.5 ± 4.8 (3)	
Fo	51.8 ± 3.0 (10)	51.8 ± 2.2 (10)	52.4 ± 3.1 (6)	49.3 ± 2.4 (6)	41.6 ± 5.5 (6)	77.8 (1)

Numbers of pairs are reported in parentheses.
Pt, *P. tesselatus* aphids; Ft, *F. tarquinius* caterpillars reared on *P. tesselatus*; FD, *F. tarquinius* reared on dogwood aphids; DA, dogwood aphids; Fm, *F. montana* ants; Fo, *F. obscuriventris* ants.

Behavioral Assays. Ant response to filter papers treated with solvent and insect extracts is shown in Figure 4, as proportion of aggressive acts. The absolute aggression measure yielded an identical pattern (Kruskal–Wallis test statistic = 18.89, $P < 0.001$; Bonferroni–Dunn *post hoc* test results were also exactly as described for relative aggression in Figure 4). *C. pennsylvanicus*

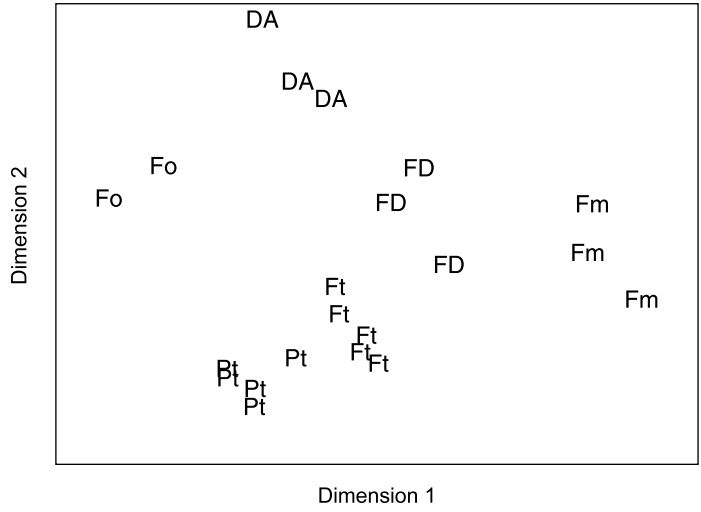

FIG. 3. Two-dimensional nonmetric multidimensional scaling ordination of 21 samples, derived from Bray–Curtis similarities between each pair of samples (stress = 0.13). Species are *P. tesselatus* aphids (Pt), *F. tarquinius* caterpillars reared on *P. tesselatus* (Ft), *F. tarquinius* reared on dogwood aphids (FD), dogwood aphids (DA), *F. montana* ants (Fm), and *F. obscuriventris* ants (Fo).

TABLE 3. PRESENCE AND ABSENCE OF "INDICATOR PEAKS" IN *F. tarquinius* CATERPILLARS AND APHIDS

Peak	Retention time (min)	Pt	Ft	DA	FD
1	28.06	x	x	0	x
2	29.15	0	x	0	x
3	29.38	x	x	x	x
4	30.34	x	x	0	0
5	30.52	x	x	(v)	(v)
6	30.91	x	x	x	x
7	32.42	(v)	x	(v)	x
8	32.67	x	x	x	x
9	33.41	x	x	0	0
10	34.47	0	x	0	x
11	34.97	(v)	x	(v)	x
12	35.46	x	(v)	0	(v)
13	36.51	x	0	0	0
14	36.83	x	x	x	x
15	37.20	x	x	x	x
16	38.18	x	x	0	0
17	41.17	x	x	x	x
18	42.43	x	x	0	0
19	43.32	x	x	x	(v)
20	44.89	x	x	0	0
21	49.35	x	(v)	x	0
22	52.51	x	x	x	(v)
23	56.31	0	0	x	0

Pt, *P. tesselatus* aphids; Ft, *F. tarquinius* caterpillars reared on *P. tesselatus*; DA, dogwood aphids; FD, *F. tarquinius* reared on dogwood aphids. x indicates presence of a peak in all individuals of a "species", 0 indicates absence of a peak in all individuals of a species, and (v) indicates variability: the peak is present in some but not all individuals of the species.

workers were continuously hostile toward extracts of other ants and the aphid predator *H. axyridis*. Ants were initially aggressive toward caterpillar and aphid extracts and blanks—it was impossible to introduce samples onto aphid colonies without ants responding to the intrusion—but they quickly accepted the samples and did not display further aggression.

Ant response did not depend on the order in which extracts were offered. A Kruskal–Wallis test with position, rather than extract identity, as the grouping variable was not significant, and we observed that ant response to a given extract was consistent regardless of when it was presented relative to other extracts. Differences in aggression toward extracts and blanks were quantitative, not qualitative. Extracts and blanks did not differ in the likelihood of eliciting the four categories of aggressive behaviors (uncorrected P values ≥ 0.7 for all 10 comparisons between extracts and blanks).

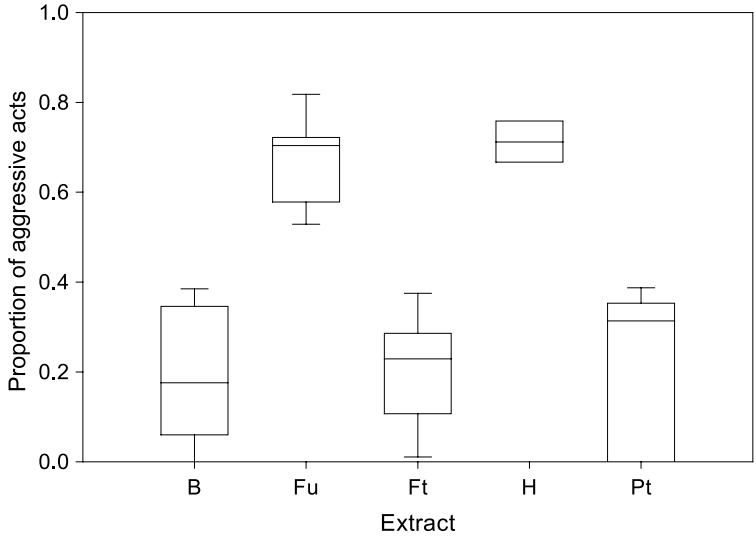

FIG. 4. *C. pennsylvanicus* response to filter paper dummies introduced to tended *P. tesselatus* aphid colonies. *Y*-axis indicates proportion of aggressive acts out of total number of encounters. Dummies were treated with solvent blank (B) or extract of one of the following insects: *F. ulkei* ants (Fu), *F. tarquinius* caterpillars reared on *P. tesselatus* aphids (Ft), *H. axyridis* beetles (H), or *P. tesselatus* (Pt). Median, box, and whiskers are as in Figure 1. Kruskal–Wallis test statistic = 19.623, $P < 0.001$. Bonferroni–Dunn *post hoc* comparisons confirmed that ant response to both *F. ulkei* and *H. axyridis* differed from response to each of the other three extracts ($P < 0.001$ for those six comparisons, ns for all others).

Caterpillar Morphology. Typical body setae of a fourth instar *F. tarquinius* caterpillar are shown in Figure 5. These setae are simple, resembling ordinary tactile and defensive body setae found in a wide range of nonmyrmecophilous lycaenid caterpillars (DeVries 1997, unpublished data).

DISCUSSION

Effects of Ants on Caterpillars. Given the prevailing notion that ants select for concealment behavior in *F. tarquinius* caterpillars (Edwards, 1886; Scudder, 1889; Atstatt, 1981; Cottrell, 1984), it was surprising to observe the reverse pattern in the field in 2001 (Table 1). It is tempting to speculate that concealment protects caterpillars from natural enemies, a function performed by ants when they are present. It is unclear why *F. montana* had no effect on

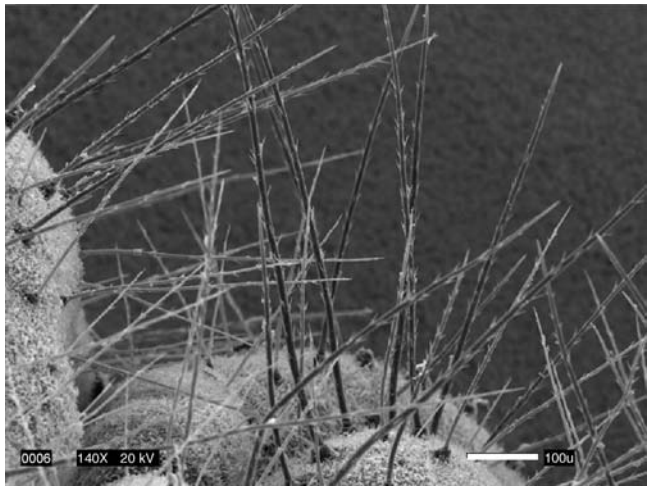

FIG. 5. Scanning electron micrograph of dorsolateral setae on the third thoracic (left) and first abdominal (right) segments of a fourth instar *F. tarquinius* caterpillar.

caterpillar concealment, but it may relate to interspecific differences in ant behavior. Although ant behavior was not quantified, it appeared that, relative to the other two species, *F. montana* had a higher rate of worker turnover on aphid colonies, was less likely to assume defensive posture, and more likely to move away or drop off the plant when disturbed. If these characteristics made *F. montana* less likely to deter intruders from aphid colonies, caterpillar behavior was consistent with the interpretation that caterpillars are concealed when not defended, albeit inadvertently, by ants. Similarly, low and sporadic density of *C. pennsylvanicus* in 2002 may have decreased its protective value and contributed to more universal caterpillar concealment that year.

In 2002, we reproduced the alder–wooly aphid system in a screened experimental garden. Alders were paired, and *C. pennsylvanicus* ants were allowed to tend aphids on one tree in each pair. Paired caterpillars were introduced to aphid colonies on paired trees. In this experiment, there was no effect of *C. pennsylvanicus* on *F. tarquinius* caterpillar growth rate, survival, or behavior. Thus, we found no evidence, in the field or garden, that ants were a threat to *F. tarquinius* caterpillars. Our suggestion that aggressive ants tending *P. tesselatus* colonies also defend resident caterpillars is speculative, but suggests testable hypotheses about interactions in the *Feniseca* system. Although the causes and cues for caterpillar concealment remain unknown, our observations indicate that ants are not aggressive toward *F. tarquinius* caterpillars and suggest that caterpillar concealment is not a defense against ants.

Chemical Resemblance Between Caterpillars and Aphids. Cuticular lipid analysis suggests a mechanism by which *F. tarquinius* invades the ant–aphid mutualism. The 76% similarity in lipid composition between *F. tarquinius* and *P. tesselatus* (Table 2) is comparable to the hydrocarbon similarity between another well-documented chemical mimicry pair. The cuticular hydrocarbon composition of the socially parasitic lycaenid caterpillar *Maculinea rebeli* is 72% similar to that of its host ant *Myrmica schencki* when similarity is calculated by the same methods used here; this level of similarity is adequate to maintain peaceful host–parasite relations within the host ant colony (Elmes et al., 2002). It seems plausible that *Feniseca–Prociphilus* similarity is also adequate to deceive ants. It is important to note that, although only hydrocarbons are specifically implicated in ant recognition systems (Singer, 1998; Lahav et al., 1999), and only hydrocarbons were considered in the case of *Maculinea*, the present study considers total cuticular lipids. Separation and identification of the hydrocarbon fraction from the total lipid extract remain for future studies of the *Feniseca* system.

Caterpillars could invade aphid resources by resembling ants, rather than aphids. However, our data do not support this scenario. Resemblance between *F. tarquinius* caterpillars and attendant ant species was ≤55%, compared to 76% similarity between caterpillars and *P. tesselatus*. Resemblance to a specific ant species would limit caterpillars to aphid colonies tended exclusively by that species. On the other hand, resemblance to the host aphids should allow caterpillars to feed on aphid colonies tended by any ant species. The latter ant–generalist strategy could explain why *F. tarquinius* coexists with many ant species across its geographic range and also with different ant species that tend the same aphid colonies at different times of day.

Behavioral assays confirmed that cuticular extracts contained behaviorally relevant information for *C. pennsylvanicus* tending *P. tesselatus* colonies. Ants responded to extracts of potential competitors for aphid resources, *F. ulkei* and *H. axyridis*, with high levels of both absolute and relative aggression (Figure 4). They responded to blanks and extracts of *P. tesselatus* with significantly lower levels of aggression. Notably, ants responded to *F. tarquinius* extract as they did to the chemically similar aphid extract, not as they did to the extracts of other competitors, suggesting that ants did not recognize *F. tarquinius* caterpillars as aphid predators. Although trace volatiles may have been present in cuticular extracts (as in Allan et al., 2002), our results support the idea that chemicals present on the caterpillar cuticle can account for low levels of ant aggression toward *F. tarquinius* compared to other insects that might "trespass" on an aphid colony.

Camouflage vs. Mimicry. Rearing experiments suggested that the *Feniseca–Prociphilus* resemblance was due to chemical camouflage, not mimicry. In this study, caterpillars reared on dogwood aphids lost their resemblance

to *Prociphilus*, a result consistent with camouflage. They did not, however, acquire a resemblance to dogwood aphids. Because caterpillars might acquire camouflage when aphid lipids rub off on them through physical contact, we suspect that this result was because of aphid size. Dogwood aphids are minute relative to *Prociphilus*, and feeding caterpillars had relatively little bodily contact with them, whereas caterpillars feeding on *Prociphilus* had constant and abundant bodily contact with their prey. Thus, dogwood aphid lipids would be unlikely to rub off on feeding caterpillars, which would, therefore, display only *Feniseca*'s native lipids. Caterpillars feeding on larger *Prociphilus* would still produce their native lipids, but additional lipids acquired from prey would alter the total lipid profile.

This interpretation is consistent with the pattern of similarities among caterpillars and aphids (Table 2 and Figure 3) where *Prociphilus*-reared caterpillars appeared intermediate between the lipid profile of their prey and that of putatively unaltered caterpillars (i.e., those reared on dogwood aphids). This pattern is also reflected in the presence and absence of "indicator peaks" (Table 3), which provide no evidence that *Feniseca* that fed on dogwood aphids (henceforth "dogwood *Feniseca*") acquired lipids from its prey. Dogwood aphids had few unique components that could serve as "labels" for tracing lipid transfer. However, the many disparities between the simple lipid profiles of dogwood aphids and dogwood *Feniseca* do not suggest hydrocarbon acquisition. The indicator peaks of normal *Feniseca* are the sum of those present on dogwood *Feniseca* and *Prociphilus*, also consistent with our suggestion that the lipid profile of normal *Feniseca* is the result of both biosynthesis and acquisition. However, to appreciate the significance of specific differences between species, chemical identifications are necessary. For instance, it is important to know whether the caterpillar-specific peaks (peaks 2 and 10, Table 3) were nonhydrocarbons or other chemicals unlikely to influence ant behavior.

Our results support the camouflage hypothesis and a hydrocarbon rub-off mechanism, but cannot exclude the possibility that observed differences in caterpillar lipid profiles were due to diet-induced shifts in biosynthesis. We can conceive of three approaches that might distinguish between these alternatives. *In vitro* hydrocarbon synthesis experiments could clarify which components are biosynthesized by caterpillars. Alternatively, aphids could be treated with a synthetic hydrocarbon normally absent from the system. If this hydrocarbon appeared unaltered on caterpillar cuticle, the rub-off mechanism would be implicated. Finally, caterpillar internal and external hydrocarbon composition could be compared. Insect cuticular and internal hydrocarbons are generally the same, so if caterpillars had cuticular hydrocarbons that were absent internally, such hydrocarbons were likely acquired by rub-off.

Some myrmecophilous butterfly caterpillars possess ant organs that appear to release semiochemicals that modify ant behavior (DeVries 1988, 1997;

Pierce et al., 2002), and when examined under high magnification, the setae associated with these organs may show flaps and convolutions that could act to disseminate volatile chemistry (DeVries 1997). Our data suggest that chemical camouflage is important to *F. tarquinius* caterpillars, but their setae showed no evidence of increased surface area or abrasive qualities that might aid in acquisition or dispersal of aphid chemistry (Figure 5). That *F. tarquinius* setae resemble the ordinary tactile and defensive body setae of nonmyrmecophilous lycaenid caterpillars is consistent with the mechanism of chemical camouflage by passive, nonspecialized lipid acquisition.

In summary, although the role of physical concealment in *F. tarquinius* caterpillars remains unclear, all evidence from this study points away from ant avoidance. Instead, acquisition of chemical resemblance to the aphid prey appears adequate to defuse ant aggression. Such a relationship admits the possibility that ants could indirectly benefit caterpillars, especially when ants are abundant, pugnacious, and effective at excluding caterpillar enemies from aphid colonies. This study points to the possibility that chemical camouflage occurs among Old World miletines and other aphid predators and may be as common among users of ant-tended resources as it is among social parasites of ant nests.

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