Journal of Chemical Ecology, Vol. 15, No. 7, 1989

TEMPORAL CHANGES IN COLONY CUTICULAR HYDROCARBON PATTERNS OF *Solenopsis invicta* Implications for Nestmate Recognition

ROBERT K. VANDER MEER,[1] DAVID SALIWANCHIK,[2] and BARRY LAVINE[3]

[1]*Insects Affecting Man and Animal Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 14565, Gainesville, Florida 32604*

[2]*Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32611*

[3]*Department of Chemistry Clarkson University, Potsdam, New York 13676*

(Received June 17, 1988; accepted November 7, 1988)

Abstract—Heritable cuticular hydrocarbon patterns of *Solenopsis invicta* workers are consistent within colonies for a given sampling time but vary sufficiently from colony to colony to distinguish the colonies from each other. In addition, cuticular hydrocarbon patterns change within colonies over time. Nestmate recognition cues found on the individual's cuticle, can be from heritable or environmental sources, and are a subset of colony odor. The cuticular hydrocarbons can be used as a model for heritable nestmate recognition cues. We propose that because potential nestmate recognition cues, both environmental and genetic, are dynamic in nature rather than static, during its lifetime a worker must continually update its perception (template) of colony odor and nestmate recognition cues.

Key Words—*Solenopsis invicta*, Hymenoptera, Formicidae, cuticular hydrocarbons, nestmate recognition, colony odor, temporal changes.

INTRODUCTION

Cuticular hydrocarbons serve several purposes in insects, such as prevention of desiccation and regulation of cuticular permeability (Hadley, 1980). In addition, this class of chemically inert compounds can have many semiochemical

functions, such as alarm, recruitment, defense, sex attractants, and host attractants (see Howard and Blomquist, 1982, for review).

Solenopsis invicta Buren cuticular hydrocarbons constitute the largest lipid class (over 70%) on the cuticle (Lok et al., 1975) and are species specific (Nelson et al., 1980; Vander Meer, unpublished). *S. invicta* and *S. richteri* have the same cuticular hydrocarbons, identified as normal, monomethyl, and dimethyl branched alkanes (Lok et al., 1975; Nelson et al., 1980). However, quantitative patterns for the two species are distinctly different (Nelson et al., 1980; Vander Meer et al., 1985). Their species specificity has been used as a reliable chemotaxonomic tool to attack several problems (Vander Meer, 1986). The heritable nature of these compounds has been clearly demonstrated in studies of hybridization between *S. invicta* and *S. richteri* (Vander Meer et al., 1985; Ross et al., 1987).

It is generally accepted that nestmate recognition in social insects involves the detection of specific odiferous cues on the cuticle (Wilson, 1971). Heritable (Mintzer and Vinson, 1985) and/or environmental (Jutsum et al., 1979) odors can be operational in ant nestmate recognition. Odors under genetic control or resulting from genetic–environment interactions are termed heritable odors. It has been demonstrated that *S. invicta* uses both heritable and environmental nestmate recognition cues (Obin, 1986; Obin and Vander Meer, 1989). Cuticular hydrocarbons have been implicated in species and caste recognition in termites (Howard et al., 1982a), and several species of termitophiles have been shown to have cuticular hydrocarbons identical to their host, which was associated with their integration into the host colony (Howard et al., 1982b). Hydrocarbons have also been implicated in nestmate recognition of the ant *Camponotus vagus* (Bonavita-Cougourdan et al., 1987).

Although there is no direct evidence for the use of cuticular hydrocarbons in nestmate recognition in *S. invicta,* we have used the cuticular hydrocarbons as a model to study the quantitative variation of heritable components of colony odor. We report here that individual colonies have distinguishable cuticular hydrocarbon patterns; however, these patterns change with time. The implications of the dynamic nature of these heritable characters in relationship to nestmate recognition mechanisms are discussed.

METHODS AND MATERIALS

Source of Colonies. Laboratory colonies of *S. invicta* were reared from newly mated queens collected near Gainesville, Florida, or Gulfport, Mississippi, and reared in the Gainesville laboratory by the procedures described by Banks et al. (1981). Workers from monogynous field colonies were sampled

from the Gainesville, Florida, area. *S. invicta* workers undergo age-related polyethism and have been categorized as nurses, reserves, and foragers (Miranda and Vinson, 1981). Only reserve workers were used in this study. Reserve workers were defined in laboratory colonies as those workers that were inactive and clustered together at the outside of the colony cell (Sorensen et al., 1981). For field colonies, reserve workers were obtained by placing a beaker (50 ml), coated on the inside lip with Fluon, partly into the surface of a mound. If any workers were observed carrying brood, the sample was discarded. In the laboratory, the collected ants were anesthetized with CO_2, and three replicate samples of 50 or 100 reserve workers were placed in a vial and weighed on a Mettler H51Ar analytical balance. Worker ants within a range of 1.5–4.0 mm in length, readily determined by visual inspection, were used for the analysis of cuticular hydrocarbons.

Analysis of Cuticular Hydrocarbons. Cuticular hydrocarbons were obtained by soaking the ant samples for 7 min in hexane (ca. 2 ml HPLC grade, Baker Chemical Co., Phillipsburg, New Jersey). The samples were gently shaken for the first and last 15 sec of the soak period. Immediately after the rinses were complete, 9 μl of a 0.1% hexane solution of *n*-pentacosane was added for quantitation as an internal standard. Samples were evaporated to a small volume under a stream of nitrogen and the residue applied to a Pasteur pipet silicic acid HA column (325 mesh particle size, Bio Rad, Richmond, California). Hydrocarbons were isolated by eluting the column with hexane (Christie, 1973). The hexane eluate was evaporated to ca. 50 μl and an aliquot was analyzed on a Varian 3700 gas chromatograph (Varian Associates, Sunnyvale, California) equipped with a flame-ionization detector. A 1.8-m × 2-mm-ID glass column packed with OV-17 on 120–140 mesh Gas Chrom Q (Applied Science Laboratories, Deerfield, Illinois) was used to separate the hydrocarbons. The oven was programmed from 175°C to 235°C at 3°C/min. Qualitative and quantitative data were obtained for the five major hydrocarbon peaks (Figure 1) using a Varian Vista 401 data processor (Varian Associates).

Quantitative Influence of Soak Time. Three replicates of 100 workers each from the same colony were weighed and allowed to soak in hexane, containing *n*-pentacosane as an internal standard, for the following time periods: 3, 5, 7, 10, 15 min, and overnight. Aliquots of the hexane were removed at the indicated time periods, and hydrocarbons were isolated and analyzed as described above.

Analysis of Data. The individual peak areas were quantitated using the internal standard and digitalized as the number of nanograms per sample. The data were normalized using the weight of the corresponding ant sample. We used principal component analysis, a pattern recognition technique (Wold, 1976), to establish relationships between the GC profiles of the ants and several

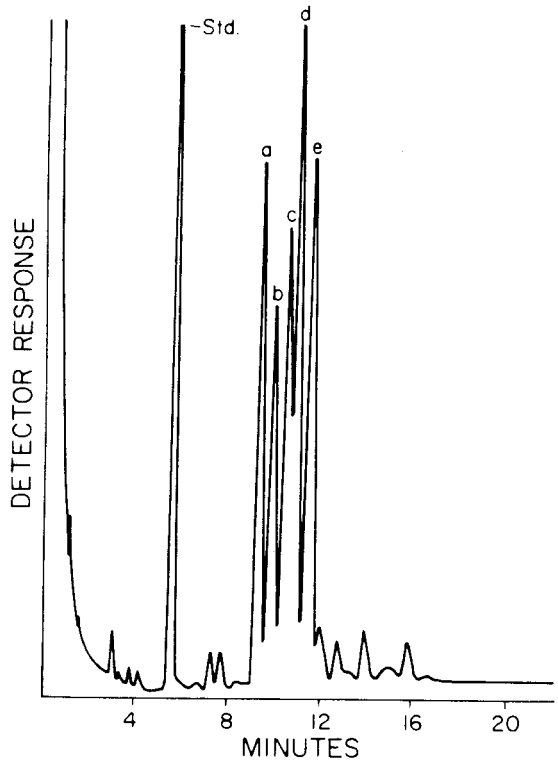

FIG. 1. Gas chromatogram of cuticular hydrocarbons from *Solenopsis invicta* workers. Peak a: n-heptacosane, b: 13-methylheptacosane, c: 13,15-dimethylheptacosane, d: 3-methylheptacosane, and e: 3,9-dimethylheptacosane.

variables, such as colony source and time. The principal component projections were made without the use of information about the class assignment of the samples.

Hydrocarbon Time Study. Four laboratory colonies were selected for a study to determine whether hydrocarbon patterns varied with time. These colonies were sampled, as described above, three to six times during a four- to eight-month period. The hydrocarbons were isolated, separated by gas chromatography, and the data analyzed as described in the previous sections.

RESULTS

The quantitative results for the removal of cuticular hydrocarbons over several soak times is shown in Table 1. The percent composition of the five hydrocarbon peaks under consideration varied most for time periods of 3 min and overnight. Consistent results were obtained for time periods 5, 7, 10, and

TABLE 1. EFFECT OF SOAK TIME ON EXTRACTIONS OF HYDROCARBONS FROM *S. invicta* WORKER ANTS

	Percent hydrocarbon peak[a]					Hydrocarbon (μg)	
Soak time	A	B	C	D	E	Per mg ant	Per 100 ants
3 min	12.4 ± 0[b]	27.6 ± 0.2	18.6 ± 0.0	15.4 ± 0.0	25.9 ± 0.2	0.62 ± 0.02	31.8 ± 1.0
5 min	11.6 ± 0.6	27.8 ± 0.2	19.2 ± 0.1	15.2 ± 0.0	26.1 ± 0.3	0.76 ± 0.11	38.7 ± 6.7
7 min	10.4 ± 0.2	27.3 ± 0.2	19.1 ± 0.3	15.6 ± 0.1	27.7 ± 0.6	0.76 ± 0.04	37.3 ± 2.5
10 min	10.3 ± 0.3	27.8 ± 0.2	19.8 ± 0.2	15.2 ± 0.1	27.0 ± 0.2	0.80 ± 0.01	39.8 ± 2.5
15 min	10.0 ± 0.0	27.1 ± 0.3	19.8 ± 0.0	15.4 ± 0.2	27.8 ± 0.0	0.88 ± 0.04	46.4 ± 4.2
Overnight	8.7 ± 0.4	26.0 ± 1.0	22.4 ± 2.4	15.6 ± 0.4	27.2 ± 0.6	2.91 ± 0.27	138.8 ± 14.2

[a] See Figure 1.
[b] Mean ± standard deviation (N = 3).

15 min. However, the quantity extracted, whether expressed as micrograms per milligram of ant or micrograms per 100 ants, was most consistent for the 5-, 7-, and 10-min soak periods. Consequently, all subsequent samples were soaked for 7 min.

The inter- and intracolony variations in cuticular hydrocarbon patterns were monitored from samples collected within two weeks of each other. A principal component analysis of a set of 27 ant samples from nine different laboratory and field colonies (three replicates each taken at the same time) was performed using the five major hydrocarbon GC peaks as descriptors (Figure 1). A plot of the first two principal components is shown in Figure 2. The first two eigenvectors account for 85.9% of the total cumulative variance.

Pattern groupings according to each individual colony are evident. As determined by inspection (Figure 2), within-colony variation is less than the between-colony variation. There were no obvious principal component plot separations based on a colony's geographic origin (Mississippi and Florida), nor was there a separation of field versus laboratory colonies.

Cuticular hydrocarbon patterns of laboratory colonies sampled at several

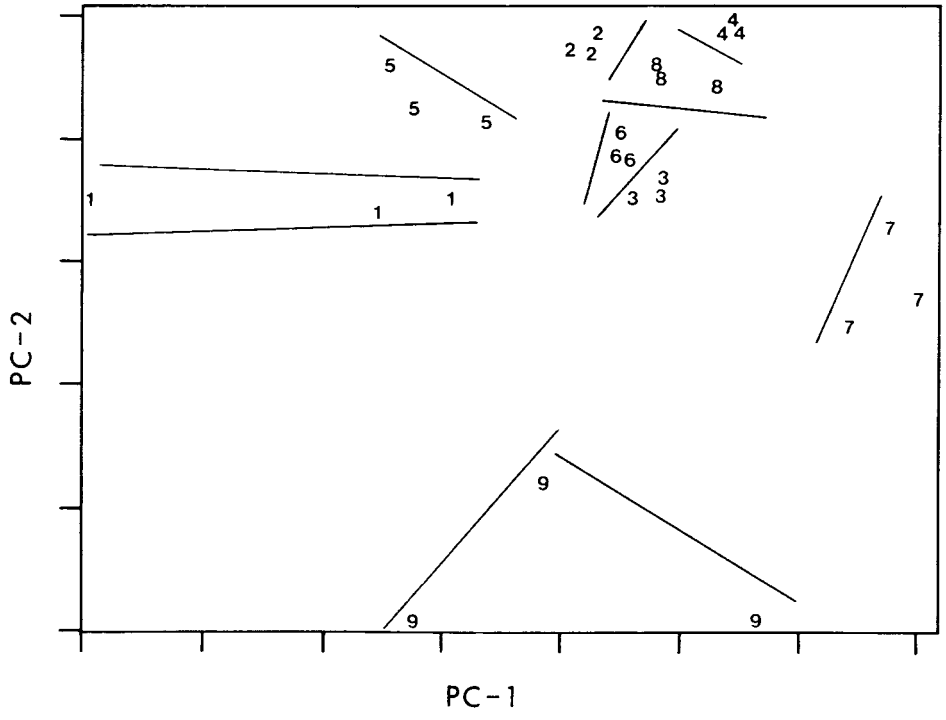

FIG. 2. A plot of the first two principle components of the five major hydrocarbons for nine *S. invicta* colonies. Colonies 1–4 are field collected from Florida; colonies 5–7 are lab-reared in Gainesville, Florida, but originated from newly mated queens from Gulfport, Mississippi; colonies 8 and 9 are lab-reared in Gainesville, Florida, collected in Florida from newly mated queens.

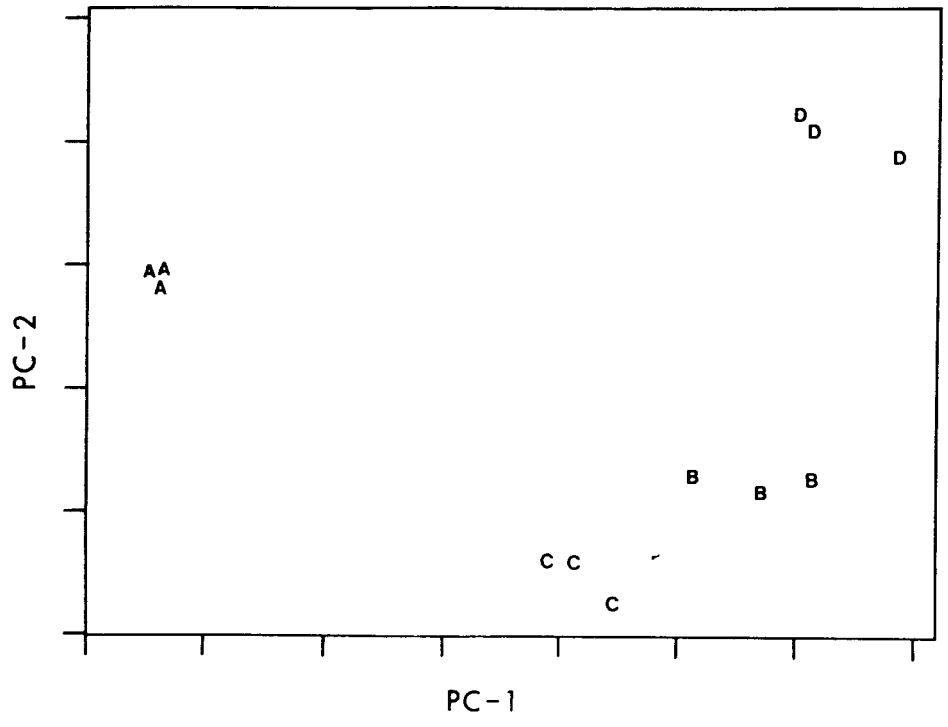

FIG. 3. A plot of the first two principle components of the five major cuticular hydrocarbons collected from field colony 3 (Figure 2) over four time periods. The letters A, B, C, and D represent collections in November 1982, January 1983, February 1983, and April 1983, respectively.

time periods were not static. Principal component maps of the first two principal components developed from the five GC peaks showed pattern groupings according to the date of collection in all four cases. The variation in replicates within a time period was less than that between time periods. An example of one colony sampled at four different times is shown in Figure 3. The first two eigenvectors account for 90% of the total cumulative variance. Use of an internal standard and careful attention to the proper peak integration baselines minimized instrument variation. When a single principal component plot was made of several colonies at several times, the pattern fluctuation of a colony with time caused overlap with other colony patterns.

DISCUSSION

Hydrocarbon Variability. It is not possible to obtain a rinse or soak of whole ants that does not contain compounds from a variety of sources other than the cuticle (Vander Meer, 1989). For example, species-specific fire ant venom alkaloids and hydrocarbons are routinely obtained by simply soaking

worker ants in hexane (Vander Meer et al., 1985; Ross et al., 1987). In this study, hydrocarbons were isolated by column chromatography to avoid contamination from other classes of chemicals. Quantitative analysis of the hydrocarbons from worker ants soaked for various times showed a dramatic increase in material when samples were soaked overnight. This probably reflects leaching of the large amount of hydrocarbon found in the postpharyngeal gland (Vander Meer et al., 1982). This material can exit the gland via the pharynx and mouth parts. A 7-min soak time was used for all subsequent analyses because of the consistent results from 5–10 min and to provide the best approximation of a cuticular hydrocarbon profile.

The separation of nine *S. invicta* colonies from diverse locations at a single collection time (Figure 2) is indicative of the quantitative intercolony variability possible in the cuticular hydrocarbons. These data also suggest that cuticular hydrocarbon variation is random throughout the distribution of *S. invicta* in the United States, since there was no obvious separation of colonies based on whether they were derived from Florida or Mississippi. Nestmate recognition studies (Obin, 1986) support this data.

The lack of separation of field-collected colonies from laboratory-maintained colonies in the principle component plots suggests that diet and other environmental factors do not have a noticeable influence on the phenotypic expression of cuticular hydrocarbon patterns (no gene–environment interaction). Similar results for hydrocarbon analysis of laboratory and field colonies were obtained by Obin (1986). The genetic origin of these compounds has been confirmed by the concordance of hydrocarbon profile data and genetic data (isozymes) in differentiating hybrid fire ants from their *S. invicta–S. richteri* parents (Ross et al., 1987).

It should be noted that the principal component projections (Figures 2 and 3) were made without the use of information about the class assignment of the samples. Therefore, distinct separations are a strong indication of real differences in the GC profiles of the ants.

The analysis of colony hydrocarbons over time demonstrated that individual colony profiles (reserve worker temporal subcaste) are dynamically changing, although for any given time period the profiles for the three replicates of a given colony are consistent (Figure 3). This is in part because the social interactions of the colony workers ensure the efficient transfer of cuticular chemicals (environmental and genetic) throughout the worker force of a colony. The transfer of compounds on the cuticle of several ant species has been clearly demonstrated by: (1) the mixing of species-specific cuticular chemical components on individuals from laboratory colonies composed of two different species (Errard and Jallon, 1987; Vander Meer, unpublished), (2) the rapid distribution of radiolabeled markers in fire ant colonies (Sorensen et al., 1985), and (3) the transfer of species-specific fire ant hydrocarbons to a myrmecophilous beetle

(Vander Meer and Wojcik, 1982). These interactions decrease the variability of all chemicals found on the worker surface and confound studies aimed at the investigation of individual variation. Although in this study we cannot address the mechanism of profile change, we can definitively state that heritable cuticular hydrocarbon profiles undergo continuous quantitative changes during the life of a colony. The magnitude of the temporal changes creates overlap with the clusters of other colony patterns.

Nestmate Recognition. Nestmate recognition occurs in ants when a worker distinguishes between workers of another colony and its own colony following a pause and sweep of the antennae over the others' body (see Breed and Bennett, 1987, for a review). The odor on the surface of a worker is a composite of environmentally and genetically derived components. In general, the odors used by a particular species or colony for nestmate recognition are a subset of all possible odors and may be composed of any combination of components (Vander Meer, 1988). *S. invicta* uses both environmental and heritable nestmate recognition cues (Obin, 1986; Obin and Vander Meer, 1988). Studies with *S. invicta* have investigated the effects of environmental components on nestmate recognition (Obin and Vander Meer 1988, 1989). However, although cuticular hydrocarbons are strongly correlated with nestmate recognition in *Camponotus vagus* (Bonavita-Cougourdan et al., 1987) and associated with the integration and survival of a myrmecophilous beetle in fire ant nests (Vander Meer and Wojcik, 1982), the composition of the heritable part of the cues has not been determined. In any event, cuticular hydrocarbons are a part of the heritable component of colony odor and can be used as a general model for heritable nestmate recognition cues. This paper establishes the dynamic nature of these compounds.

Nestmate recognition cues are only half of the nestmate recognition process. It is generally considered that colony-specific chemical patterns or templates (Breed and Bennet, 1987) are learned by workers soon after eclosion (Morel, 1983; Errard, 1984; Morel et al., 1988). Although newly eclosed workers may not be completely olfactorally naive in terms of nestmate recognition (Isingrini et al., 1985), they learn to recognize the first odors they experience, whether from their own colony or, in the case of laboratory adoption experiments, from another colony or species (Carlin and Hölldobler, 1983; Errard, 1986; Morel and Blum, 1988).

Our results indicate that early template formation must be modified by continuous updating. The dynamic nature of the environmental part of colony odor is obvious. The variation in cuticular hydrocarbons demonstrates that the heritable component of colony odor is not fixed either. Because of the dynamic nature of colony odor and, by implication, nestmate recognition cues, we propose that workers are not hard-wired to a specific chemical or pattern of chemicals but have a great deal of flexibility through a process of continuous, iterative

learning that provides a mechanism for colony members to cope with a constantly changing chemical environment, as well as within-colony individual genetic variations (hydrocarbon patterns vary temporally as widely as between colony patterns). This concept was alluded to by Wallis (1963), who likened the recognition process to odor habituation and suggested that an ant "is continuously habituating to the slight variations in the odour of its nest-mates." Our work supports the necessity for such a process in nestmate recognition.

REFERENCES

BANKS, W.A., LOFGREN, C.S., JOUVENAZ, D.P., STRINGER, C.E., BISHOP, P.M., WILLIAMS, D.F., WOJCIK, D.P., and GLANCEY, B.M. 1981. Techniques for Collecting, Rearing and Handling Imported Fire Ants. USDA, SEA-AATS-S-21:1-9.

BONAVITA-COUGOURDAN A., CLEMENT, J.L., and LANGE, C. 1987. Nestmate recognition: Cuticular hydrocarbons and colony odor in the ant *Camponotus vagus* Scop. *J. Entomol. Sci.* 22:1-10.

BREED, M.D., and BENNETT, B. 1987. Kin recognition in highly eusocial insects, pp. 243-286, *in* D.J.C. Fletcher and C.D. Michener (eds.). Kin Recognition in Animals. John Wiley & Sons, New York.

CARLIN, N.F., and HÖLLDOBLER, B. 1983. Nestmate and kin recognition in interpecific mixed colonies of ants. *Science* 222:1027-1029.

CHRISTIE, W.W. 1973. Lipid Analysis. Pergamon Press, Oxford. 338 pp.

ERRARD, C. 1984. Evolution en fonction de l'age des relations sociales dans les colonies mixtes heterospecifiques chez des fourmis des genres *Camponotus* et *Pseudomyrmex*. *Insectes Soc.* 31:185-194.

ERRARD, C. 1986. Role of early experience in mixed-colony odor recognition in the ants *Manica rubida* and *Formica selysi*. *Ethology* 72:243-249.

ERRARD, C., and JALLON, J.M. 1987. An investigation of the development of the chemical factors in ants intra-society recognition, p. 478, *in* J. Eder and H. Rembold (eds.). Chemistry and Biology of Social Insects. Verlag J. Peperny, Munchen.

HADLEY, N.F. 1980. Surface waxes and integumentary permeability. *Am. Sci.* 68:546-553.

HOWARD, R.W., and BLOMQUIST, G.J. 1982. Chemical ecology and biochemistry of insect hydrocarbons. *Annu. Rev. Entomol.* 27:149-172.

HOWARD, R.W., MCDANIEL, C.A., NELSON, D.R., BLOMQUIST, G.J., GELBAUM, L.T., and ZALKOW, L.H. 1982a. Cuticular hydrocarbons of *Reticulitermes virginicus* Banks and their role as potential species and caste-recognition cues. *J. Chem. Ecol.* 8:1227-1239.

HOWARD, R.W., MCDANIEL, C.A., and BLOMQUIST, G.J. 1982b. Chemical mimicry as an integrating mechanism for three termitophiles associated with *Reticulitermes virginicus* (Banks). *Psyche* 89:157-167.

ISINGRINI, M., LENOIR, A., and JAISSON, P. 1985. Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proc. Natl. Acad. Sci. U.S.A.* 82:8545-8547.

JUTSUM, A.R., SAUNDERS, T.S., and CHERRETT, J.M. 1979. Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. *Anim. Behav.* 27:839-844.

LOK, J.B., CUPP, E.W., and BLOMQUIST, G.J. 1975. Cuticular lipids of the imported fire ants, *Solenopsis invicta* and *richteri*. *Insect Biochem.* 5:821-829.

MINTZER, A., and VINSON, S.B. 1985. Kinship and incompatibility between colonies of the accacia ant *Pseudomyrmex ferruginea*. *Behav. Ecol. Sociobiol.* 17:75-78.

MIRENDA, J.T., and VINSON, S.B. 1981. Division of labour and specification of castes in the red imported fire ant *Solenopsis invicta* Buren. *Anim. Behav.* 29:410–420.

MOREL, L. 1983. Relation entre comportement agressif et privvation sociale précoce chez les jeunes immatures de la Fourmi *Camponotus vagus* Scop. *C.R. Acad. Sci. Paris, Ser. D* 296:449–452.

MOREL L., and BLUM, M.S. 1988. Nestmate recognition in *Camponotus floridanus* worker ants: Are sisters or nestmates recognized? *Anim. Behav.* 36:718–725.

MOREL, L., VANDER MEER, R.K., and LAVINE, B.K. 1988. Ontogeny of nestmate recognition cues in the red carpenter ant (*Camponotus floridanus*): Behavioral and chemical evidence for the role of age and social experience. *Behav. Ecol. Sociobiol.* 22:175–183.

NELSON, D.R., FATLAND, C.L., HOWARD, R.W., MCDANIEL, C.A., and BLOMQUIST, G.J. 1980. Reanalysis of the cuticular methylalkanes of *Solenopsis invicta* and *Solenopsis richteri*. *Insect Biochem.* 10:409–418.

OBIN, M.S. 1986. Nestmate recognition cues in laboratory and field colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae): Effect of environment and the role of cuticular hydrocarbons. *J. Chem. Ecol.* 12:1965–1975.

OBIN, M.S., and VANDER MEER, R.K. 1988. The cue hierarchy of nestmate recognition in *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Anim. Behav.* 36:1361–1370.

OBIN, M.S., and VANDER MEER, R.K. 1989. Mechanism of template-label matching in fire ant, *Solenopsis invicta* Buren, nestmate recognition. *Anim. Behav.* In press.

ROSS, K. G., VANDER MEER, R.K., FLETCHER, D.J.C., and VARGO, E.L. 1987. Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution* 41:280–293.

SORENSEN, A.A., MIRENDA, J.T., and VINSON, S.B. 1981. Food exchange and distribution by three functional worker groups of the imported fire ant, *Solenopsis invicta* Buren. *Insectes Soc.* 28:383–394.

SORENSEN, A.A., FLETCHER, D.J.C., and VINSON, S.B. 1985. Distribution of inhibitory queen pheromone among virgin queens of the ant, *Solenopsis invicta*. *Psyche* 92:57–69.

VANDER MEER, R.K. 1986. Chemical taxonomy as a tool for separating *Solenopsis* spp., pp. 316–326, *in* C.S. Lofgren and R.K. Vander Meer (eds.). Fire Ants and Leaf-cutting Ants: Biology and Management. Westview Press, Boulder, Colorado.

VANDER MEER, R.K. 1988. Behavioral and biochemical variation in the fire ant, *Solenopsis invicta*, pp. 223–255, *in* R.L. Jeanne, (ed.). Interindividual Behavioral Variation in Social Insects. Westview Press, Boulder, Colorado.

VANDER MEER, R.K., and WOJCIK, D.P. 1982. Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticolis*. *Science* 218:806–808.

VANDER MEER, R.K., GLANCEY, B.M., and LOFGREN, C.S. 1982. Biochemical changes in the crop, oesophagus and postpharyngeal gland of colony-founding red imported fire ant queens (*Solenopsis invicta*). *Insect Biochem.* 12:123–127.

VANDER MEER, R.K., LOFGREN, C.S., and ALVAREZ, F.M. 1985. Biochemical evidence for hybridization in fire ants. Fla. *Entomol.* 68:501–506.

WALLIS, D.I. 1963. A comparison of the response to aggressive behaviour in two species of ants, *Formica fusca* and *Formica sanguinea*. *Anim. Behav.* 11:164–171.

WILSON, E.O. 1971. The Insect Societies. Harvard University Press, Cambridge, Massachusetts. 548 pp.

WOLD, S. 1976. Pattern recognition by means of disjoint principal component models. *Pattern Recog.* 8:127–139.