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CHEMICAL MIMICRY IN A PARASITOID (HYMENOPTERA: EUCHARITIDAE) OF FIRE ANTS (HYMENOPTERA: FORMICIDAE)

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Abstract—A wasp (*Orasema* sp.) parasitic on the fire ant, *Solenopsis invicta* Buren, develops to the adult stage within the ant colony, where wasp larvae are ectoparasitic on ant pupae. This phase of the parasite's life cycle requires a mechanism of integration into the host colony. Gas chromatographic profiles of hexane soaks of various stages of the parasite and host suggest that during development within the ant colony the parasite acquires the colony odor of the host through a passive mechanism, based on simple contact and other social interactions. No parasite-specific components were observed. After leaving the host nest as adults, the parasite biosynthesizes a parasite-specific cuticular compound, while retaining residual amounts of the host acquired components. This complicated scenario is consistent with current knowledge of nestmate recognition and the preferential treatment of ant workers to their brood.

Key Words—Parasite, fire ant, *Solenopsis invicta*, *Orasema*, Hymenoptera, Formicidae, mimicry, nestmate recognition, cuticular hydrocarbons, Eucharitidae.

INTRODUCTION

The search for natural enemies of the fire ant, *Solenopsis invicta* Buren, has revealed a number of diseases, parasites, and inquilines (Jouvenaz, 1986; Wojcik et al., 1987). One of these is a wasp, *Orasema* sp. (Hymenoptera: Chalcidoidea: Eucharitidae), that parasitizes *S. invicta* in Brazil. Species of Eucharitidae only parasitize ants (Das, 1963; Johnson et al., 1986; Williams

and Whitcomb, 1974) and have an unusual life cycle. Female wasps oviposit in plants (leaves, buds, fruit) that are frequented by ants. The planidia (newly hatched larvae) attach themselves to foraging ants and are carried to the nest (Wheeler, 1907; Johnson et al., 1986). The planidia then make their way to ant brood and complete their development as ectoparasites (Wojcik, 1986).

The apparent inability of fire ants to distinguish between wasp brood and their own brood suggested that the *Orasema* sp. mask their odor in some way to confuse the ants' nestmate-recognition mechanism. There is ample precedence for chemical mimicry among myrmecophiles and termitophiles. Adults of the myrmecophilous scarab beetle [*Martinezia duterteri* Chalumeau = *Myrmecaphodius excavatiocollis* (Blanchard)] integrates into host colonies of fire ants, *Solenopsis* spp., by passively acquiring the colony odor of the host (Vander Meer and Wojcik, 1982). Integrated beetles move unmolested among host ants and obtain food directly from workers by trophallaxis, by predation on ant brood, and by feeding on dead ants (Wojcik, 1975). Chemical mimicry also occurs in syrphid flies (*Microdon* sp.) that live in ant nests (R. Howard, USDA, Manhattan, Kansas, personal communication). In addition, it has been proposed that myrmecophilous lycaenid larvae successfully associate with their hosts through mimicry of volatile secretions produced by the host ant and its brood (Henning, 1983). The termitophilous beetle, *Trichosenius frosti* Seevers, biosynthesizes the same cuticular hydrocarbons as its host, *Reticulitermes flavipes* Kollar (Howard et al., 1980). These compounds are believed to be important in termite nestmate recognition. Three other termitophiles have been shown to have a cuticular hydrocarbon profile identical to that of their hosts (Howard et al., 1982).

We report the results of studies that compare chemical profiles of cuticular washes of an *Orasema* sp. parasite and its host during parasite development within the host colony and of adult parasites captured away from their host colony.

METHODS AND MATERIALS

Source of Parasite and Host. Colonies of *S. invicta* were collected for biocontrol screening in the vicinity of Cáceres, Mato Grosso, and Campo Grande, Mato Grosso do Sul, Brazil, in 1985 and 1986, and the ants were separated from the soil by flotation (Jouvenaz et al., 1977). In addition to the adult and immature wasps and ants collected from nest soil (Cáceres), adult male and female wasps were captured with an insect net as they swarmed over fire ant nests (Campo Grande). Queenless, miniature laboratory colonies composed of conspecific adult and immature worker ants, with and without wasp parasites (wasps were always maintained with the host colony from which they

were collected), were maintained in soil-free nests for up to one week by methods described by Banks et al. (1981). To facilitate observation, colonies were limited to fewer than 200 individuals.

Sample Preparation. Pupae and adults of both wasps and ants were placed individually and in groups in 7-ml vials that contained ca. 0.5 ml HPLC-grade *n*-hexane (Merck, Darmstadt, West Germany). The vials were capped with aluminum foil-lined lids and allowed to stand at room temperature for 45 min or 24 hr. The hexane was transferred by Pasteur pipet to 2-ml vials. The vials were loosely capped and the hexane allowed to evaporate. The cap was then tightened. Great care was taken to avoid cross-contamination of the samples. All samples were externally labeled and shielded from sunlight and foreign matter. They were hand carried to our laboratory in Gainesville, Florida, where they were reconstituted with HPLC-grade hexane (Burdick and Jackson, Muskegan, Michigan) prior to analysis by gas chromatography.

Chemical Analysis. Gas chromatographic (GC) analyses were carried out on a Varian 3700 gas chromatograph (Walnut Creek, California) equipped with a flame ionization detector and a 30-m × 0.032-mm-ID DB-1 fused silica capillary column (J&W Scientific, Inc., Rancho Cordova, California). Helium was used as the column carrier gas, and nitrogen was used as the makeup gas. The following two oven temperature programs were used: (1) 50°C for 1 min then increased to 285°C at 5°/min and held for a total run time of 70 min; (2) 150°C for 1 min then increased to 285°C at 4°/min for total run time of 35 min. The chromatograms were printed and peak areas calculated on a Vista 401 data processor (Varian). If injection of 1 μl of the reconstituted samples gave a weak chromatogram, then they were concentrated under a stream of nitrogen and run again. The accuracy of peak integration was checked by replotting the chromatograms with integration baselines.

Quantitative Analysis of Orasema *sp. Adults.* Samples of evaporated hexane soaks of individual *Orasema* sp. adults were reconstituted with hexane. Three microliters of a 0.01% hexane solution of *n*-pentacosane was added as an internal standard. The samples consisted of three replicates of each of the following: (1) males collected from the host ant nest; (2) females collected from the host ant nest; and (3) males collected from outside potential host ant nests. Gas chromatograms were obtained for each sample as described above and the data quantified using the Varian Vista data processor. The quantitative data was statistically analyzed using the Newman-Keuls test.

Mass Spectral Analysis. Mass spectra were obtained on a Hewlett-Packard 5988A GC-MS (Hewlett-Packard Co., Palo Alto, California) that was operated in the EI mode (70 eV) and equipped with an HP 9000/300 Chemstation and interfaced with an HP 5890 GC operated in the splitless mode. An HP methyl silicone fused silica capillary column (12 m × 0.20 mm ID) was used for the chromatographic separation. The GC oven was operated at 60°C for 1 min then

to 225°C at 25°/min and finally to 300°C at 7°/min. The mass spectra of GC-separated components derived from pooled samples of *Orasema* sp. pupae and host colony pupae were compared. Comparison of the chromatograms of *Orasema* sp. pupae and adults with the corresponding host ant chromatograms was accomplished using the Newmans-Keuls test, comparing, one at a time, the percentages of the five dominant peaks in the chromatograms.

RESULTS

Parasitism in fire ant colonies by *Orasema* sp. (identified as *Orasema* sp. by L. de Santis, Universidad Nacional de La Plata, La Plata, Argentina) can be common, with up to 41% of the colonies sampled containing the parasites and 1–598 wasps per colony (Wojcik et al., 1987). Wasp larvae and pupae were visually distinguishable from host ant larvae and pupae. In the disturbed conditions of isolating ants and parasites by flotation (Jouvenaz et al., 1977), worker ants carried wasp larvae and pupae around in the same way ant brood was carried. Wasp pupae were readily adopted by conspecific colonies of fire ants. No aggressive behavior toward wasp pupae was observed. One female wasp eclosed in an observation nest. She did not move about the nest, but remained immobile and was tended with the ant brood. After two days, the adult wasp was presumed dead and was removed from the nest for examination, whereupon she immediately flew away.

Gas chromatograph (GC) traces of host worker ants show the characteristic venom alkaloid pattern of *S. invicta* (Figure 1A). These piperidine alkaloids are found in large amounts (10–20 μg) in the fire ant poison sac and are released into the solvent during the hexane soak period. They are composed of 6-methylpiperidine alkaloids with 2-substituted alkyl or alkenyl side chains (Brand et al., 1972). The structures are simply defined by the chain length of the 2-position side chain and whether or not it contains a double bond; i.e., $C_{13:1}$ = 2-tridecenyl-6-methylpiperidine; see Figure 1A. In addition, Figure 1A illustrates the species-specific hydrocarbon patterns characteristic of *S. invicta* (section A of Figure 1A) (Vander Meer, 1986). These components have been identified as a series of normal, monomethyl and dimethyl branched hydrocarbons (Lok et al., 1975; Nelson et al., 1980). The patterns and peak retention times were directly compared with authentic samples of *S. invicta* venom alkaloids and cuticular hydrocarbons.

Gas chromatograms of hexane soaks from male and female *Orasema* sp. adults collected from their host net (Figure 1B and C) showed an identical qualitative hydrocarbon pattern to that of its ant host (Figure 1A) established by comparison of retention times and mass spectra of the five major components. The mass spectra were completely in accord with published spectra (Lok et al.,

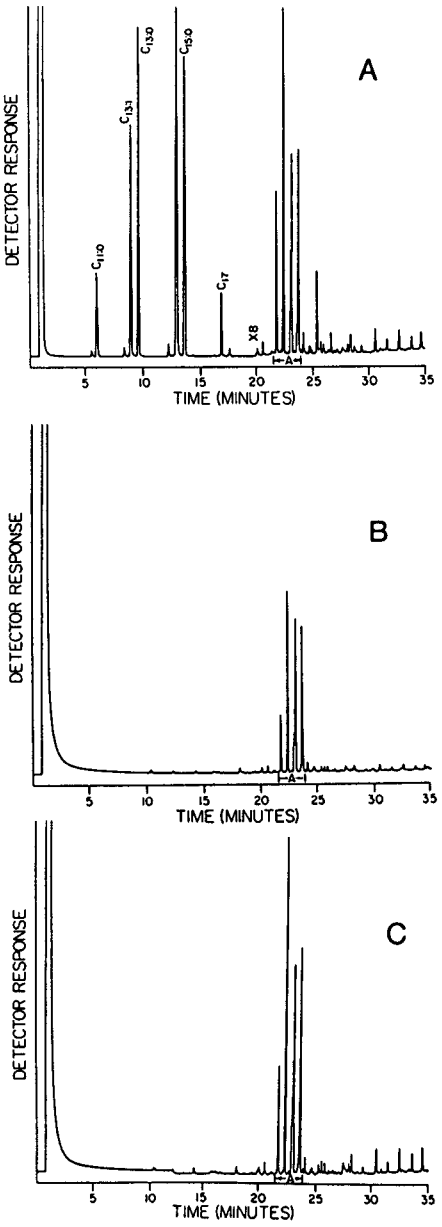

FIG. 1. Total gas chromatograph volatiles from hexane soaks of (A) host fire ant worker; (B) *Orasema* sp. female adult from host ant colony; (C) *Orasema* sp. adult male from host ant colony. Alkaloids are designated by their side-chain length and whether or not they contain a double bond. The five species-specific hydrocarbons associated with *S. invicta* are defined by chromatogram section A.

1975; Nelson et al., 1980; Thompson et al., 1981) of the five major *S. invicta* hydrocarbon components identified as *n*-heptacosane, 13-methylheptacosane, 13,15-dimethylheptacosane, 3-methylheptacosane, and 3,9-dimethylheptacosane. Venom alkaloids leached from the poison sac of the host during the hexane soak are evident. Although it is known that *S. invicta* aerosols venom on its brood (Obin and Vander Meer, 1985), the amount of alkaloids present on individuals is too small to measure (<1 ng) by GC; consequently, alkaloid peaks are absent or not detectable from the *Orasema* sp. soaks. Volatile components were not found when the starting GC oven temperature was 50°C (temperature program 1) and no additional peaks were found on maintaining the maximum oven temperature for a prolonged period of time (285°C for 22 min).

Gas chromatographic analysis of host colony *S. invicta* pupae and parasite wasp pupae were qualitatively identical (Figure 2A and B) as demonstrated by comparison of retention times and comparison of the mass spectrum of each peak. As in the case of the parasite adults, no additional highly volatile or nonvolatile peaks were found when different chromatographic conditions were used.

Several replicates of individual *Orasema* sp. pupae and adults from one colony were analyzed by GC. The percentage of each of the five major GC peaks were compared for host pupae and adult workers, and parasite pupae and adults of both sexes. The results are given in Table 1. Host and wasp pupae were indistinguishable for all five peaks. Host pupae and adult workers were significantly different in four of five hydrocarbon peaks. The male and female adult wasps were identical in all but one hydrocarbon peak; however, they were both significantly different from adult ants and wasp and ant pupae in three of five hydrocarbon peaks (not all the same peaks).

Adult *Orasema* sp., collected flying above *S. invicta* colonies, were analyzed by GC. A representative chromatogram is shown in Figure 3. Peaks characteristic of *S. invicta* hydrocarbons are evident (defined by retention times A on the chromatogram). However, in contrast to the chromatograms of the parasite taken directly from the host nest (Figure 1B and C; Figure 2A and B), the *Orasema* sp. adults captured outside host nests had complex GC peak patterns of both lower and higher retention times.

The results of the quantitative analysis of male and female adults from the host nest and males captured outside of the nest are shown in Table 2. The total amounts of GC detectable compounds from female and male *Orasema* sp. adults collected in the host nest are not statistically different; however, *Orasema* sp. males collected outside host nests had significantly more material than their within-nest counterpart (male or female). When only the hydrocarbon peaks characteristic of the host, *S. invicta*, are considered, males and females col-

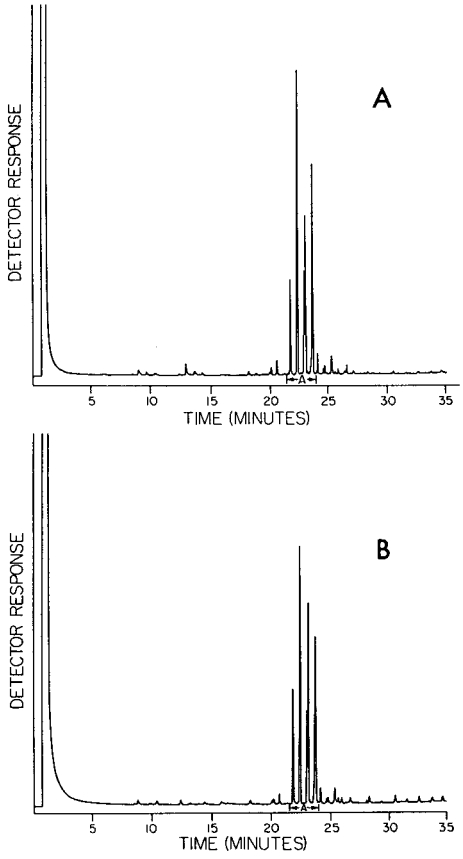

FIG. 2. Total gas chromatograph volatiles from hexane soaks of (A) host ant pupae and (B) *Orasema* sp. pupa from host colony. Chromatograph section A defines the species-specific hydrocarbons of the *S. invicta* host.

lected within the colony are indistinguishable; however, the *Orasema* sp. males collected outside the nest contain significantly less host hydrocarbon than the within-nest *Orasema* sp. The percentage of host-specific hydrocarbons to all GC components was 76.6% and 74.2% for the within-nest *Orasema* sp. females and males, respectively, and 14.5% for the *Orasema* sp. males collected outside the host colony.

The variance of the percentage of four new compounds (see Figure 3 and

TABLE 1. COMPARISON OF FIVE MAJOR GC COMPONENTS FOR PARASITE AND HOST PUPAE AND ADULTS[a]

Sample (N)	Hydrocarbon Peak (in order of elution)				
	1	2	3	4	5
Host pupae (3)	10.4 ± 0.2 (A)[b]	34.4 ± 0.2 (A)	12.3 ± 0.2 (A)	16.5 ± 0.2 (A)	26.4 ± 0.2 (AB)
Wasp pupae (10)	8.9 ± 0.8 (A)	34.7 ± 0.7 (A)	11.7 ± 0.3 (A)	17.4 ± 0.6 (A)	27.3 ± 0.4 (AB)
Host adult (3)	16.4 ± 0.8 (B)	31.1 ± 0.3 (B)	9.9 ± 0.1 (B)	19.0 ± 0.4 (A)	23.6 ± 0.9 (C)
Wasp female (7)	9.2 ± 0.3 (A)	29.9 ± 0.6 (B)	10.9 ± 0.2 (B)	22.4 ± 0.5 (B)	27.6 ± 0.3 (A)
Wasp male (5)	10.6 ± 0.3 (A)	29.3 ± 0.7 (B)	10.2 ± 0.3 (B)	23.8 ± 1.0 (B)	26.1 ± 0.5 (B)

[a] Mean and standard error of the percent of each of the five hydrocarbon peaks.
[b] Means with different letters are significantly different ($P < 0.05$), Newman-Kuels test. Comparisons are made only within each column.

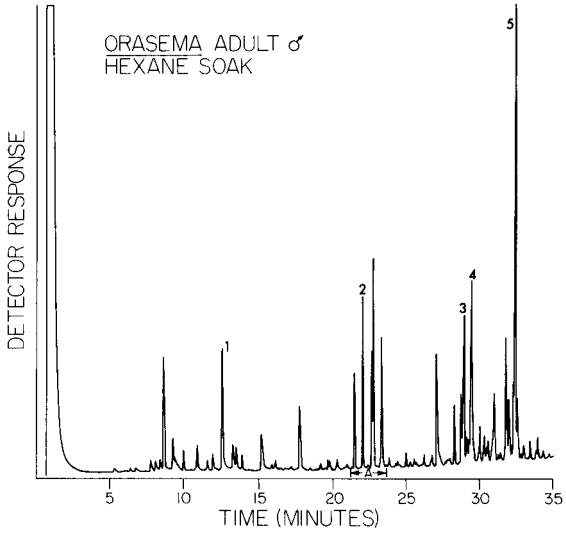

FIG. 3. Total gas chromatograph volatiles from a hexane soak of an *Orasema* sp. adult collected outside the host colony. Chromatogram section A defines the species-specific hydrocarbons of the *S. invicta* host. Peaks 1–5 were used to compare the variance of host- and nonhost-derived components.

Table 3) and one host-specific compound were compared. The relative proportions of the four new components were not significantly different from each other; however, in all cases the variance of the host-specific compound was greater than the four new components and in two cases significantly so.

TABLE 2. QUANTITATIVE COMPARISON OF GC COMPONENTS DERIVED FROM *Orasema* SP. ADULTS COLLECTED FROM WITHIN AND OUTSIDE HOST NEST

Orasema sp. type	All components (ng)[a]	Host-specific components (ng)[a]
Female from nest	602.3 ± 122.5 a[b]	461.4 ± 101.6 a[b]
Male from nest	450.3 ± 62.1 a	334.2 ± 42.8 a
Female outside nest	1340.9 ± 198.1 b	194.4 ± 122.1 b

[a] Mean ± SD ($N = 3$); see Figures 1 and 3 for GC profiles.
[b] Column results with a different letter are significantly different. Newman-Keul's test ($P < 0.05$).

TABLE 3. COMPARISON OF PERCENT COMPOSITION OF FOUR NONHOST COMPONENTS AND ONE HOST-SPECIFIC COMPONENT FROM *Orasema* SP. CAUGHT OUTSIDE HOST NEST

Component[a]	X (%) ± SD (N = 9)[b]	Variance
1	8.83 ± 2.33	4.82
2	8.77 ± 5.32	25.11
3	15.60 ± 1.90	3.18
4	24.46 ± 3.50	10.90
5	42.44 ± 3.06	8.31

[a] See Figure 3.
[b] Comparison of variance showed no significant difference between components 1, 3, 4, and 5; however, two (host-specific compound) was significantly different from components 1 and 3 ($P < 0.05$).

DISCUSSION

A great diversity of myrmecophiles have evolved a wide range of mechanisms for survival among their ant hosts (Wilson, 1971). The release of appeasement substances used to mitigate ant attacks and to gain entrance to a host colony is common (Hölldobler, 1971, 1972). Repellent and other defensive secretions are also employed (Brand et al., 1973; Gnanasunderam et al., 1981). One staphylinid beetle even mimics the alarm pheromone of its hosts, which aids in eluding worker ant attacks (Kistner and Blum, 1971). All of the above integration methods involve the release of myrmecophile-produced exocrine gland products. There are no reports of defensive or appeasement secretions for *Orasema* spp. or Eucharitidae in general. All information on the biology of the parasite states that they are not aggressively treated by the ants (Arye, 1962; Clausen, 1940; Wojcik, 1989). Past studies and observations of *Orasema* spp. parasites in the laboratory indicate that there are no defensive or appeasement secretions involved in their acceptance into fire ant colonies. We assume that initial entry into the colony by planidia is facilitated by their extremely small size (0.12–0.20 × 0.04–0.07 mm; Heraty and Darling, 1984).

Morphological mimicry is also used by certain myrmecophiles (Wilson, 1971); however, to the human eye *Orasema* sp. larvae, pupae, and adults are easily distinguished from their host. Current evidence indicates that integration involves the nestmate-recognition mechanism of their ant host.

Colony odor is composed of heritable and environmental odors. Nestmate-recognition cues are a subset of colony odor and may be any combination of the heritable and environmental factors (Vander Meer, 1988). Colony odors of all types are continually being transferred to and from the cuticle of an individ-

ual ant via simple movement within the colony and social interactions with other workers, queen, and brood. This has been demonstrated with *S. invicta* (Sorensen et al., 1985) and other ant species (Errard, 1986). *S. invicta* and probably all *Solenopsis* spp. utilize a combination of environmentally and heritably derived nestmate-recognition cues (Obin, 1986).

The surface of an insect's cuticle is coated with lipids (Blomquist and Dillwith, 1985), which are ideal for the absorption of both environmental and heritable odors. Thus, each ant is enveloped in chemicals that identify it at species and colony levels (Vander Meer, 1983). The cuticular hydrocarbons of *S. invicta* are species specific and have been used as chemotaxonomic markers in several studies (Vander Meer et al., 1985; Vander Meer, 1986). The hydrocarbons represent a heritable component of colony odor; however, there is no direct evidence that they play a role in *S. invicta* nestmate recognition (Obin, 1986). Most important, however, is the fact that these easily analyzed (by gas chromatography) cuticular components can be used as markers for the movement of odors in a colony (Vander Meer, 1988). Vander Meer (unpublished) used the technique to demonstrate the dynamic nature of nestmate-recognition cues. This is further exemplified by the ability of the myrmecophilous scarab, *Martinezia duterteri*, to integrate into the nests of several *Solenopsis* sp. (Vander Meer and Wojcik, 1982). The mode of initial entrance into a *Solenopsis* colony is unknown; however, the beetle uses its armored exoskeleton and thanatosis to survive worker attacks long enough to acquire the host's colony odor. The acquisition of colony odor was monitored by GC analysis of ant cuticular hydrocarbons transferred to the cuticle of the beetle.

Based on our knowledge of the life history of *Orasema* sp., and their cuticular chemistry, we can postulate two possible integration mechanisms, one of which is analogous to that of *M. duterteri*. In this case the tiny planidia are transported undetected to the host colony. By some mechanism (probably through worker transfer during trophallaxis) they find their way to a *Solenopsis* brood chamber where they proceed to parasitize fire ant brood and develop into adults. *Orasema* sp. brood are treated by host workers as ant brood and, through social interaction with host workers and contact with host brood, they acquire host colony odors, ergo nestmate-recognition cues. This scenario is supported by comparative GC analysis of *Orasema* sp. life stages with those of their host (Figures 1 and 2). The GC profiles of the parasite collected within host colonies are remarkable in that they contain only components found in the host. This is in contrast to *M. duterteri*, which, in addition to the host components, had its own characteristic profile (Vander Meer and Wojcik, 1982).

Perhaps the *Orasema* sp. does not produce cuticular hydrocarbons in sufficient quantity to be GC detectable and is masked by the copious amount of hydrocarbons transferred from the host (hydrocarbons comprise 75% of the total cuticular lipid for *S. invicta*; Lok et al., 1975). Cuticular lipids are known to

be important in preventing desiccation in insects (Blomquist and Dillwith, 1985). This function can be carried out by hydrocarbons, wax esters, or combinations of these or other lipid classes. The *Orasema* sp. may rely on nonhydrocarbon lipids for prevention of desiccation, or they may require the acquisition of host lipids.

An alternative possibility involves biosynthesis by *Orasema* sp. of the exact hydrocarbon profile of the host, as reported for the termitophile *Trichosenius frosti* (Howard et al., 1980). Mediating against this hypothesis is the fact that environmental odors play an important role in *Solenopsis* spp. nestmate recognition (Obin, 1986; Obin and Vander Meer, 1988). This detracts from the adaptive advantages of biosynthesis by *Orasema* sp. of genetically derived colony odor components. One would predict that in ant species where genetically derived nestmate-recognition cues dominate, i.e., *Pseudomyrmex ferruginea* (Mintzer and Vinson, 1985), the probability of finding myrmecophiles that biosynthesized host recognition cues would be much greater.

In addition, results of the colony study (Table 1) demonstrate that GC profiles of host workers and pupae are distinguishable within a given colony. Although the profiles of parasite pupae are identical to those of host pupae, the adult parasite corresponds to a profile between that of the host pupae and adult. This can be readily explained by a preponderant transfer of brood cuticular components to other brood or objects among the brood (i.e., *Orasema* sp. brood). After eclosure of *Orasema* sp. adults, their contacts are predominantly with host workers.

The thanatotic behavior on the part of adult *Orasema* sp. in the host nest fits well with the hypothesis that nestmate-recognition cues, along with the lack of bidirectional agonistic behavior, explain the preferential treatment of ant brood by workers and the successful interspecific adoption of brood (Morel and Vander Meer, 1988). Similarly, when ant callow workers are transferred to a conspecific colony, or that of a closely related species, they respond by taking on a nonaggressive posture (Morel and Vander Meer, 1988; Jaisson, 1985).

The GC profile of adult *Orasema* sp. captured outside host colonies still contains host-specific hydrocarbons; however, they now also have additional complex patterns at both higher and lower molecular weights (Figure 3). Quantitative analysis of adult *Orasema* sp. (Table 2) demonstrates that the total amount of detectable compounds increases for parasites collected outside the nest by more than a factor of two and that the host-specific compounds decrease significantly after the parasite leaves the host nest. The loss of host-specific compounds on parasite adults caught outside the nest supports our contention that they are acquired and not biosynthesized by the parasite while it is living in the host nest. As in the case of the within colony parasite, the additional compounds found in parasites captured outside the nest could be acquired or biosynthesized.

If the new compounds are biosynthesized, the relative ratios of the new components to each other should be consistent, whereas the ratio of new components to host-specific components (assuming they are acquired) should be more variable. Alternatively, after leaving the ant colony, the adult wasp may acquire the new components from its new environment. Since *Orasema* spp. are not host-plant specific (Lloyd R. Davis, Jr., USDA-ARS, Gainesville, Florida, personal communication), the sources and therefore the compounds acquired from the environment would be expected to be variable. Chemical analysis of 10 individual *Orasema* sp. collected with a sweep net outside a fire ant colony showed remarkable consistency, both qualitatively and in percent composition (Figure 3, Table 3). Given the two possibilities outlined above, this implies that the new compounds found on *Orasema* sp. outside the host nest are probably biosynthesized by the parasite. In addition, the greater variance in percentage of the host-specific compounds on *Orasema* sp. collected outside the nest supports our contention that these compounds are acquired from the host and diminish when the parasite leaves the nest.

The data support a complex scenario for parasite cuticular chemistry. The immature parasites appear to acquire the recognition-masking colony odors of the host. In contrast, the GC profiles of adults found outside the mound suggest that the biosynthesis of parasite-specific cuticular compounds commences with adult eclosion.

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