

# Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*)

## Behavioral and chemical evidence for the role of age and social experience

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**Summary.** A combination of behavioral and chemical analyses was used to investigate the nature of nestmate recognition cues and the effects of worker age and social experience on these cues in the ant *Camponotus floridanus*. Five categories of workers were tested: foragers, 5-day old and 0-day old callows, 5-day old and 0-day old naive callows. Bioassays consisted of introductions of dead workers from these categories into their own colonies or into an alien colony after the following treatments: 1) killed by freezing, 2) solvent-washed, 3) solvent-washed and coated with a nestmate soak, 4) solvent-washed and coated with a non-nestmate soak. Soaks were obtained from individual ants immersed in hexane and were applied individually to washed workers from the same category. Soaks were analyzed by gas chromatography (GC) and compared by multivariate analyses. Freeze-killed workers from each category elicited more aggressive behavior in the alien colony than in its own. By comparing GC profiles, a worker from any category can be assigned to its colony of origin. Thus all studied worker categories are colony-specific. Solvent-washed ants did not induce more aggressive behaviors in the alien colony than in their own, but they induced significantly less aggressivity in an alien colony than non-treated dead ants from the same category. Washed ants induced more aggressive behaviors when coated with a soak from a different colony as opposed to a soak from the colony in which they were introduced. The combination of behavioral and chemical results lead to the following conclusions: 1) Information contained in soak derived from workers was sufficient to allow nestmate recognition. 2) Nestmate recognition cues, and conse-

quently the recognition response displayed to their bearer, change with age. 3) Social experience is necessary to develop or acquire a colony-specific label. The role of age and social experience on nestmate recognition in social Hymenoptera is discussed.

## Introduction

An increasing number of papers have been published in the past few years about kin and nestmate recognition in social Hymenoptera (see review in Breed and Bennett 1988). In the special situation where a haplodiploid species is monogynous and monoandrous, the relatedness between workers within a colony is predicted to be 0.75. In this case nestmate and kin recognition are indistinguishable. In situations of polygyny and/or polyandry, kin and nestmate recognition can be distinguished, since kin recognition would occur within the colony between matriline and/or patriline, and nestmate recognition would occur between colonies. If the intra-colonial relatedness between workers is not known, as in *Camponotus floridanus*, and if discrimination is measured between colonies, then the term nestmate recognition must be used and no extrapolation to kin recognition is possible.

The recognition mechanism is composed of two distinct components (Beecher 1982): 1) the cues provided by a donor animal to be recognized, referred to as “labels” and 2) the process of the recipient animal decoding these cues to recognize, often referred to as “using a template”. The ontogeny of nestmate recognition, with special attention to the template component, has been studied in several social wasp species (see review in Gam-

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boa et al. 1986b). In ants the ontogenetical studies have not distinguished between the two recognition components. In *Camponotus vagus* (Morel 1983) and *C. floridanus* (Morel 1988), interactions with older nestmates during the first hours of adult life are necessary to be completely recognized as a nestmate and to have the ability to recognize nestmates. In *Camponotus* again, Carlin and Hölldobler (1986) showed that colony-specific recognition labels are learned by workers after their eclosion. As is known in many ant species, callow workers of *C. floridanus* can be successfully cross-fostered between colonies. The resulting young workers are recognized as nestmates by the sisters of their foster nurses. This suggests that colony-specific recognition cues were transmitted from the nurses to the adopted callows (Morel and Blum 1988).

Chemical signals are the only nestmate (or kin) recognition cues known in ants and bees (Hölldobler and Michener 1980) and certainly play a major role in social wasp recognition (Gamboa et al. 1986b). These chemical signals are present on the insect's cuticle as the result of genetically controlled production (e.g. in the ant *Pseudomyrmex ferruginea*, Mintzer 1982) and/or adsorption from the environment (e.g. in the ant *Solenopsis invicta*, Obin 1986). In small colonies of *Camponotus*, queen derived labels are dominant, but worker derived labels are clearly noticeable when the queen is removed or has a reduced fertility (Carlin and Hölldobler 1986, 1987a, b). Both environmentally derived and individually produced (by queens and/or workers) chemical cues may be exchanged among other colony members through social interactions. Chemical analyses of artificial groups of *Manica rubida* and *Formica selysi* (Errard and Jallon 1987) or of several North American *Camponotus* species (Vander Meer and Carlin unpublished) indicated that members of these groups had mixed chemical profiles characteristic of the species present. Therefore chemicals are shared and acquired among the members of these artificial groups. A study of the integration mechanism of a myrmecophilous beetle into fire ant (*Solenopsis* spp.) colonies also demonstrated a transfer of cuticular components from host to myrmecophile. This transfer was correlated with the beetle's acceptance into the ant colony (Vander Meer and Wojcik 1982).

We report the results of a behavioral and chemical analysis of the ontogeny of nestmate recognition cues in the ant *Camponotus floridanus*. Such a combined ontogenetical study has not been reported in social insects. The criterion used for nestmate recognition was the aggressive behavior dis-

played by workers toward conspecific alien intruders. The role of chemicals was tested using solvent-washed ant dummies coated with soaks from either nestmates or non-nestmates. The role of age was investigated by comparing the chemistry and aggressive responses displayed to three age groups: newborn, 5-day old callow workers, and foragers. Naive callow workers were compared to normal ones of the same age to investigate the role of social experience.

## Methods

### Study animals

Colonies of *Camponotus floridanus* were collected in the Appalachian National Forest and in Gainesville, Florida. In the laboratory, ants were housed in Petri dish cells (135 mm diameter) that had a layer of moistened dental plaster (Castone®). For each colony or group of workers, the cells were contained in a plastic arena (55 × 40 × 18 cm), the sides of which were coated with Fluon® to prevent escapes. Ants were fed regularly with honey and immature insects. Water was supplied in test tubes closed with cotton wool. The following worker categories were tested:

- 1) Foraging workers (F) collected at the honey source in the foraging area.
- 2) Normal callow workers less than 12-h old (referred to as 0-day old, C0). They were defined by their pigmentation, mobility, and presence of some pupal skin, and were taken directly from the source colony cells.
- 3) Normal 5 day-old callow workers (C5) were initially collected as described for C0 then maintained separately in Petri dish cells (50 mm diameter) with other C5 callows and always with an equal number of nurses (1:1).
- 4) Naive callow workers were artificially removed from their cocoon with fine forceps and tested between 3 and 12 h old (N0).
- 5) Naive callow workers artificially emerged from their cocoon were maintained separately with other naive callows of the same age, without nurses, in small Petri dish cells. They were tested at 5-days old (N5).

### Behavioral assays

Dead workers from each of these five categories were tested after one of the following treatments:

- 1) Freeze-killed ants: workers were frozen until dead (ca. 3 h) then warmed to room temperature.
- 2) Washed ants: Each live individual was soaked in 150 µl of hexane for 3 h then rinsed with additional hexane and allowed to air dry for 15 min. The ants were dead after this procedure. The soak was transferred to a clean vial, concentrated under nitrogen to about 20 µl, and used in the two following treatments.
- 3) Washed ants coated with a nestmate soak: A total nestmate soak was applied with a capillary pipet in three equal aliquots to the head, thorax and abdomen of a washed worker of the same category. Treated ants were allowed to air dry for 15 min before testing.
- 4) Washed ants coated with an alien soak: The above procedure was used to apply a soak from a non-nestmate to a washed worker.

Two queenless groups of ca. 600 workers, each coming from a different colony, were tested between the second and fourth week after they were collected from the field. Just after treatment (one of the four described above), individual ants were introduced into the foraging area of their own colony or into the foraging area of the alien colony. Active foraging and patrolling allowed the discovery of the introduced ant within the first minute, as well as a large number of potential encounters between the introduced and resident ants. For each of the five categories of worker, for each treatment, and for each kind of introduction (nestmate or alien), 10 replicates were performed (total of 800 introductions). The aggressive behavior displayed by resident workers in *Camponotus* spp. has already been described (Morel 1983; Carlin and Hölldobler 1986). The aggressive acts used in this study were opening of the mandibles, grasping, and flexing of the gaster to spray formic acid. Nibbling and light, short-duration seizing were not included in the analysis when freeze-killed ants were tested, since these behaviors were often associated with transportation rather than aggression. However, in bioassays with live ants (see below for chemical analysis), these behaviors could be unambiguously used.

For each assay, the number of residents performing aggressive acts to the introduced ant was recorded at the end of every minute for a total of 10 min. The score of each assay was the total number of ants performing the three aggressive acts mentioned above during these 10 recordings. The distributions of these scores were very variable, so their medians rather than means, were selected for graphic representation (Fig. 2). Comparisons between the scores corresponding to different treatments and worker categories were performed with Mann-Whitney *U* tests. The type of aggressive act was also recorded for each observation. In some cases, as mentioned in the results section, the total number of ants performing the most aggressive acts (i.e. grasping and gaster flexion), instead of all aggressive acts pooled together, was used.

### Chemical analysis

Chemical analyses were performed on four queen-right colonies A, B, C, and D. Colonies A and B were raised from foundress queens in the USDA-ARS Fire Ant Project Laboratory, Gainesville, Florida. Colony C and D were collected from the field. Foragers were analyzed from these four colonies (15 from A, 10 from B, and 5 each from C and D). The four categories of callow workers (C0, C5, N0, and N5) were analyzed in colonies A and B (10 replicates for each category and for each colony). In addition, 16 samples (10 from colony A, 6 from colony B, 7 being foragers, 3 C5, 2 C0, 3 N5, and 1 N0) were analyzed blind.

A series of bioassays were performed on colony A and B with live callows according to the same procedure described for dead ants, except the introduction procedure which was specific for live ants: Each ant was introduced into the foraging area under a glass tube and maintained like this for 10 min to allow the alarm behavior induced by manipulation to dissipate. At the end of the assay, the ant was removed and used for chemical analysis.

Individual ants were soaked in 150  $\mu$ l of hexane. After three hours, the ant was removed and the solution was transferred via a Pasteur pipet to a clean vial. Prior to analysis, soaks were concentrated to about 20  $\mu$ l under a stream of nitrogen. Gas chromatography (GC) was performed on a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 30 meter DB-1 fused silica capillary column (J & W Scientific Inc.; 0.322 mm ID, 0.25  $\mu$ m film thickness). The oven

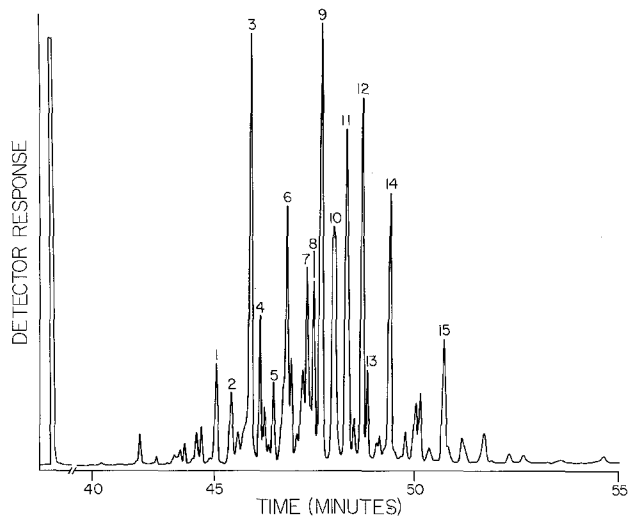


Fig. 1. A representative GC trace of a forager from colony A showing the 15 peaks on which multivariate analyses were based

temperature was programmed from 50° C (1 min hold) to 285° C at 5° C/min, then held at 285° C for 12 min. Chromatograms were analyzed with a Varian Vista 401 data processor.

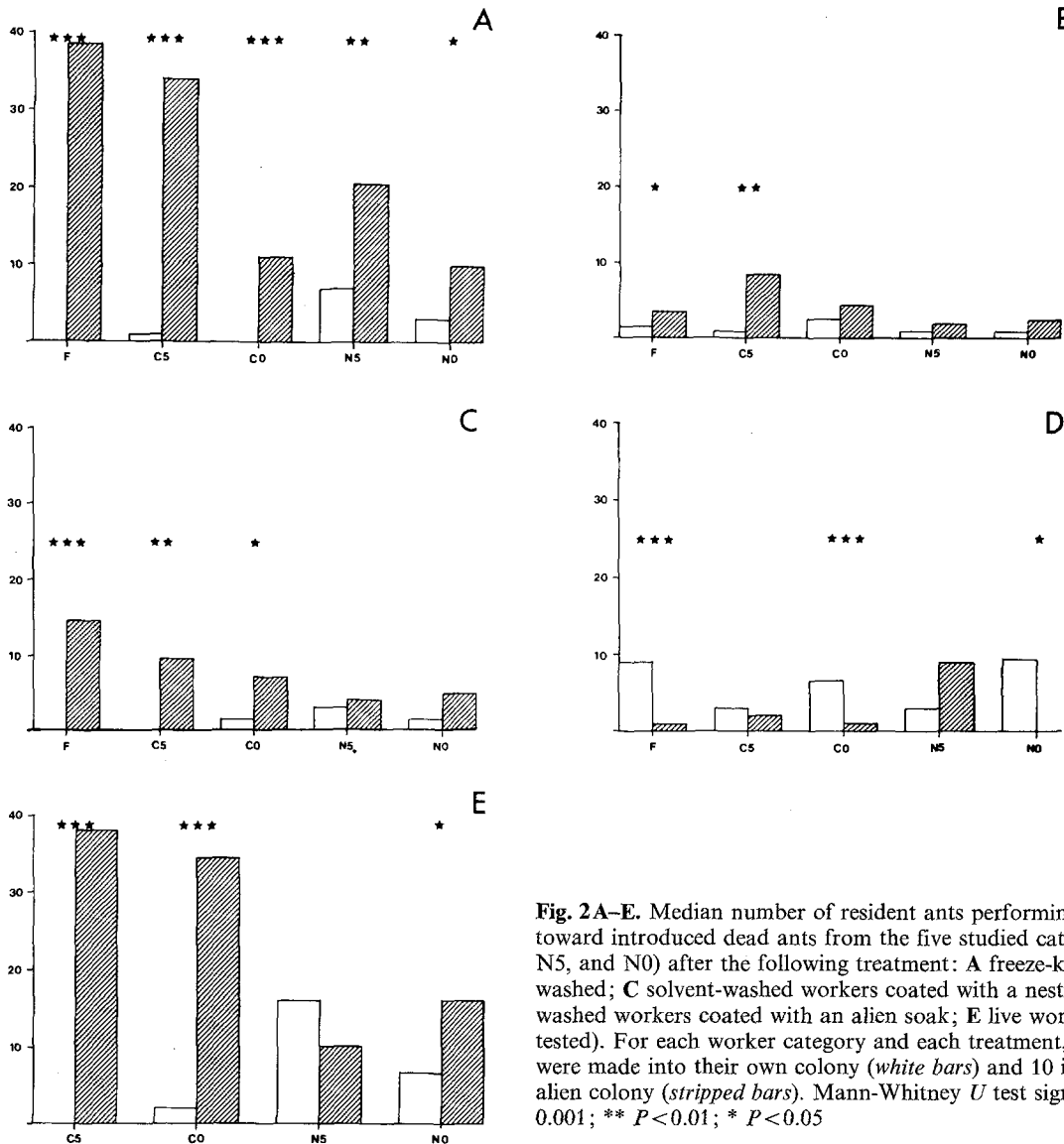
Pattern recognition methods were used to analyze the GC data. In this study we used principal component analysis (Jolliffe 1986) and the K-nearest neighbor classification method (Tou and Gonzalez 1974) to seek relationships between the GC profiles of the ants and the biological variables, i.e. age, social experience and colony of origin. Each chromatogram was represented by a data vector  $X = (x_1, x_2, x_3, \dots, x_j, \dots, x_n)$  where component  $x_j$  is the area of the  $j$ th peak. In our study each GC peak was normalized and expressed as percent of total area to indicate relative concentrations. Of the more than 40 peaks present in each chromatogram, only 15 were considered for pattern recognition analysis (Fig. 1). Each of these peaks has an area representing more than 1% of the total. Computer peak integration always yielded unambiguous results. The normalized chromatographic data were also auto-scaled so that each variable (peak) had a mean of zero and standard deviation of one.

## Results

### Behavioral assays

Freeze-killed ants from all test categories induced significantly more aggressive acts in the alien colony than in their own (Fig. 2A). In addition, there were significant differences in the behavior induced by the five worker categories, whether they were introduced into their own colony or into the alien colony.

When introduced into their own colony, freeze-killed foragers did not induce aggressive behavior. All four categories of callow workers elicited some aggressive acts, but naive callow workers caused significantly more aggressive acts than normal callows of the same age. The most intense aggressive



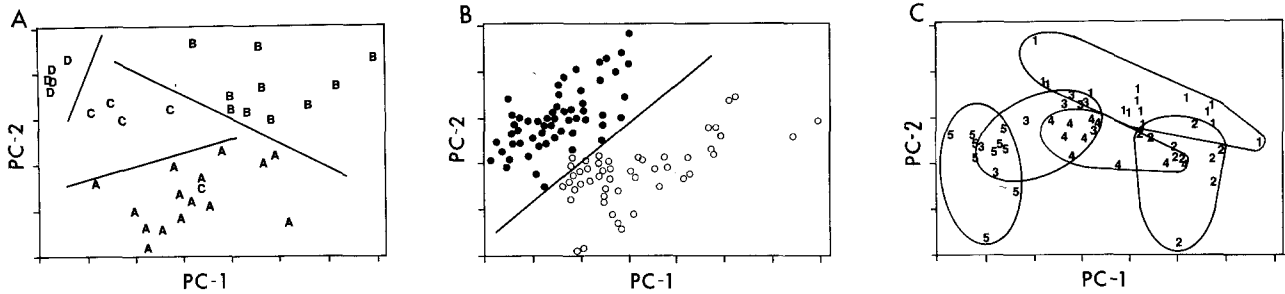
**Fig. 2A-E.** Median number of resident ants performing aggressive acts toward introduced dead ants from the five studied categories (F, C5, C0, N5, and N0) after the following treatment: **A** freeze-killed; **B** solvent-washed; **C** solvent-washed workers coated with a nestmate soak; **D** solvent-washed workers coated with an alien soak; **E** live workers (foragers not tested). For each worker category and each treatment, 10 introductions were made into their own colony (white bars) and 10 introductions into the alien colony (stipped bars). Mann-Whitney *U* test significance: \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$

act, gaster flexion with formic acid spray, was never displayed toward normal callows: however, it occurred in 2 of the 20 introductions of N0 callows and in 8 of the 20 introductions of N5 callows. No significant difference was observed between C0 and C5 callow introductions. N0 and N5 callow introductions differed only on the number of gaster flexions given above ( $X^2_1 = 4.08$ ,  $P < 0.05$ ). Thus only foragers were recognized immediately by their nestmates; however, the small number of aggressive acts displayed by nestmates toward C0 callows was not significantly different from zero.

When introduced into the alien colony, freeze-killed foragers induced the largest number of aggressive acts, which was not significantly different from the results obtained with C5 callows. But the number of aggressive acts induced by foragers was

significantly larger than the number induced by N5, N0 and C0 callows ( $P < 0.01$ ). There was no significant difference in response to N5 and C5 callows; neither was there a difference in response to N0 and N5 callows. However, normal callows (C0 and C5) induced significantly more aggressive acts at 5-days old than at 0-day old ( $P < 0.001$ ). The number of the most aggressive acts (grasping and gaster flexion) was significantly larger toward C0 than toward N0 ( $P < 0.01$ ).

There was no significant difference between introductions of washed ants into the source colony and into the alien colony for N5, N0, and C0 callows (Fig. 2B). Washed foragers and washed C5 callows still induced more aggressive acts in the alien colony than in their own, but significantly less than freeze-killed ants from the same categor-



**Fig. 3A–C.** Plot of the two first principal components of the 15 GC peaks obtained from individual workers. The lines between groups or the envelope of each group were drawn arbitrarily for clarity. **A** Foragers from colony *A* ( $N=15$ ), colony *B* ( $N=10$ ), colony *C* ( $N=5$ ), and colony *D* ( $N=5$ ). **B** Pooled workers from all studied categories (F, C5, C0, N5, and N0) from colony *A* (black circles) and colony *B* (open circles). **C** Foragers (1), C5 callows (2), C0 callows (3), N5 callows (4), and N0 callows (5) from colony *A*

ies ( $P < 0.001$  for F;  $P < 0.01$  for C5). In addition, more aggressive acts were displayed toward washed nestmate foragers than toward freeze-killed nestmate foragers ( $P < 0.05$ ). This difference was not significant for C5 callows. The wash treatment also eliminated the differences between test categories obtained with freeze-killed ants described above.

The number of aggressive acts was significantly larger toward ants coated with a soak than for washed ants of the same categories in the following cases: 1) Foragers coated with a nestmate soak introduced into the alien colony and 2) foragers and N0 callows coated with a soak from the alien colony, and introduced into their own colony. Similarly, a greater number of differences between test categories were observed for introduced ants coated with either a nestmate or an alien soak when compared to washed ants. However, some differences obtained between categories of freeze-killed ants were not observed in ants coated with a soak.

When coated with a soak from a nestmate, washed ants induced more aggressive acts in the alien colony than in their own. The difference was significant for the categories F, C5, and C0 (Fig. 2C). When washed ants were coated with soaks from the alien colony, they induced more aggressive acts in their own colony than in the alien colony (Fig. 2D). The difference was significant for F, C0 and N0 workers. However, the reverse occurred for N5 callows, although the difference was not significant.

The four categories of callow workers were tested alive in colonies *A* and *B* (Fig. 2E), after which the same workers were analyzed chemically. The total number of resident ants displaying aggressive acts toward C0 and C5 callows was significantly larger in the alien colony than in their own. For N0 callows, the difference was significant

when only grasping and gaster flexion were considered ( $P < 0.05$ ). N5 callows tended to elicit more aggressive behavior when introduced into their own colony, although the difference was not significant. When only grasping and gaster flexion were considered, normal callows were significantly less attacked in their colony than naive callows of the same age ( $P < 0.05$ ). Within naive workers, N5 callows induced more aggressive acts than N0 callows ( $P < 0.05$ ). In the alien colony, C0 callows were more attacked than N0 callows ( $P < 0.05$ ). The difference was not significant between N5 and C5 callows, even with a large difference in the medians (Fig. 2E), due to an asymmetrical distribution of the scores for the N5 category.

#### Chemical analysis

Using principal component mapping techniques developed from the 15 GC peaks (Fig. 1), the chemical profiles of the foragers obtained from four different colonies *A*, *B*, *C*, and *D* were compared. This same technique was used to compare the GC profiles of ants from colony *A* to those from colony *B*. For each colony, principal component mapping experiments of this nature were also performed for the purpose of determining whether or not a relationship exists between the chemical profile of a worker ant and its category (F, C5, C0, N5, or N0).

The chemical profiles were found to be characteristic of the colonies. In Fig. 3A the results of a principal component mapping experiment are shown for the foragers from the four different colonies. Each ant is represented as a point in the principal component map. It is evident that the four colonies are well separated in the two-dimensional principal component space. It is also important to note that this projection is made without

the use of information about the class assignment of the samples. The resulting separation is, therefore, a strong indication of real differences in the hydrocarbon profiles of the ants. The first two principal components account for 68.8% of the total cumulative variance.

In Fig. 3B the results of a principal component mapping experiment are shown for ants from all five categories in colonies A and B. Again, the colonies are well separated in the two-dimensional map. The first two principal components account for 60.8% of the total cumulative variance. The first nearest neighbor classification procedure was also used to analyze the data. In this phase of the study the data set was divided into four categories according to the colony of origin of the ant samples. A 98% correct classification success rate was achieved with the training set members. Sixteen ant samples comprising a prediction set were then added to the training set, and the colony of origin was determined for the unknowns, again using the first nearest neighbor classification rule. All of the members of the prediction set were correctly classified.

The chemical profiles were also found to be characteristic of the worker's category. In Fig. 3C the results of a principal component mapping experiment are shown for colony A. Pattern groupings according to worker category can be seen in the figure. The first two principal components account for 75.1% of the total cumulative variance. A mapping experiment of this nature was also carried out for colony B, and again pattern groupings with respect to worker type were observed. Principal component maps were also used to predict the worker category of the sixteen blind samples. To accomplish this task, the data set was again divided into two groups according to the colony of origin: A or B (none of the blind specimens were identified as colony C or colony D members). Blind samples identified as being members of colony A were added to the group that included the known colony A samples. A principal component analysis was then performed on the enlarged colony A training set, and the worker type of each unknown sample was determined on the basis of its position in the principal component space relative to the other known workers. This same experimental sequence was repeated for the blind samples that were identified as being members of colony B. Using this approach, 14 of the 16 (85.5%) blind specimens were correctly assigned to their respective worker category. The two samples that were misidentified with this technique were a forager and a C5 callow worker.

## Discussion

Nestmate recognition has been reported in a large number of eusocial Hymenoptera (see reviews in Gadagkar 1985; Breed and Bennett 1988). In ants, recognition cues produced by workers, the queen, and/or acquired from the environment have been reported in several different species (see review in Breed and Bennett 1988). The importance of the queen in *Camponotus* recognition systems has been stressed by Carlin and Hölldobler (1983, 1986, 1987a). Our behavioral results were obtained with queenless colonies. If queen labels last more than 4 weeks (maximum duration of the absence of the queen) it is possible that a queen effect was still present when the ants were tested in our assays. In any event, our results showed that the presence of the queen is not necessary for young callows to acquire the colony label.

The role of chemical cues in nestmate recognition in social Hymenoptera has been suggested for a long time by many behavioral studies (see Hölldobler and Michener 1980). From another direction, cuticular chemicals (hydrocarbons in particular) have been associated, but not directly correlated, with nestmate recognition (Vander Meer and Wojcik 1982). Recently, a direct correlation between worker chemical rinse and nestmate recognition has been demonstrated in the ant *Camponotus vagus* (Bonvavita-Cougourdan et al. 1987). Our results with *C. floridanus* show that chemicals derived from worker soaks are involved in nestmate recognition. However, ant rinses or soaks contain exocrine products, regurgatory products, and/or excretory products in addition to the expected cuticular chemicals (Vander Meer 1988). Also, although hydrocarbons are the major class of GC detectable compounds in *C. floridanus* soaks (Morel and Vander Meer unpublished), we cannot say at this time that they are responsible for nestmate recognition. A three hour hexane soak was sufficient to suppress or to significantly diminish a worker's ability to be recognized as a nestmate or a non-nestmate. It also suppressed or lowered the differences induced by age and social experience. Solutions obtained from individual worker soaks applied to solvent-washed ant dummies clearly demonstrated that chemicals from the worker soak carried information about its colony of origin. Soaks applied to washed ants gave variable results for callow workers. This may be due to the small amount of material obtained from callow soaks as compared to forager soaks (Morel and Vander Meer unpublished). However, some behavioral cues normally associated with callows

may be missing in dead test callows. In addition to these results, all of the worker categories tested (0- and 5-day old normal or naive callow workers, and foragers) could be assigned to their colony based on their GC profiles.

Even if the ontogeny of labels and templates occurred simultaneously, it is essential for an understanding of the recognition process to study these components independently (Gadagkar 1985; Gamboa et al. 1986b). Our results concerned only the ontogeny of the colony label. This label can be carried by an animal without changes during its life-time or alternatively, it can be modified during development. Honeybees (Breed 1983) and gynes of *Polistes fuscatus* wasps (Gamboa et al. 1986a) possess the colony label just after emergence due to pre-imaginal acquisition or to endogenous production. In both cases, no social experience, at least at the adult stage, was shown to be necessary for label production and/or acquisition. Quantitative and qualitative changes in cuticular hydrocarbon patterns have been shown in *Drosophila virilis* (Jackson and Bartelt 1986). In *C. vagus*, variations in the relative proportions of some hydrocarbons were correlated with the different aggressive responses induced by nurses and foragers (Bonavita-Cougourdan and Clement 1987).

The behavioral variations we obtained between 0-, 5-day old callows and foragers in *C. floridanus* are reflected by different relative proportions of the GC peaks. Therefore, *C. floridanus* colony labels are modified with age, at least through the early adult stage. Newborn *C. floridanus* workers have a very different profile compared to older workers of the same colony. Normal newborn workers were significantly less attacked in an alien colony than older callows or foragers. This well known phenomenon (see Jaisson 1971) allows the adoption of newborn workers into alien colonies, or even colonies from closely related species. However, the small amount of aggressive behavior that was induced by newborn callows in alien colonies implies that these workers do carry a colony-specific label. These cues could be individually produced as an expression of their genotype or acquired earlier, at larval, pupal or early adult stage. In *Cataglyphis cursor*, although newborn workers are readily adopted into alien colonies, they share fewer social interactions with other colony members than resident workers of the same age (Lenoir et al. 1982). Our chemical analyses showed that profiles from all test categories, including newborn ants, were colony-specific. However, the small amount of aggressive behavior directed at some newborn callows by their nestmates indicates that their col-

ony label may not be complete. Five-day old normal *C. floridanus* workers induced as much aggressive behavior as foragers when they were introduced into an alien colony. But when this category was introduced into their own colony, more aggressive behavior was directed to them than to foragers. The 5-day old callow label may be significantly different from that of nestmate foragers, yet they could both be sufficiently different from any alien colony label such that the sensitivity of the bioassay can not distinguish between the responses induced by the two worker categories in an alien colony. However, when introduced back into their colony, the difference between zero aggression (for foragers) and some aggression (for callows) can be detected. The nestmate acceptance mechanism appears to require more information than the rejection mechanism. These results favor a recognition mechanism similar to the "genotype matching" model proposed by Getz (1982) in which acceptance requires complete label matching between intruders and residents. Our results argue in favor of diminished aggressive behavior as the recognition label of the introduced ant becomes closer to that of encountered residents, and therefore against a strictly defined genotype matching mechanism. Five-day old workers induced more aggressive acts in their own colony than foragers, although 0-day old workers did not. This can be explained by at least two factors. 1) Five-day old workers were isolated in sub-colonies; therefore their environment and social interactions were different from that of foragers. 2) Newborn callows may have special pheromones (see Jaisson 1972a, b; Hölldobler and Michener 1980) which do not exist after five days. In fact, the duration of the "callow" stage is not well-defined in social insects. It could be an accelerated phase of a continuously changing process, as appears to be the case for trophallactic behavior in *C. vagus* (Morel 1986) or even for chemical profiles in *Solenopsis invicta* (Vander Meer et al. submitted).

In ants, most of the previous studies did not demonstrate whether or not social experience is necessary for label acquisition. However, results on *Camponotus* (Morel 1983, 1988; Morel and Blum 1988), and in heterospecific colonies (Errard 1984, 1986; Le Moli and Mori 1984, 1985) strongly suggest that social experience plays a role in this phenomenon. Our results on *C. floridanus* demonstrate clearly that social experience with older nestmates is necessary for callow workers to develop the colony label needed for complete integration into the colony. Naive and normal callows of the same age showed different chemical profiles

and their introduction induced different behavioral responses, either in their own colony or an alien colony. In their own colony, both 0- and 5-day old naive callows were more attacked than normal callows. In the alien colony, 0-day naive callows were less attacked than 0-day normal callows. Thus the different label carried by naive newborn workers allows them to be accepted into their own colony and to induce less aggressive behavior in an alien colony than normal workers of the same age. No difference in aggression was noted in 5-day old naive and normal callows when introduced into alien colonies, probably because of the development of a more complete colony label, which may combine food and nestmate odors. More aggressive acts were displayed toward 0-day naive workers by alien workers than by nestmates in all treatments (washed ants not included). However, 5-day old naive workers were more attacked by alien workers only for the freeze-killed worker treatment. In addition to the comparison of the chemical profiles, these results indicate that a naive newborn worker individually produces a part of the colony label, or acquired it at the larval or pupal stage (it is not possible at this time to choose between the two hypotheses). But it is not sufficient for complete integration. After five days isolated together from the colony, naive workers could develop a group odor that begins to diverge from their parent colony odor. This could account for aggression directed at them in both alien and their own colonies. Induction of aggressivity between nestmates after a long separation is known in ants (Wallis 1962; Provost 1985; Errard 1986).

A combined behavioral and chemical study of the ontogeny of nestmate recognition demonstrated that the recognition cues, and consequently the recognition response displayed to their bearer, change with age in the ant *Camponotus floridanus*. It also showed that social experience with older nestmates is necessary to develop and/or acquire a complete colony-specific label.

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