

NESTMATE RECOGNITION AND TERRITORIAL
BEHAVIOUR IN THE ANT *ODONTOMACHUS BAURI*
EMERY (FORMICIDAE : PONERINAE)

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SUMMARY

Odontomachus bauri workers recognize conspecifics from other colonies and ants from different species as aliens. Colony specific recognition is based on endogenous odours, present in the different body parts. The chemicals responsible for these odours are volatiles, insoluble in water and soluble in CH₂Cl₂. No influence of the environment on the recognition signals could be detected. Evidence of intercolony differences in the relative proportions of the different volatile substances of cephalic and abdominal pheromones are presented. Thus, it is postulated that nestmate recognition is based on relative proportions of volatiles produced by the ant in different parts of the body.

On the other hand, evidence of territorial defence is presented. Territorial pheromones, in case they exist in this species, are not necessary for recognition of territories, as is the case in other ant species. The possible relationship between nestmate recognition systems and territorial behaviour is discussed and an evolutionary hypothesis of their development is presented.

RESUME

**Reconnaissance des compagnons du nid et comportement territorial
chez la fourmi *Odontomachus bauri* Emery (Formicidae : Ponerinae)**

Les ouvrières chez *Odontomachus bauri* reconnaissent les homosspécifiques d'autres colonies et les fourmis d'espèces différentes comme étant étrangères. La reconnaissance spécifique de la colonie est basée sur la présence d'odeurs endogènes dans les différentes parties du corps. Les substances responsables de ces odeurs sont volatiles, insolubles dans l'eau et solubles dans CH₂Cl₂. On n'a pas pu détecter une influence du milieu sur le système de reconnaissance. On démontre l'existence de différences dans les proportions relatives des substances volatiles des phéromones céphaliques et abdominales entre les colonies. On propose donc que la reconnaissance des compagnons du nid est basée sur quelques différences dans les proportions relatives des substances volatiles produites dans les différentes parties du corps.

On présente aussi des preuves de défense territoriale. Contrairement à ce que l'on trouve chez d'autres espèces, le marquage chimique du territoire n'est pas nécessaire pour la reconnaissance du territoire. Finalement, on analyse la possibilité d'une relation entre les systèmes de reconnaissance des compagnons du nid et le comportement territorial, et on présente une hypothèse évolutive sur leurs développements.

INTRODUCTION

Social insects in general and specifically social hymenoptera require, in order to maintain the cohesiveness of their society, mechanisms of identification and colony specific discrimination of conspecifics. On the other hand, kinship selection theory (HAMILTON, 1964) postulates social discrimination on the basis of differential degrees of genetic relatedness. Thus, genetically regulated colony specific recognition mechanisms should exist in social insects. The common assumption is that odours are responsible for the recognition (FOREL, 1874 ; FIELDE, 1904 ; HOWSE, 1975 ; HÖLLDOBLER and MICHENER, 1980). Few experimental evidence exist supporting the above assumptions. Works done with the bee *Lasioglossum zephyrum* (BELL, 1974 ; GREENBERG, 1979 ; BUCKLE and GREENBERG, 1981) hint on genetic determined odours which are learned by the bees. They show evidences of a positive linear relationship between the genetic coefficient of relationship of the bee and the probability of acceptance to a colony. From the existing literature (WILSON, 1971 ; DUMPERT, 1979 ; CROZIER and DIX, 1979 ; HÖLLDOBLER and MICHENER, 1980) it is evident that the specific nature of the cues involved in nestmate recognition in Hymenoptera is not known. HÖLLDOBLER and MICHENER (1980) postulate a genetic and an environmental odour component responsible for nestmate recognition. CROZIER and DIX (1979) present two theoretical models, one assuming colony odours distributed all over the cuticle of the insects, i.e. the gestalt model ; the other one assuming individual odours with no possible contamination between nestmates. In both cases they assume odours to be genetically determined. HÖLLDOBLER and MICHENER (1980) add two other possible nestmate recognition systems to the ones named above ; one based on a " queen odour " which is distributed among the workers, and another one based only on environmental odours.

In ants, it has been shown that imprinting mechanisms work in brood recognition (JAISSON, 1975) in some species. JAISSON (1980) also presented evidence of imprinting in nestmate recognition. The evidence is based on the possibility of producing mixed colonies using young workers, which he could produce even with ants of different genera or subfamilies. With ants of the subfamily Ponerinæ, he was not able to produce mixed colonies using young workers of different genera of the same subfamily (*Ectatomma*, *Neoponera* and *Odontomachus*), nor of other subfamilies. On the other hand,

LE MASNE (1952) referred to intraspecific aggression and to the possibility of forming artificial colonies with individuals from different origins in his classification of open and closed societies. The closed societies were those with a marked intraspecific aggression. This kind of approach is not helpful in studying nestmate-recognition systems, as the relation between nestmate-recognition mechanisms and the openness of a society is not direct because of the intervention of many other factors in the determination of a society (PROVOST, 1979).

In any case, most ants are able to recognize their nestmates and distinguish them from alien ants. When alien ants are encountered, they are generally attacked and eventually killed. As to the possible cues allowing for these discrimination, it has been proposed that they are odours related to the "nest odour". For example, *Pogonomyrmex badius* is able to recognize nest materials from its own nest and differentiates them from nest materials from other colonies of the same species or from different species (HANGARTNER *et al.*, 1970). In *Formica polyctena* it has been shown that the nest odour is related to the diet of the colony (LANGE, 1960). HOWSE (1975) proposed that the recognition signals were associated with the cuticle. LONGHURST (1977) was further able to demonstrate in *Megaponera fæstens* that ants or ant dummies with cuticular waxes of the workers were attacked when offered to alien conspecific colonies, but were accepted or attacked much less if presented to their colony of origin. JAFFE (1982) showed that workers of *Atta cephalotes* have a colony specific recognition system which originates from colony differences in the relative quantities of the different chemical components of the mandibular alarm pheromone.

Territorial behaviour has been reported for only a few ant species (HÖLLDOBLER, 1979). JAFFE *et al.* (1979) reported colony specific territorial marking in *Atta cephalotes* and the presence of a territorial marking pheromone in the valves gland (BAZIRE-BENAZET and ZYLBERBERG, 1979). Also for *Cecophylla longinoda* territorial marking with rectal sac secretions has been reported (HÖLLDOBLER and WILSON, 1977). CAMMAERTS *et al.* (1977) postulates a function in territorial marking for the Dufour's gland secretion in *Myrmica rubra*. For *Pogonomyrmex badius* (HÖLLDOBLER, 1976), *Formica rufa* (SKINNER, 1980) and *Solenopsis saevissima* (WILSON *et al.*, 1971) evidences of territorial behaviour have been presented although no references to chemical marking of territories were made.

Territorial behaviour and nestmate recognition systems are two of the most important mechanisms available to ants to maintain the cohesiveness of their colonies. For the more primitive ant species as regards to social behaviour, LE MASNE (1952) reports for example absence of intraspecific aggression and even fusion of colonies of *Hypoponera eduardi*, contradicting JAISSE's (1980) report mentioned earlier. Reports on marked hostility among colonies, seemingly based on odours, exist for *Myrmecia* (HASKINS and

HASKINS, 1950). But detailed studies on territorial behaviour and nestmate recognition systems for Ponerinae are missing. Therefore, the study of these behaviours in *Odontomachus bauri* (Ponerinae), considered primitive as regards to colony size, polymorphism and social structure, seems to be promising in understanding the evolution of colony segregation mechanisms.

O. bauri is a monomorphic ant, with a simple social structure and small colonies of up to 300 ants, living in rudimentary subterranean nests. Solitary individuals forage on the surface for live arthropod prey using no recruitment. Workers in queenless colonies lay eggs from which alate males emerge. This work explores the mechanisms by which individuals recognize their nestmates and their territory and considers a hypothesis about the evolution of recognition mechanisms in the Formicidae, relating it to territorial behaviour.

MATERIALS AND METHODS

40 colonies of *Odontomachus bauri* were collected at the University campus of Sartenejas, Estado Miranda, Venezuela in an area of about 1 km² of gardens. Colonies were reared in plastic containers (23 × 45 × 15 cm) with small chambers made of plaster of Paris which served as nests. Colonies were fed with living insects captured with a light trap, and were maintained with sun light on the natural 12 h light / 12 h dark cycle. Experiments were carried out 2 h before the light cycle finished, at the time of maximum foraging activity. Before starting an experimental series, colonies were checked for their aggressiveness. Colonies not attacking an intraspecific intruder were eliminated.

Two test ants, selected at random, were placed in front of the plaster of Paris chambers using plastic forceps. In experiments with dead ants, these were rapidly killed by plunging them into finely crushed dry-ice. They were used in experiments after the body temperature equilibrated with the room temperature (25 °C). In experiments with freeze-dried ants, ants were killed as just described and freeze-dried using a vacuum pump and a refrigerated supporting plate. Pressures of about 0.5×10^{-3} mmHg at - 40° C during 6 h were applied.

For the extraction of volatile substances, ants were washed three times by shaking an individual in 5 ml of solvent for 3 min. The extract obtained in the first wash was then used to impregnate some washed test ants, and the solvent was evaporated by blowing dry air on the test ant. For experiments with impregnated freeze-dried ants, ants from a different colony of those to be tested were freeze-dried and then placed in small closed glass vials together with ten crushed heads, thoraces or gasters, but with no direct contact with them, for 15 min at room temperature. In this way, only volatile substances could be absorbed by the freeze-dried ants cuticle.

In experiments testing the influence of the environment on the recognition mechanism, colonies were split in two queenless groups (minimum of 30 ants each) and were fed on different diets or were treated with vanilla solutions. 1 % vanilla extract (Mc Cormick) solutions were used for impregnating cottons with 5 ml of solution. The cotton was then introduced in the plaster of Paris chamber of the queenless groups of 5 colonies. The 5 remaining groups were supplied with cottons impregnated with 5 ml of distilled water. The cottons were left in the chambers for 7 days before testing aggression between members of different colonies as described above. Other 4 colonies were split in the same way but some of them were fed with honey and beef meat,

whereas the rest of the groups were fed with sugar water and dead crickets, for 30 days.

Gas chromatography was on 5 % Carbowax columns on 100-120 mesh Diatomite CLQ in 12 mm internal diameter, 3 m steel columns. The solid sample technique (MORGAN and WADHAMS, 1972) was used for studying the relative amount of pheromone components in a single ant. Solvent extracts were concentrated by blowing dry nitrogen on the surface of the extract. Ants for these experiments were randomly drawn from laboratory colonies and fed on the same diet for at least 30 days.

For the study of territorial behaviour, open Petri dishes (9 cm diameter) with a filter paper (Whatman Nr 1) covering the bottom, were placed in the foraging area of the colonies and were left there for a minimum of 48 h. Two ants, selected at random, one from each of two different colonies, were placed into one of the dishes. The number of attacks initiated by each ant, was recorded during the two min following the introduction of the ants into the dish. The variables of the experiment were the following: *Ants*: "Resident ants" were those *O. bauri* workers which were taken 5 min before the assay from the colony where the assay was performed. When the assay was performed outside the nests, the "resident ant" was the one which came from the colony which had explored the dish before. The "Intruder ant" was an *O. bauri* worker taken 5 min before the assay from a colony which was different from that where the assay was performed.

Petri dishes: The position of the dish during the assay was important. It could be inside the nest which had explored it at least during the 48 h before the assay. In this case, the dish was removed 5 min before the assay and reintroduced just for the assay. The dish used for the assay could also come from the intruder's colony. In this case, the dish was removed from one of the colonies 5 min before the assay and introduced in the « resident » colony just for the assay. In this case, the "intruder ant" was of the same origin as the Petri dish. The last possibility was the one where the assay was performed with a Petri dish placed outside the nests. Also in this case, the dish was removed from the respective colony 5 min before the assay.

Only one experiment per colony per day was performed. All non parametric tests used for the analysis of the data were taken from STEIGEL (1956).

RESULTS

Odontomachus bauri workers attack their opponents using their open mandibles, which they snap on their enemies. This attack is not only observed against other arthropods and ants of different species (*Camponotus*, *Atta*, *Acromyrmex*, *Odontomachus chelifer*), but also against ants from different conspecific colonies. These attacks eventually lead to the death of the intruder.

Some colonies maintained in the laboratory for long periods, lost their aggressiveness towards workers from other colonies, regardless if they came directly from the field or from other laboratory colonies. Other laboratory colonies maintained a high level of aggressiveness during all the time they were studied, in some cases over a year. These colonies were normally those with a highly active queen, i.e. a queen producing many eggs and therefore the colony had many larvæ and cocoons. These observations suggested that aggression was somehow related to the physiology of the colony. As the

bioassays used for evaluating nestmate recognition were based on aggressive behaviour, colonies not attacking at least two intraspecific intruders selected at random were not used for starting an experimental series.

On the meeting of two *O. bauri* workers, antennation nearly always occurs, with both ants front to front and thus touching their opponents antennæ or head with their antennæ. Antennation may then lead to four different behavioural patterns: *Basic alertness*: in which the ants open their mandibles to about 180°; *Alarm*: the same as basic alertness but together with fast random body movements and probably with release of alarm pheromone; *Attack with mandibles*: as described earlier; and *Attack with sting*: which occurs infrequently and consists of the ant trying to sting the opponent, bending its abdomen ventrally forwards.

The time ants spend antennating each other on their first contact does not differ between test ants from the same colony (control) or from a different conspecific colony (experimental) ($p > 0.1$, Student's t-test, $n = 12$). The behaviour which occurred most frequently and which showed the greatest difference between the two type of test ants, was attack with mandibles. Therefore, the number of test ants attacked was taken as a measurement for nestmate-recognition (table I). We observed that live experimental ants, freshly killed ones, or live experimental ants isolated for 5 days or more were attacked significantly more than their respective control. Even experimental ants, kept before the test in a wire mesh capsule inside the control colony, were attacked much more than controls, kept inside the colony of the experimental ant. This suggests that the recognition signal is produced by the ant and not taken from the environment. The fact that *O. bauri* recognizes dead nestmates suggest that the nestmate recognition signal is not a sound or a specific behaviour.

Freeze dried ants were never attacked (table I), nor were ants presented in a plastic wire mesh capsule. These results strongly suggest that odours are responsible for the nestmate recognition and that these odours are detected on contacting the cuticle of the ant. Although the fact that test ants have to be directly accessible to the workers in order to produce attack may also be explained by a lack of secondary cues (vision, tactile stimuli, etc.), necessary to direct the attack.

In order to further test the possible influence of environmental odours on nestmate recognition, the following experiments were performed. Four colonies were split in eight queenless groups of workers. Four groups of four different colonies were fed on one diet, and the remaining four groups on a different one. When testing aggression towards nestmates from groups fed on a different diet, one month after the colonies were split, not a single attack could be recorded ($n = 16$). The ants from groups fed on the same diet, but from different colonies were attacked in 75 % of the cases ($n = 16$). The statistical difference between both results is highly significant ($p < 0.001$).

if compared with a Fisher's exact probability test. If similar groups of ants were left before the test with cottons impregnated in vanilla solution for one week, the following results were obtained. Test ant with vanilla odours in colonies without vanilla: Controls elicited attack in 8 % of the cases vs 100 % of experimental test ants ($n = 12$, $p < 0.001$, Fisher's test). Test ants with vanilla odours in colonies also submitted to the same odours: Controls were never attacked but experimentals were attacked in 75 % of the cases ($n = 12$, $p < 0.001$, Fisher's test). If the results from experiments between groups with different odours but from the same colony were compared to those of groups submitted to the same odour from different colonies, a highly significant difference was obtained ($p < 0.01$, Fisher's test). That is, no evidence of an environment influence on the nestmate recognition system could be found.

In order to find out the nature of the recognition cue, the test ant was washed with distilled water. This washing did not alter the recognition pattern (table I). Washing with CH_2Cl_2 did remove the colony specific recognition signals. That is, CH_2Cl_2 washed ants are treated the same, regardless if they are controls or experimentals. The colony specific recognition signals was at least partially restored when CH_2Cl_2 extracts (washings from 3 ants) were allowed to evaporate on a CH_2Cl_2 washed ant from a third colony (table I).

Table I. — Percentage of *O. bauri* workers attacked when placed into an *O. bauri* colony.

Tableau I. — Pourcentage d'ouvrières d'*O. bauri* attaquées lorsqu'elles sont placées dans une autre colonie d'*O. bauri*.

Treatments	n	Control ¹	Experi- mental ²	Cont. p ³ vs. Exp.
Live ants	16	0	100	< 0.001
Dead ants	13	16	77	< 0.01
Freeze dried ants	12	0	0	NS
Live ants isolated singly for 5 days	12	0	92	< 0.001
Live ants isolated for 15 days in an artificial colony	12	0	83	< 0.001
Live ants isolated for 20 h in a wire mesh capsule placed in a foreign colony	14	0	93	< 0.001
Live ants in a wire mesh capsule	12	0	0	NS
Dead ants washed with water	12	0	100	< 0.001
Dead ants washed with CH_2Cl_2	14	36	36	NS
Dead ants washed and later impregnated with washings of CH_2Cl_2	13	25	71	< 0.03

1 Test ants and host ants from the same colony.

2 Test ants and host ants from different colonies.

3 Fisher exact probability test ($\alpha = 0.1$), NS = not significantly different.

Table V – Ratio between peak areas of 4-methyl-3-heptanone and 2-heptanone in gas chromatograms of *A. cephalotes* media workers heads.

Tabla V – Relación entre las áreas de los picos de 4-metilo-3-heptanona y de 2-heptanona en cromatogramas de cabezas de obreras medianas de *A. cephalotes*.

A : Mean and standard deviation

Colony	Ratio of peak-area
A	18.2 ± 1.6 a
B	11.8 ± 2.0 b
C	8.6 ± 2.2 c
D	6.6 ± 1.7 c

a, b, and c indicate means which are different ($p < 0.05$) by Hartley's test.

B : ANOVA

Source of variability	Sum of squares	Degrees of freedom	Mean squares	F
Treatments	278.0	3	92.7	23.0 ***
Error	52.3	13	4.0	
Total	330.4	16		

*** indicate $p < 0.001$

DISCUSSION

From these experiments we may conclude that *A. cephalotes* workers recognise ants of their species by chemical cues spread on the cuticle which probably originate in the head. Colony-specific recognition seems to be achieved by perception of cephalic volatile substances, but it seems that ants react to these only if the intruder shows movement. That is, dead nestmates are not distinguished from those of other colonies of the same species. It is also possible that the way the volatiles are released from the head is different in an anaesthetised ant or an isolated head from a live ant or a live ant with the legs and gaster removed. There could be different rates of release of the alarm pheromone from the mandibular glands. The biological assays and the chemical analysis of the alarm pheromones from workers of different *A. cephalotes* colonies suggest that the ants use the alarm pheromone complex for recognising their nestmates or alien ants. Major differences in the alarm pheromone such as exist between *A. cephalotes* and *Acromyrmex octospinosus*,

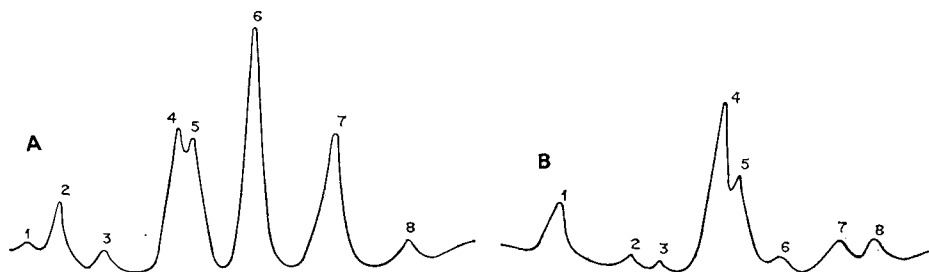


Fig. 1. — Gas chromatograms of: A, the head of a single *O. bauri* worker and B, the gaster of a single worker; showing at least eight different peaks. Temperature program: 4° per min. increase from 120° to 190° C. Graph reads from right to left.

Fig. 1. — Chromatogrammes en phase gazeuse de: A, la tête d'une seule ouvrière d'*O. bauri* et B, l'abdomen d'une seule ouvrière d'*O. bauri* (au moins huit pics différents). Programme de température: accroissement de 4° par min. depuis 120° jusqu'à 190° C. La figure se lit de droite à gauche.

from the thorax with our method, even when CH_2Cl_2 extracts of 50 thoraxes were run through the gas chromatographic columns. Gas chromatograms of the CH_2Cl_2 washings of workers showed the same compounds detected from single head and gaster chromatograms. Each colony showed a characteristic pattern of the relative proportion of the different chemicals present in the head and gaster. In order to check for inter-colony differences in the proportion of the volatiles from the head and the gaster, two components (peaks of the gas chromatograms) from both, the head and the gaster, were tested for their constancy in ants from the same colony and their variations between colonies (*table III*). The analysis of variance of two

Table III. — Ratio of areas of peaks 6 and 7 and of peaks 4 and 5 of gas chromatograms of single heads and gasters respectively, of *O. bauri* workers from different colonies.

Tableau III. — Relation de surfaces des pics 6 et 7 et des pics 4 et 5 des chromatogrammes en phase gazeuse de têtes seules et abdomens respectivement d'ouvrières d'*O. bauri* de différentes colonies.

Colony	\bar{x} of area 6/7 \pm sd (Head)	n	\bar{x} of area 4/5 \pm sd (Gaster)	n
A	1.1 \pm 0.1	4	0.71 \pm 0.03	5
B	1.3 \pm 0.4	4	0.80 \pm 0.20	4
C	1.2 \pm 0.6	6	1.34 \pm 0.26	5
D	5.0 \pm 3.6	4	0.88 \pm 0.08	3
E	17.7 \pm 3.4	6	1.45 \pm 0.26	7
Analysis of variance, $F_{4,20}$	16.58 *		14.67 *	

* indicate $p << 0.001$.

arbitrarily selected peak ratios showed highly significant bigger intercolony variations compared to the intracolony variations ($F_{4,20} = 14$, $p < 0.001$). Great intracolony variations had to be expected because of methodological difficulties in capturing, killing and dissecting the ants in exactly the same way each time. The way ants are handled will undoubtedly affect the proportions of volatiles lost during the process. In spite of these potential difficulties, the results demonstrate the presence of statistically significant colony differences of odours, i.e. volatile substances detectable by gas chromatographic methods, in these ants.

As regards to territorial behaviour, results in *table IV* clearly show the presence of differences in aggressiveness of the workers, depending on the location of the Petri dish where the bioassay was performed. That is, inside their own nest, *O. bauri* workers are significantly more aggressive than an intruder, independently if the substrate has been explored before by its own colony or by the intruders colony. Outside the nest, aggressivity of *O. bauri* workers is also independent on the substrate, and no significant differences between the aggressivity of resident and intruder ants can be observed. These results suggest the absence of chemical cues on the substratum used for colony specific territorial recognition. At least it is clear if a chemical cue exists on the territory, it is less important than other type of signals which are present in their nest area, and which make the workers defend their area more actively than an intruder. Thus, *O. bauri* workers recognize their territory, probably using visual cues or environmental odours in the air.

DISCUSSION

From these experiments we may conclude the following :

- a) *O. bauri* workers recognize the surroundings of their nest, which they defend more actively than other areas. The main cue for the recognition of the territory is not a chemical mark on the substratum.
- b) *O. bauri* workers recognize their nestmates and differentiate them from conspecifics of different colonies, using odours which are present on all body parts and which are produced by the ant. These odours are volatile chemicals, insoluble in water and soluble in CH_2Cl_2 .
- c) Volatile chemicals of *O. bauri* workers, detectable by gas chromatography, present intercolony variations in their relative proportions, and are relatively constant in their proportions between individuals of the same colony.

The possibility of the existence of a territorial pheromone, which did not adsorb on the filter papers used in the experiments, can be reasonably excluded. The natural environment of these ants are substrates covered with dry leaves which have approximately the same physical properties regarding

adsorption and absorption of chemicals compared to filter papers, as both consist mainly of cellulose. Colonies of *O. bauri* change their nest sites in nature very frequently, sometimes staying less than 24 h in one place. Thus, if a territorial pheromone exists, this should be deposited by the ants relatively fast. On the other hand, the fact that territorial behaviour can be demonstrated inside the foraging area but not outside it on the same substrate, is an evidence of the unimportance of an eventual territorial pheromone compared to other cues which have to be used by the ants in order to explain the results on *table IV*.

Table IV. — Median (range) of the number of attacks observed during the first two minutes for each of two different ants placed into a Petri dish explored by one of the colonies for a minimum of 48 hours.

Tableau IV. — Médiane (rang) du nombre d'attaques observées pendant les deux premières minutes pour chacune des deux fourmis placées dans une boîte de Pétri explorée par une des colonies pendant 48 heures.

Position of the dishes during the test	n	Attacks by resident ant	Attacks by intruder	p ¹	p ²
Inside the nest of the resident on dish explored by the resident's colony	21	2 (0-3)	0 (0-2)	< 0.01	< 0.05
Inside the nest of the resident on dish explored by the intruder's colony	12	2 (0-3)	0 (0-3)	< 0.05	< 0.10
Outside the nests	14	3 (0-6)	2 (0-4)	NS	NS

1 Wilcoxon's matched pairs test ($\alpha = 0.10$).

2 Binomial test ($\alpha = 0.10$).

From experiments on nestmate recognition, we know that these ants recognize their nestmates using CH_2Cl_2 — soluble volatile substances produced by the ants. On the other hand, we showed that different colonies of these ants produce different relative proportions of volatile substances. Thus, we propose that *O. bauri* uses the information given by the relative proportion of volatile chemicals produced by the ant and/or adsorbed on the cuticle of the insect for nestmate recognition. Although no direct proof to this hypothesis is available, such as experiments with ant-dummies impregnated with synthetic compounds in a precise proportion, indirect evidences supporting this hypothesis exist. These evidences are :

In the case of *O. bauri*, the source of the nestmate recognition signal (or of the main signal) is not exogenous. Different authors postulated environmental factors as determinant in the colony odour which is used for nestmate recognition (LANGE, 1960 ; WILSON, 1971 ; HÖLLDOBLER and MICHENER, 1980, for example). The experiments with isolated ants (*table I*) show that different

environments (natural or unnatural) do not change the recognition cues on the ant. Also food do not seem to influence decisively the recognition cue. In spite of this, it is evident from the results that the recognition cue is a volatile substance and that visual and auditory stimuli are not indispensable for nestmate recognition. Nor seem tactile stimuli alone allow workers to recognize their nestmates as experiments with freeze dried ants showed. Thus, only chemical cues can explain results on *tables I and II*.

The source of the chemical signal for nestmate recognition is not clear from these results. The lack of volatiles detectable by gas chromatography in the thorax, in spite of its ability to give recognition signals (*table II*) seems contradictory, although it can be explained in the following way: Very volatile components and very polar components can not be detected with the methods used. On the other hand, the sensibility of the gas chromatograph in detecting chemicals is about 0.1 ng, whereas an insect antenna is able to detect a few molecules of a chemical. That is, the bioassay is much more sensitive in detecting the presence of colony specific chemicals than the gas chromatograph. In any case, from results on *table II* it is clear that the volatile chemicals responsible for nestmate recognition are present in all three body parts of the insect.

The gas chromatograms from ants of different colonies clearly show that relative proportions of volatile chemicals vary from colony to colony. We do not know, whether the compounds detected by the gas chromatograph are the same ones used by the ants in nestmate recognition. What this fact shows is that the possibility of a colony specific odour, based on differences in the relative proportions of different chemicals, is a real one. These results support the individualistic odour model of CROZIER and DIX (1979). This model of nestmate recognition seems to be the most plausible one, as colony differences in the relative proportions of pheromone components seems to be a common phenomenon in ants (CAVILL and HINTENBERGER, 1960; BRADSHAW *et al.*, 1979; JAFFE, 1982, for example).

One interesting feature of this study is that if we compare *O. bauri* with the more socially advanced ant species, such as the Formicinae *Camponotus rufipes* or the Myrmicinae *Atta cephalotes*, the former seem to have a more sensitive recognition system in spite of their relatively social primitiveness. *Atta cephalotes* recognize their nestmates via the alarm pheromone complex from the mandibular gland (JAFFE, 1983), the same as *C. rufipes* (SANCHEZ, 1982; JAFFE and SANCHEZ, in preparation). These two ant species do not recognize headless conspecifics as aliens, even if they come from different colonies. The Ponerinae *O. bauri* on the other hand, recognizes nestmates and differentiates them from aliens even when other body parts are presented (*table II*), and thereby has a greater ability for nestmate recognition. In order to interpret this phenomenon we postulate the following evolutionary hypothesis:

All ants recognize their nestmates by imprinting the odours produced by their nestmates. In the case of primitive ants, all odours produced by the pheromone secreting glands are used as recognition signals. These primitive ants lack mechanisms by which they could mark their territory with chemicals, and thus have no intra-specific defence mechanisms through advertisement of their defended area. In this case, the probability of meeting an intruder is high, and thus efficient nestmate recognition mechanisms are required. This seems to be the case of *O. bauri* and of the fungus growing Myrmicinae *Trachymyrmex urichi* which uses visual cues to recognize its territories (VILLEGAS, 1982; JAFFE and VILLEGAS, in preparation). More advanced ant species such as *A. cephalotes* (JAFFE *et al.*, 1979; JAFFE, 1983) and *C. rufipes* (SANCHEZ, 1982; JAFFE and SANCHEZ, in preparation), *Conomyrma biconis* (COLMENARES, 1982; JAFFE and COLMENARES, in preparation) and *Solenopsis geminata* (PUCHE, 1982; JAFFE and PUCHE, 1984), do mark their territories with chemicals, advertising it to conspecifics from different colonies. These ants recognize their nestmates mainly through their alarm pheromones, which show highly significant inter-colony differences in the relative proportion of volatile chemicals. In these ants, the individualistic model of CROZIER and DIX seems also to be valid, but restricted to one pheromone. In this case, the probability of a foreign conspecific appearing in the defended area of a colony is reduced, due to the advertising of the territory, and thus, detection of foreign ants and recognition of nestmates is only required in intraspecific combats, where alarm pheromones play a mayor role in directing the attack and organizing the combat (WILSON, 1971). Thus, the same alarm pheromone seems to be the more appropriate signal for nestmate recognition (JAFFE, 1984).

No examples of the "gestalt" model (CROZIER and DIX, 1979), in which colony odours are transferred between individuals resulting in a gestalt colony odour is known, although CROZIER and DIX supposed that this model was favored for most ant species, specially for the more evolved ones. One reason for the improbability of the gestalt model in big ant colonies is that the bigger the colony, the more improbable that every member of the group interact with all other members, producing the gestalt colony odour. In *Atta cephalotes* for example, colonies with over 10 million individuals are known (WEBER, 1972). In this case, the gestalt colony odour seems to be impractical as ants are separated inside the nest in thousand different chambers, some of them separated by distances of various meters. Similarly, no experimental evidence for the other two alternative models proposed by HÖLLDOBLER and MICHENER (1980), i.e. the queen odour model and environmental odour model are known. Although an environmental effect on the production of endogenous chemicals can not be completely excluded. These conclusions seem to be in agreement with the nestmate recognition system proposed for the bee *Lasioglossum zephyrum* (BARROWS *et al.*, 1975). In any case, more

experimental work with different species is required in order to determinate the evolutive pathway of territorial behaviour and nestmate recognition systems in ants.

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