

ON THE NESTMATE-RECOGNITION SYSTEM AND
TERRITORIAL MARKING BEHAVIOUR IN THE ANT
CAMPONOTUS RUFIPES

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SUMMARY

Camponotus rufipes (Fab.) workers recognize conspecifics from other colonies and ants from different species as aliens. Colony specific recognition is based on odours produced by the mandibular gland which also serve as alarm pheromone.

This ant marks its territory with chemicals secreted by an unidentified abdominal gland, which is thus advertised to conspecifics. Marked territories near to the nest induce recruitment or at least prolonged exploration if marked by a foreign conspecific colony. The same territory outside the nest area induces a prolonged permanence of workers from the colony giving the mark.

The possible relationship between nestmate recognition systems and territorial behaviour is discussed in an evolutionary context by stating the hypothesis that alarm pheromones are used for nestmate recognition by those ants possessing territorial marking behaviour.

RESUME

**Sur le système de reconnaissance des membres de la même société
et le comportement de marquage du territoire chez la Fourmi
*Camponotus rufipes***

Les ouvrières de *Camponotus rufipes* (Fab.) reconnaissent comme étrangers les individus de même espèce appartenant à d'autres sociétés et les Fourmis d'autres espèces. La reconnaissance des membres de leur propre société repose sur les odeurs produites par la glande mandibulaire des ouvrières, qui sert également de phéromone d'alarme.

Cette espèce marque son territoire par des substances chimiques provenant d'une glande abdominale non identifiée, qui avertissent les membres de la même espèce. Tout territoire marqué aux alentours du nid induit un recrutement ou au moins, dans le cas où il est marqué par les ouvrières d'une société étrangère, une exploration prolongée. Le même territoire, situé en dehors de la zone de nidification, entraîne la présence prolongée des ouvrières de la société qui l'a marqué.

L'éventuelle relation entre la reconnaissance du nid et le comportement territorial est discutée dans un contexte évolutif, en posant l'hypothèse suivante : les phéromones

d'alarme sont utilisées pour la reconnaissance des membres de leur société par ces Fourmis qui possèdent en même temps un comportement de marquage du territoire.

INTRODUCTION

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 this discrimination, it has been proposed that they are odours related to the "nest odour" as in *Pogonomyrmex badius* (HANGARTNER *et al.*, 1970). HOWSE (1975) proposed that the signals used by ants to detect their nestmates are associated with the cuticle. LONGHURST (1977) was able to demonstrate in *Megaponera foetens* that ants or ant dummies with cuticular waxes of the workers were attacked when offered to alien colonies of the same species, but were accepted or attacked much less if presented to their colony of origin. JAFFE (1983) showed that workers of *Atta cephalotes* have a colony specific recognition system which originates from colony differences in the relative quantities of the different chemicals of the alarm pheromone produced by the mandibular gland. In the ponerine *Odontomachus bauri*, volatile chemicals produced in the different body parts of each worker are held responsible for nestmate recognition (JAFFE and MARCUSE, 1983).

CROZIER and DIX (1979) presented two theoretical models trying to explain nestmate recognition in the social insects and specifically the social Hymenoptera. One model assumes colony odours distributed all over the cuticle of the insect, i.e. the gestalt model, the other one assumes individual odours with no possible contamination between nestmates. In both cases they assume the odours to be genetically determined. HÖLLDOBLER and MICHENER (1980) postulated a genetic and an environmental odour component responsible for nestmate recognition in Hymenoptera, and they add two other possible nestmate recognition systems to those named by CROZIER and DIX, one based on a "queen odour" which is distributed among the workers, and another one based only on environmental odours.

Territorial behaviour has been reported for some ant species. A territorial pheromone which is used in marking parts of the home range is known for *Atta cephalotes* (JAFFE *et al.*, 1979), *Ecophylla longinoda* (HÖLLDOBLER and WILSON, 1977), *Myrmica rubra* (CAMMAERTS *et al.*, 1977) and *Solenopsis geminata* (JAFFE and PUCHE, 1984), which is secreted by the valves gland (BAZIRE-BENAZET and ZYLBERBERG, 1979), rectal sac, Dufour's gland and metapleural gland respectively. For *Pogonomyrmex badius* (HÖLLDOBLER, 1976), *Formica rufa* (SKINNER, 1980) and *Solenopsis saevissima* (WILSON *et al.*, 1971), evidences for territorial behaviour have been presented although no references to possible

chemical marking of the territories were made. (See also review in HÖLLEDOBLER, 1979).

In the case of *Odontomachus bauri* (JAFFE and MARCUSE, 1983) and *Trachymyrmex urichi* (VILLEGAS, 1982, JAFFE and VILLEGAS, in preparation) territorial behaviour not related to chemical marking of the territory has been observed. The workers of these species recognise its territory with the aid of visual cues or landmarks, defending it more vigorously than a foreign one.

Territorial behaviour and nestmate recognition are two of the most important mechanisms available to ants in order to maintain the cohesiveness of their colonies. Thus, a relation between the nestmate recognition systems and territorial behaviour in ants is likely to exist (JAFFE, 1982; JAFFE, 1985; JAFFE and MARCUSE, 1983). One trend noted from the existing data is that ant species which mark their territories chemically have nestmate recognition systems based on the alarm pheromones. On the other hand, species which do not mark their territories have nestmate recognition systems based on a variety of pheromones. Therefore, the study of nestmate recognition systems and of territorial behaviour in different ant species seems to be appropriate in order to gain some insight into the evolution of social behaviour related to the cohesiveness of a colony and colony segregation mechanisms. Here we present the first report of a specific pheromone being used as nestmate recognition signal.

Camponotus rufipes (Fab.) is one of the most common Formicinae in the neotropics. It is an insectivorous ant, which also collects honey-dew. It is active at night and has a recruitment system which includes the use of a trail pheromone from the rectal sac, serving as an orientation cue and attractive signal. *C. rufipes* also uses tactile information in recruitment (SANCHEZ, 1982; JAFFE and SANCHEZ, 1984). It can be regarded as one of the more evolved Formicinae with respect to the degree of sophistication of the communication system used in recruitment, and to the degree of worker polymorphism. Like most Formicinae, ants of the genus *Camponotus* use the mandibular gland, poison gland and Dufour's gland as a source of alarm pheromones (AYRE and BLUM, 1971; BERGSTROM and LOFOVIST, 1971, 1972; BROPHY *et al.*, 1973; HASKINS *et al.*, 1973; PARRY and MORGAN, 1979).

MATERIALS AND METHODS

Colonies of *Camponotus rufipes* (Fab.) were collected at the Universidad Simón Bolívar campus, Sartenejas, Estado Miranda, Venezuela, in an area of about 1 km². 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.

For the study of nestmate-recognition, test ants were placed in front of the plaster of Paris chambers using plastic forceps. Ants were rapidly killed by plunging them

into finely crushed dry-ice. Dead ants were used in experiments after the body temperature equilibrated with the room temperature ($25 \pm 4^\circ \text{C}$). Freeze-drying was carried out after killing the ants as just described, using a vacuum pump and a refrigerated supporting plate. Pressures of about 0.5×10^{-3} mm Hg at -40°C during 6 h were applied to the ants during this process.

For testing the mandibular gland secretion, ants were taken from a nest with plastic forceps. During this process, the worker became alarmed. With the help of a glass capillary, the droplet of secretion which appeared on the mouthparts of the worker was quickly collected. A cylindrical plastic-foam dummy (Polyurethan, 1 cm long and 0.5 cm in diameter) was then contaminated with the secretion by including the glass capillary inside the dummy. Two dummies, one (the control) contaminated with the secretions of an ant from the same test colony, the other (experimental) contaminated with secretions from a worker of a different colony, were placed simultaneously inside the nest, as described above. The whole process lasted less than 3 min.

For this study of territorial behaviour, two different bioassays were used. In bioassay I, two ants from two different colonies were placed into a Petri dish (9 cm diameter) which contained a filter paper (Whatmann Nr 1). The filter paper had been placed into the foraging area of one of the two colonies for 24 h before the bioassay. The first ant to exit the Petri dish was noted.

In bioassay II, two filter papers were placed simultaneously in front of the nest of a *C. rufipes* colony. One minute later, the number of ants on each filter paper and the number of them dipping the abdomen on the ground were counted. Different treatments were applied to those filter papers. Some of them were placed on the foraging area of the colonies for 24 h before they were used in the bioassay. Others were impregnated with methanol (Fisher P.A.) extracts of body parts or of glands from single *C. rufipes* workers. The extract of one gland or one body part was used to impregnate one filter paper (9 cm diameter). When the paper had been explored by the same colony where the bioassay was performed, or if the paper had been impregnated with extracts from ants of this colony, the paper was referred to as Control. When it had been explored by a different colony or was impregnated with extracts of ants from a different colony, it was called Experimental. More details are given in the tables and in the results.

All non-parametric statistical tests used for the analysis of the data were taken from SIEGEL (1956).

RESULTS

Nestmate recognition

On the meeting of two *Camponotum rufipes* workers, antennation nearly always occurs, with both ants front to front and thus touching their opponents antennae or head with their antennae. Antennation may then lead to the subsequent behavioural patterns: *Alarm*, in which the ants make fast random body movements and release probably alarm pheromone; *Biting*, in which the ant jerks forward towards the opponent and bites it with the mandibles; and *Stinging*, in which the ant bends the abdomen ventrally forwards and attempts to apply its defence secretion to the opponent.

Each one of these parameters, with the exception of antennation, increases when an ant from a different species is introduced into a *C. rufipes* colony (table I). Here we also observed that conspecific intruders were

Table I. — Mean number and standard deviation of ants observed during a 30 min period, showing different behaviours towards an introduced live ant of various origins. *n* stands for the number of replicate colonies tested.

Tableau I. — Nombre moyen (et déviation standard) de Fourmis observées durant une période de 30 minutes, montrant différents comportements envers une intruse vivante, d'origine diverse. *n* représente le nombre de colonies testées.

Type of the intruder ant	Alarm	Bites	Stingings	Antennation	<i>n</i>
From the same colony (control)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	30.5 ± 23.6	12
From a different conspecific colony	2.0 ± 1.5 a	7.4 ± 2.9 a	0.0 ± 0.0	23.6 ± 21.6	12
From a different <i>Camponotus</i> species	1.2 ± 1.0 a	12.5 ± 6.3 a	1.3 ± 1.1 a	13.2 ± 12.1	6
<i>Atta laevigata</i>	4.8 ± 2.2 a	15.2 ± 9.4 a	1.8 ± 0.6 a	27.0 ± 26.7	12

a indicates values which are significantly different ($\alpha = 0.05$) from the control (Mann-Whitney U-test).

bitten but not stung and workers contacting the intruder also became alarmed. Thus, the reaction towards interspecific intruders was much stronger than towards an intraspecific intruder from a foreign colony ($p < 0.01$, Mann-Whitney test comparing Nr of bites and Nr of stingings). Colonies maintained under the same diet and under the same environmental conditions in the laboratory for over 6 months always showed more biting and more alarm towards intraspecific intruders as compared to nestmates ($p < 0.01$, Wilcoxon's matched-pairs test, $n = 12$).

These experiments (*table I*) show that *C. rufipes* is able to recognize nestmates and differentiate them from conspecifics of different colonies. On the other hand, these results show that interspecific aggression is much more intense than intraspecific aggression, and that the diet or environmental factors do not decisively affect intraspecific aggression.

The source of the recognition signal

In order to find the source of the intraspecific recognition signal, the experiments in *table II* were performed. Here, conspecific ants were presented to *C. rufipes* colonies in different forms. Live ants and heads of ants dissected alive were bitten significantly more and elicited significantly more alarm behaviour when they were taken from a different colony as compared to nestmates. Gasters and thoraces of ants dissected alive were treated the same, independently of their colony of origin. Similar results were obtained when tests were carried out on dead ants (*table II*). We again observed that complete ants and heads of dead ants were treated differently, depending on the origin of the test ant, but not so thoraces and gasters.

In another test, headless ants and gasterless ants were presented to the colonies. The results (*table II*) show clearly that headless ants, i.e. ants with thorax, legs and gaster but without head, were treated the same, independently of their colony of origin, whereas gasterless ants, i.e. ants with head, thorax and legs but without gaster, elicited more alarm behaviour when they originated from a different conspecific colony. Headless experimental ants elicited 1.6 ± 1.3 sd bites against 0.3 ± 0.9 bites of headless controls ($p > 0.1$, Mann-Whitney U-test, $n = 8$), whereas gasterless experimental ants elicited 26.8 ± 11.2 bites against 3.6 ± 3.0 of the controls ($p < 0.005$, $n = 8$). Thus, we may confidently assume that the head is the important factor in nestmate recognition and not the form of presentation of the ant.

These results suggest that nestmate recognition is not based on movements or sound as dead ants are recognized as nestmates or aliens respectively. Thus, tactile or olfactory stimuli seem to be important. Experiments with freeze-dried ants (*table II*) show that they are not recognized, i.e. they are treated the same, independently of their colony of origin, suggesting that odours are the cues responsible for nestmate recognition.

Thus, cephalic odours are responsible for nestmate recognition. Since

Table II. — Probability (%) of observing a behaviour towards each of two *C. rufipes* workers or parts of them introduced simultaneously into a conspecific colony. C indicates introduced nestmates whereas E indicates workers from a different colony. n stands for the number of replicate colonies tested.

Tableau II. — Probabilité (%) d'observer un comportement envers deux ouvrières, ou parties d'ouvrières, introduites simultanément dans une société de même espèce. C : ouvrière du même nid. E : ouvrière provenant d'une société différente. n : nombre de sociétés testées.

Type of presentation	Bites			Alarm			n
	E	C	p	E	C	p	
Dissected alive							
Whole ant	100	0	< 0.001	83	8	< 0.001	12
Head	75	0	< 0.001	83	0	< 0.001	12
Thorax	42	8	NS	17	0	NS	12
Gaster	0	0	NS	17	8	NS	12
Dead ants							
Whole ant	50	0	NS	83	0	< 0.01	6
Head	100	0	< 0.005	67	0	< 0.05	6
Thorax	0	0	NS	0	0	NS	6
Gaster	0	0	NS	17	0	NS	6
Dissected alive							
Headless	50	13	NS	25	13	NS	8
Gasterless	100	88	NS	100	38	< 0.05	8
Freeze-dried ants	0	0	NS	0	0	NS	8

p indicates statistically significant differences between C and E using Fisher exact probability test. NS indicates non significant differences ($\alpha = 0.05$).

the main source of volatiles in the head is the mandibular gland, we tested the mandibular gland secretion, which is known to work as an alarm pheromone in these ants. The following experiment was performed. Two plastic dummies, one contaminated with mandibular secretion of nestmates (control), the other one contaminated with secretions of a different *C. rufipes* colony (experimental), were presented simultaneously to a colony. Both dummies released the same amount of alarm behaviour in the workers when presented to a colony ($p > 0.10$, Wilcoxon's matched pairs test, $n = 15$). When we counted the number of bites in 15 replicates, a mean of 1.2 ± 0.9 sd was observed for control dummies, whereas 3.5 ± 1.8 bites were observed for the experimental dummies in a one minute observation period. That is, experimental dummies were bitten significantly more often than control dummies ($p < 0.01$, Wilcoxon's matched pairs test). This result shows that the alarm pheromone has colony-specific properties, where alarm pheromones from ants from different colonies elicit significantly more aggressive behaviour than the pheromone from nestmates.

Territorial marking behaviour

When two workers from different conspecific colonies were placed into a petri dish containing a filter paper which had formerly been explored by one of the colonies (Bioassay I), the following behaviour was observed. Both ants tended to escape from the Petri dish, but nearly always one of the ants took longer to escape than the other. If we noted which ant was the first one to exit the dish we found the following results. In 25 replicates the first ant to escape was on 5 occasions the ant from the colony from which the paper was taken (resident) and on 20 occasions the ant from a different colony (intruder) ($p < 0.005$, Binomial test for one sample). In this experiment, the territories of each of 5 colonies were tested on 5 different occasions with ants of 5 different colonies (6 colonies in total), thus eliminating any possible effect due to differences in activity or aggressivity of the workers of the different colonies.

The experiments with Bioassay I showed that *C. rufipes* workers are able to differentiate substrates formerly explored by their colony from substrates explored by another colony. As both substrates were of the same kind, the only reasonable explanation for this behaviour is that the ants mark their territory with colony specific chemicals. This experiment also showed that for any worker, the probability of staying on its own territory is greater than the one on a foreign territory.

The bioassay just described had some methodological difficulties. The ants got very "excited" and alarmed, i.e. they ran very fast in an irregular pattern when they were taken from their colony and placed into the petri dish. Therefore a different bioassay was developed. In Bioassay II, territories, i.e. filter papers formerly explored during 24 h by one of the colonies, were

Table III. — Median number of ants showing different behaviours during a 10 min interval over two different filter papers presented simultaneously to a *C. rufipes* colony (Bioassay II). *Control* indicates papers which had been placed for 24 h in the foraging area of the colony where the test was performed. *Experimental* indicates papers placed on the foraging area of a different conspecific colony.

Tableau III. — Médiane du nombre de Fourmis montrant différents comportements, en 10 minutes, devant deux types de papier-filtre, présentés simultanément à une société de *C. rufipes* (Test de type II). *Control* indique un papier qui a été placé pendant 24 h dans la zone de récolte de la société où le test a été effectué. *Experimental* indique un papier placé dans l'aire de récolte d'une société différente, de même espèce.

n	Pairs of papers	Nr. of ants on the paper	Nr. of ants dipping the abdomen on the ground	Speed of a randomly selected ant on the paper (mean value in s/cm)
11	New paper	10.0	3.0	3.8
	Control	3.0	1.0	3.5
	p	< 0.05	< 0.05	NS x
11	Experimental	6.5	3.5	4.5
	Control	4.0	0.0	4.6
	p	< 0.01	< 0.01	NS x
12	Control after 2 h outside the nest	7.0	2.0	4.3
	Control	5.0	1.5	4.3
	p	NS	< 0.01	NS x
11	Control after 24 h outside the nest	12.0	3.0	5.3
	Control	7.0	1.0	6.1
	p	< 0.01	< 0.05	NS x

p indicates the two tailed probability ($\alpha = 0.05$) given by the Wilcoxon matched-pairs signed-ranks test, or if marked x by the Student's t-test.

placed into the foraging ground next to the nest entrance of a colony. The number of ants exploring the paper and the number of them dipping the abdomen on it were counted. The results are shown in *table III*. Here we see that new papers and papers formerly explored by a different colony (experimental) were explored more intensively than papers formerly explored by the same colony (control). No differences in the speed of movement of the ants on the different papers could be detected. When testing similar filter papers which were allowed to remain at room temperature for 2 or 24 h before the bioassay was performed, the results in *table III* were obtained. That is, differences between a recently explored control paper and a control paper left at room temperature for two hours could be detected. This difference was much greater between recently explored control papers and control papers left at room temperature for 24 h. This last difference was

not different to the one observed between a new paper and a recently explored control paper (*table III*) ($p > 0.70$, 2×2 Chi-square test).

The source of the territorial pheromone

In order to localize the source of the territorial pheromone, different extracts were tested using Bioassay II. The results (*table IV*) show that the territorial pheromone is produced in the gaster, possibly by the Dufour's gland and/or a tergal gland. All glands with the exception of the Dufour's gland could be dissected without submerging the gaster in Ringer solution. That is, all glands could be dissected directly under a stereoscopic microscope. In the case of the Dufour's gland, the gaster had to be submerged in Ringer solution in order to be able to find the gland. Thus, contamination with other secretions could not be avoided. Experiments with sternal glands dissected in Ringer solution gave the same results as those reported in *table IV*. This suggests that if contamination between exocrine glands exists when dissecting, it is still possible to observe the behavioural responses of a specific pheromone.

Table IV. — Median value of different behaviours shown by ants during a 10 min interval over two filter papers presented simultaneously to a *C. rufipes* colony. Bioassay II was used to measure the reaction of the workers towards papers impregnated with different extracts. *Control* papers are those impregnated with extracts from nestmates, whereas *Experimental* papers are those impregnated with extracts from ants of a different conspecific colony. Each value is the median of 24 replicates.

Tableau IV. — Médiane du nombre des différents comportements manifestés par des Fourmis en 10 minutes, devant deux types de papiers-filtre présentés simultanément à une société de *C. rufipes*. Le test de type II a été utilisé afin de mesurer la réaction des ouvrières vis-à-vis de papiers imprégnés de différents extraits. Les papiers *Control* sont ceux qui sont imprégnés d'extraits de Fourmis du même nid, alors que les papiers *Experimental* sont ceux imprégnés d'extraits d'ouvrières d'une société différente. Chaque valeur est la médiane des 24 colonies testées.

Extract	Nr. of ants on the paper			Nr. of ants dipping the abdomen on the ground		
	Control	Experimental	p	Control	Experimental	p
Second half of the gaster	8.0	15.0	< 0.02	2.0	5.0	< 0.02
Poison gland	10.0	7.5	NS	1.5	2.5	NS
Rectal sac	9.5	11.5	NS	1.5	3.0	NS
Thorax	9.0	9.5	NS	1.0	2.0	NS
Head	10.0	13.5	NS	1.5	3.0	NS
Dufour's gland	9.5	13.0	< 0.05	—	—	—
Last three sternal segments	12.0	11.0	NS	—	—	—
Last three tergal segments	9.0	15.0	< 0.01	—	—	—

p indicates the two tailed probability ($\alpha = 0.1$) of the two groups being equal (Wilcoxon matched-pairs signed-ranks test).

In all experiments reported in *table IV*, the concentration of the extracts was one ant equivalent per paper (one gaster, thorax or head was used to impregnate the paper). Higher concentrations of extract produced repulsion and alarm behaviour in the workers. Papers with one ant equivalent produced alarm only in the cases of poison gland and head extracts, whereas papers impregnated with two ant equivalents produced alarm also for the extracts of the sternal segments, tergal segments and Dufour's gland. Therefore, the concentration of one equivalent per paper was considered to be the highest pheromone concentration in which the normal behavioural effects could be observed.

DISCUSSION

The nestmate recognition system

The experiments suggest that odours from the mandibular gland play a significant role in nestmate recognition in *C. rufipes*. A similar system has been reported for *Atta cephalotes* (JAFJE, 1983) and for *Conomyrma sp.* (COLMENARES, 1982, JAFJE and COLMENARES, in preparation). In both cases, the ants use a cephalic pheromone as nestmate recognition signal, but no direct tests with the mandibular gland secretion were performed. The nestmate recognition system may be also influenced by other factors such as environmental odours, queen odours, etc., but no evidence for this has been found for *C. rufipes*. In spite of the lack of evidence, a secondary role in nestmate recognition for cues different from the mandibular alarm pheromone can not be completely excluded.

C. rufipes shows more aggression towards interspecific intruders as compared to conspecific foreign ants. This difference in aggression suggests that *C. rufipes* workers are able to recognize conspecifics and differentiate them from ants from different species. It is possible that the mandibular alarm pheromone of the different species gives the information required by *C. rufipes* workers in order to recognize them as aliens. In any case, the use of other cues in interspecific recognition can not be excluded.

The fact that *C. rufipes* uses an alarm pheromone as recognition signal suggests that inter-colony variations of the alarm pheromone should exist. Studies with different ant species showed that in spite of the existence of individual variations, inter-colony differences in the proportions of the different components of alarm pheromones can be detected (CAVILL and HINTENBERGER, 1960; BRADSHAW *et al.*, 1979; JAFJE, 1983; WINTERBOTTOM, 1982; JAFJE and MARCUSE, 1983). In the case of *C. rufipes*, gas chromatographic analysis of the alarm pheromone revealed the presence of more than 20 different compounds (JAFJE, unpublished observations). Thus, colony differences in the relative proportion of those compounds are to be expected. On the other hand, the findings of PROVOST (1979) in *Camponotus lateralis*,

who showed that these ants form closed societies where no mix between colonies was possible, suggest that these ants have genetically determined colony differences. We therefore propose that *C. rufipes* uses genetically determined colony differences in the proportion of volatiles in the mandibular alarm pheromone for nestmate recognition. This model would be equivalent to the "individualistic" model of CROZIER and DIZ (1979) and to that proposed for the bee *Lasioglossum zephyrum* (BARROWS *et al.*, 1975 ; BUCKLE and GREENBERG, 1981).

No definitive evidence for the other alternative models proposed by HÖLLDOBLER and MICHNER (1980) are known. JUTSUM *et al.* (1979) for example found in *Acromyrmex octospinosus* that both endogenous (genetic) and exogenous (environmental) factors contribute to the colony odour. If endogenous factors determine the colony odour even partially, they will play the major role in nestmate recognition as environmental differences in colonies living close together and thus competing between them, are not to be expected.

Territorial marking behaviour

Any behaviour which affects the probability of being successful in a defensive strategy or in the exploitation of the home range compared to other areas, and which is affected by some feature from the particular surrounding of a colony is related to territorial behaviour (see JAFFE and PUCHE, 1984). In this context we may postulate the existence of a territorial pheromone in *C. rufipes*.

Experiments on *table III and IV* show the existence of an odour cue on the substratum which regulates recruitment and/or exploratory behaviour in a colony-specific manner. Bioassay I showed that this odour cue has an effect on the time of permanence of workers on areas far from their nest. That is, a piece of territory with a chemical mark from a different colony induces increased exploration when presented to the whole colony near to their nest, but induces an increased escape response in solitary workers far from their nest. These results suggest that a territorial pheromone exists indeed, but that at least near to the nest, other cues, probably visual cues, play a role in territorial recognition. A similar situation has been described for the Formicinae *Ecophylla longinoda* (HÖLLDOBLER and WILSON, 1978).

The source of this territorial pheromone is not clear. We may postulate that the probable source of the territorial pheromone is a tergal and/or the Dufour's gland. Since no detailed study of the abdominal exocrine glands exist for *Camponotus*, a histological examination of the possible abdominal glands is needed before any definitive conclusion about the possible territorial pheromone secreting gland can be drawn. In any case, it is clear that the source is not the rectal sac as is the case in *O. longinoda*. All ants studied so far use different exocrine glands to produce the territorial pheromone. *Atta* uses the valves gland (JAFFE *et al.*, 1979 ; BAZIRE-BENAZET and ZYLBERBERG,

1979); *Myrmica* uses the Dufour's gland (CAMMAERTS *et al.*, 1977); *Solenopsis* the metapleural gland (JAFFE and PUCHE, 1984); and *Ecophylla* the rectal sac. Even species belonging to the same subfamily (*Ecophylla* and *Camponotus*; *Atta*, *Myrmica* and *Solenopsis*) differ in the exocrine glands used. This fact suggests that the use of exocrine secretions in marking the territory of a colony is new in ant phylogeny. We might expect that the absence of a common ancestor using exocrine secretions for territorial marking will have produced a great diversity of exocrine gland secretions used in territorial behaviour in the different species.

The relation between territorial marking behaviour and the nestmate recognition system

If we compare *C. rufipes* with other ant species whose nestmate recognition system and territorial behaviour is known we find that those ant species with territorial pheromones have a nestmate recognition system based on alarm pheromones. This is not the case in species which lack a territorial pheromone. *Atta cephalotes* (JAFFE, 1983), *Conomyrma sp.* (COLMENARES, 1982; JAFFE and COLMENARES, in preparation); *Solenopsis geminata* (PUCHE, 1982; JAFFE and PUCHE, 1984); and *C. rufipes* mark their territories with chemicals, advertising it to conspecifics from different colonies. These ants recognize their nestmates mainly through their alarm pheromones (JAFFE, 1985). In the case of *Odontomachus bauri* (JAFFE and MARCUSE, 1983) and of the Attini *Trachymyrmex urichi* (VILLEGAS, 1982; JAFFE and VILLEGAS, in preparation) no territorial pheromone seems to exist. These ants recognize their territories mainly using visual cues and/or environmental odours. The nestmate recognition system of these ants is based on non-specific intrinsic odours, i.e. probably the relative proportions of the different compounds of different pheromones of the workers serve as the recognition signal.

An interesting recognition system is that reported for *Neoponera apicalis* (FRESNEAU, 1980). In these ants, the recognition of an intruder seems to be based on the contrast between its own odour and the territorial odour of the colony. This would represent a situation which is intermediate between the two systems described above.

In the light of these findings we propose that ants with no intraspecific defence mechanism through advertisement of their defended area need a nestmate recognition system which allows immediate recognition of alien conspecifics. The probability of meeting an intruder in an unadvertised area is high, and thus efficient nestmate recognition mechanisms are required. In the case of species marking their territory with chemicals, the probability of a foreign conspecific ant appearing in the defended area of a colony is reduced due to the advertising of the territory. Thus, detection of foreign ants and recognition of nestmates is required only in intraspecific combats, where alarm pheromones play a major role in triggering the attack and

organizing the combat (WILSON, 1971; BRADSHAW *et al.*, 1975). Therefore, the same alarm pheromone seems to be the most appropriate signal for nestmate recognition. It is also curious to note that ants entering a foreign marked territory generally become alarmed, releasing alarm pheromones and thus denouncing themselves as intruders (personal observations).

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References

- AYRE G.L., BLUM M.S., 1971. — Attraction and alarm of ants (*Camponotus* spp. Hymenoptera: Formicidae) by pheromones. *Physiol. Zool.*, 44, 77-83.
- BARROWS E.M., BELL W.J., MICHENER C.D., 1975. — Individual odor differences and their social function in insects. *Proc. Nat. Acad. Sci. U.S.A.*, 72, 2824-2828.
- BAZIRE-BENAZET M., ZYLBERBERG L., 1979. — An integumentary gland secreting a territorial marking pheromone in *Atta* sp.; morphology, histochemistry and ultrastructure. *J. Insect Physiol.*, 25, 751-765.
- BERGSTRÖM G., LÖFQVIST J., 1971. — *Camponotus ligniperda* Latr. A model for the composite volatile secretions of Dufour's gland in formicine ants. *Chemical Releasers in Insects* (A.S. Tahori ed.). *Proc. 2nd Int I.U.P.A.C. Congr. Pest. Chem. Tel Aviv*, 3, 195-223. Gordon & Breach, N.Y.
- BERGSTRÖM G., LÖFQVIST J., 1972. — Similarities between the Dufour gland secretions of the ants *Camponotus ligniperda* (Latr.) and *Camponotus herculeanus* (L.). *Entomol. Scandinavica*, 3, 225-238.
- BRADSHAW J.W.S., BAKER R., HOWSE P.E., 1975. — Multicomponent alarm pheromone of the weaver ant. *Nature*, 258, 230-231.
- BRADSHAW J.W.S., BAKER R., HOWSE P.E., HIGGS M.D., 1979. — Caste and colony variations in the chemical composition of the cephalic secretions of the African weaver ant, *Cecophylla longinoda*. *Physiol. Entomol.*, 4, 27-38.
- BROPHY J.J., CAVILL G.W.K., SHANNON J.S., 1973. — Venom and Dufour's gland secretions of an Australian species of *Camponotus*. *J. Insect Physiol.*, 19, 791-798.
- BUCKLE G.R., GREENBERG L., 1981. — Nestmate recognition in sweat bees (*Lasioglossum zephyrum*): does an individual recognize its own odour or only odours of its nestmates? *Anim. Behav.*, 29, 802-809.
- CAMMAERTS M.C., MORGAN E.D., TYLER R., 1977. — Territorial marking in the ant *Myrmica rubra* L. *Biol. Behav.*, 2, 263-272.
- CAVILL G.W.K., HINTENBERGER H., 1960. — The chemistry of ants IV-Terpenoid constituents of some *Dolichoderus* and *Tridomyrmex* species. *Australian J. Chem.*, 13, 514-519.
- COLMENARES O., 1982. — Estudio del comportamiento de reclutamiento, reconocimiento individual y territorialidad en una hormiga perteneciente a la subfamilia Dolichoderinae. *Tesis de Licenciatura*, Universidad Simón Bolívar, Caracas.
- CROZIER R.H., DIX M.W., 1979. — Analysis of two genetic models for the innate components of colony odor in social hymenoptera. *Behav. Ecol. Sociobiol.*, 4, 217-224.
- FRESNEAU D., 1980. — Fermeture des sociétés et marquage territorial chez des fourmis ponérines du genre *Neoponera*. *Biol. Ecol. Méditerranéenne*, 7 (3), 205-206.
- HANGARTNER W., REICHSON J.M., WILSON E.O., 1970. — Orientation to nest-materials by the ant *Pogonomyrmex badius*. *Anim. Behav.*, 18, 331-334.
- HASKINS C.P., HEWITT R.E., HASKINS E.F., 1973. — Release of aggressive and capture behaviour in the ant *Myrmecia gulosa* F. by exocrine products of the ant *Camponotus*. *J. Entomol. A*, 47, 125-139.

- HÖLLDOBLER B., 1976. — Recruitment behavior, home range orientation and territoriality in harvester ant *Pogonomyrmex*. *Behav. Ecol. Sociobiol.*, 1, 3-44.
- HÖLLDOBLER B., 1979. — Territoriality in ants. *Proc. Am. Philos. Soc.*,
- HÖLLDOBLER B., MICHENER C.D., 1980. — Mechanism of identification and discrimination in social hymenoptera. In *Evolution of Social Behavior*, H. Markl ed., Weinheim, Verlag Chemie GmbH, p. 35-58.
- HÖLLDOBLER B., WILSON E.O., 1977. — Colony specific territorial pheromone in the African weaver ant. *Proc. Nat. Acad. Sci. U.S.A.*, 74, 2072-2075.
- HÖLLDOBLER B., WILSON E.O., 1978. — The multiple recruitment system of the African weaver ant *Cecophylla longinoda*. *Behav. Ecol. Sociobiol.*, 3, 19-60.
- HOWSE P.E., 1975. — Pheromones and defensive secretions in social insects. *Proc. VII Congr. I.U.S.S.I.*, Dijon.
- JAFFE K., 1982. — Nestmate recognition systems in the Formicidae. The Biology of Social Insects, Breed, Michener & Evans eds. *Proc. IX Congr. I.U.S.S.I.*, Boulder, 1982.
- JAFFE K., 1983. — Chemical communication among workers of the leaf-cutting ant *Atta cephalotes*. Social Insects in the Tropics, P. Jaisson ed, Université Paris-Nord. *Proc. I Symp. I.U.S.S.I. on social insects in the tropics*, Mexico, 1980.
- JAFFE K., 1985. — *Evolucion de los sistemas de comunicacion quimicos en hormigas*. Folia Entomologica Mexicana (in press).
- JAFFE K., BAZIRE-BENAZET M., HOWSE P.E., 1979. — An integumentary pheromone secreting gland in *Atta sp.*: Territorial marking with a colony-specific pheromone in *Atta cephalotes*. *J. Insect Physiol.*, 25, 833-839.
- JAFFE K., MARCUSE M., 1983. — On nestmate recognition and territorial behaviour in the ant *Odontomachus bauri* (Emery) (Formicidae: Ponerinae). *Insect. Soc.*, 30, 466-481.
- JAFFE K., PUCHE H., 1984. — Colony-specific territorial marking with the metapleural gland secretion in the ant *Solenopsis geminata* (Fabr.). *J. Insect Physiol.*, 30, 265-270.
- JAFFE K., SANCHEZ C., 1984. — Comportamiento alimentario y sistema de reclutamiento en la hormiga *Camponotus rufipes* (Hymenoptera: Formicidae). *Acta Cientifica Venezolana*, 35, 270-277.
- JUTSUM A.R., SAUNDERS T.S., CHERRETT J.M., 1979. — Intraspecific aggression in the leaf-cutting ant *Acromyrmex octospinosus*. *Anim. Behav.*, 27, 839-844.
- LONGHURST C., 1977. — Behavioural, chemical and ecological interactions between West-African ants and termites. *Ph. D. Thesis*, University of Southampton, 1978.
- PARRY K., MORGAN E.D., 1979. — Pheromones of ants: a review. *Physiol. Entomol.*, 4, 161-189.
- PROVOST E., 1979. — Etude de la fermeture de la société de fourmis chez diverses espèces de *Leptothorax* et chez *Camponotus lateralis*. *C.R. Acad. Sci. Paris*, 288, 429-432.
- PUCHE H., 1982. — Algunos aspectos del comportamiento en la hormiga *Solenopsis geminata*. *Tesis de Licenciatura*, Universidad Simón Bolívar, Caracas.
- SANCHEZ C., 1982. — Estudio del comportamiento agonístico en hormigas del genero *Camponotus*. *Tesis de Licenciatura*, Universidad Simón Bolívar, Caracas.
- SIEGEL S., 1956. — *Nonparametric statistics for the behavioral sciences*. McGraw-Hill Kogakusha Ltd, 312 p.
- SKINNER G.J., 1980. — Territory, trail structure and activity patterns in the wood-ant *Formica rufa* in limestone woodland in north-west England. *J. Anim. Ecol.*, 49, 381-394.
- VILLEGAS G.J., 1982. — Estudio comparativo de algunos aspectos del comportamiento social de hormigas de los generos *Trachymyrmex*, *Cyphomyrmex* y *Myrmecocripta*. *Tesis de Licenciatura*, Universidad Central de Venezuela, Caracas.
- WILSON E.O., 1971. — *The insect societies*. Belknap Press, Harvard University, Cambridge, Mass., U.S.A.
- WILSON N.L., DILLER J.H., MARKIN G.P., 1971. — Foraging territories of imported fire ants. *Ann. Entomol. Soc. Am.*, 64, 660-665.
- WINTERBOTTOM S., 1982. — The chemical basis for species and colony recognition in three species of myrmicine ants. *Ph. D. Thesis*, University of Southampton, England.