NESTMATE RECOGNITION

It has been known for a long time that ants recognize and differentiate their nestmates from alien ants of conspecific or heterospecific colonies mainly on the basis of chemical cues. Species-specific recognition of queens has been reported for Pogonomyrmex badius (Hölldobler 1976), Solenopsis invicta (Glancey et al. 1981 and Chapter 20), Solenopsis spp. (Jouvenaz et al. 1974), and Neivamyrmex spp. and Labidus spp. (Watkins and Cole 1966). In these cases, a queen pheromone was reported to induce the recognition signal.

Contact pheromones appear to be responsible for brood recognition. Examples can be found in S. invicta (Walsh and Tschinkel 1974), Myrmica spp. (Brian 1975), Atta cephalotes (Robinson and Cherrett 1974), and Formica spp. (Jalison 1975; Jalison and Fresneau 1978; Le Moêl and Passetti 1977, 1978; Le Moêl and Mori 1982). Ants learn the chemical signal shortly after eclosion with a type of imprinting mechanism (see references for Formica spp. above; Isingrini and Lenoir 1984). Thus, ants that eclose to adults among foreign brood may recognize the foreign brood as nestmates and treat brood from their original colony as aliens.

Another example of chemically-based recognition is found in the necrophoric behaviour of ants. Examples include P. badius (Wilson et al. 1958), S. saevissima and S. invicta (Blum 1970; Howard and Tschinkel 1978), Myrmecia vindex (Haskins and Haskins 1974), and Monomorium pharaonis (Legakis 1979). In the case of Solenopsis, myristic acid, palmitic acid, oleic acid, and linoleic acid seem to be the recognition cues. Triethanolamine is active as a recognition signal in dead Myrmecia, and oleic acid is active in Pogonomyrmex. Oleic acid induces waste recollection but not necrophoric behaviour in Monomorium.
Nestmate recognition among adult workers has been studied less. Odours have always been assumed to be responsible for this recognition (Forel 1874; Fieide 1904; Howse 1975; Holldobler and Michener 1980). However, experimental evidence for this appeared only recently. In Megaponera foetens (Longhurst 1977), it was shown that dummies with cuticular waxes from nestmates were accepted into the colony or attacked much less than dummies with waxes from conspecific aliens. In the Ponerinae Odontomachus bauri (Jaffe and Marcuse 1983), and Ectatomma ruidum (Marquez 1984), it was shown that volatile substances produced by workers in the different body parts are used as recognition signals. These volatiles are absorbed on the cuticle and thus can be active in nestmate recognition as both contact and volatile pheromones. The Formicinae Camponotus rufipes (Jaffe and Sanchez 1984) uses the cephalic alarm pheromone as a nestmate recognition signal, the same as the Myrmicinae A. cephalotes (Jaffe 1983). Crematogaster sumichrasti (Pino 1984) utilizes cephalic and abdominal alarm pheromones as nestmate recognition signals as do some of the Dolichoderinae (Colmenares 1982; Alvarez 1984; Jaffe et al., unpublished). In the Pseudomyrmicinae, we find examples of both; e.g., Pseudomyrmex triplarinus uses the alarm pheromone as a recognition signal (Aragort 1984), whereas P. termitarius (Lopez 1984) uses non-specific, intrinsically produced odours, the same as the Ponerinae, for nestmate recognition.

As with brood recognition, adult nestmate recognition appears to be achieved through an imprinting process (Le Moli and Passetti 1977; Jaisson 1980; Le Moli and Mori 1984); i.e., ants that eclose in an environment of adult workers will recognize them as nestmates, even if they are from a different species. Although this relation has been studied most in slave ants and their slave makers, this seems to be a more or less general phenomenon in the Formicidae (Le Masne 1952; Provost 1979; Jaisson 1980).

The Nestmate Recognition System of Solenopsis geminata and Some Attini

Two types of bioassays have been used to study nestmate recognition. In the first, two workers were drawn at random, one from the nest where the test was to be performed (control), and the other from a different conspecific colony (experimental). The workers were picked up with clean plastic forceps and placed at random on the foraging area of the control colony. The behaviour of the resident workers towards the intruders was described verbally on a tape recorder for later quantification. If statistical differences could be detected in the response of ants of the resident colony towards either the experimental or control ants for any particular behaviour, colony-specific recognition was presumed to exist.
The data from these tests (Table 1) revealed that most species differentiated between their nestmates and the intruder, although aggression towards the latter was not very strong in *Acromyrmex landolti* and *Ac. octospinosus*. The lower Attini did not exhibit nestmate recognition, but this may be because disturbance of the nests is always too intense and thus no appropriate behavioural variables can be used to measure recognition.

In the second bioassay, dead workers (killed by freezing in dry ice) or parts of them were placed near the entrance to the foraging area of the colonies. Two workers or worker parts were always presented simultaneously to the colonies. One of them was a nestmate from the test colony, whereas, the other came from a different conspecific colony. The time resident workers took before removing each of the dead workers or worker parts was measured.

In other tests, dead workers were freeze-dried by exposing them at pressures of 0.001 mm Hg at -20°C using a vacuum pump. The freeze-dried workers were impregnated with odours from different body parts of other workers by placing them together in a 2 ml glass vial with freshly crushed body parts. Care was taken in order to avoid direct contact between the freeze-dried ants and the crushed body parts. The vial was sealed and left at room temperature for 30 minutes. After this period, the freeze-dried ants were taken out and used for tests. Statistically significant differences in any behaviour of the resident workers towards live ants, bodies or body parts of dead ants, and/or impregnated freeze-dried ants were taken as evidence for colony-specific recognition. As shown in Table 2, there are a number of possibilities in Attini nestmate recognition systems. The primitive Attini, *Trachymyrmex urichi*, use non-specific odours absorbed on the cuticle for nestmate recognition, *Acromyrmex* use both alarm pheromones and environmental odours (Jütsum et al. 1979), and *Atta* use only the alarm pheromone. *Solenopsis* seems to be located just under *Atta* in this gradient, using various cues, but all are alarm pheromones (Püche 1982).

**TERRITORIAL BEHAVIOR**

A territory, as defined by Holldobler and Wilson (1977), is "an area occupied more or less exclusively by an animal by means of overt defense or advertisement." Four operational criteria for defining territorial marking pheromones (Jaffe and Püche 1984) are as follows:

1. The organism must secrete a chemical(s) onto the substrate of a portion of the home range.

2. The organism has to recognize its own mark and has to differentiate it from marks of conspecifics. Therefore, the chemical(s) must have intraspecific differences detectable by the organism.
TABLE 1. Results of nestmate recognition tests with conspecific colonies of several species of Atta and S. geminata.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Species</th>
<th>Intraspecific aggression</th>
<th>Colony specific behaviour indicated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atta</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cephalotes laevigata</td>
<td>++</td>
<td>x</td>
<td>Jaffe 1983; Vilela 1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Naccarata 1983; Vilela 1983</td>
</tr>
<tr>
<td>Acromyrmex octospinosus</td>
<td>+</td>
<td>x</td>
<td>Jaffe 1980; Jutsum et al. 1979</td>
</tr>
<tr>
<td>landolti</td>
<td>+</td>
<td>x</td>
<td>Navarro 1983; Jaffe and Navarro, in press</td>
</tr>
<tr>
<td>Trachymyrmex urichi</td>
<td>–</td>
<td>x</td>
<td>Jaffe and Villegas, in press</td>
</tr>
<tr>
<td>Mycocepurus sp.</td>
<td>–</td>
<td>–</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Myrmicorypta sp.</td>
<td>–</td>
<td>–</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Mycetophylax sp.</td>
<td>–</td>
<td>–</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td><strong>Solenopsis geminata</strong></td>
<td>++</td>
<td>x</td>
<td>Puche 1982; Jaffe and Puche 1984</td>
</tr>
</tbody>
</table>

\textsuperscript{a}++: very aggressive; +: occasionally aggressive; x: behaviour present.
TABLE 2. Results of bioassays for detection of nestmate recognition.

<table>
<thead>
<tr>
<th>Species</th>
<th>Source of pheromone inducing colony-specific behaviour</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Head</td>
<td>Thorax</td>
</tr>
<tr>
<td>Atta cephalotes</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>laevigata</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Acromyrmex</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>octospinosus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>landolti</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Trachymyrmex</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>urichi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solenopsis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>geminata</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = behaviour present

3. The presence of a territorial signal has to give some advantage to the organism producing the signal.

4. An area marked with a territorial pheromone should be respected by conspecifics. This is a sufficient, but not necessary, condition for territoriality. Criterion 3, for example, could be fulfilled without criterion 4.

The end result of territorial marking should be a reduction in aggressive interactions between the organisms because colonies are separated more than would be expected from a random occupation of suitable habitats.

The subject of territorial behaviour in ants has been partially reviewed by Baroni-Urbani (1979) and Holldobler (1979). Evidence for territoriality has been reported for a number of species, including P. badius (Holldobler 1976), Formica rufa (Skinner 1980), and S. saevissima (Wilson et al. 1971). The gland secreting the territorial pheromone varies for each species. Thus, the rectal sac secretes the territorial mark in Oecophylla longinoda (Holldobler and Wilson 1977) and the Dufour's gland in Myrmica ants (Cammaerts et al. 1977, 1978, 1981). An abdominal gland, other than the rectal
sac, Dufour's gland, or poison gland, is suspected as the pheromone source in the Formicinae, C. rufipes (Jaffe and Sanchez 1984). The metapleural gland is used by two species of Pseudomyrmicinæ (Lopez 1984; Aragort 1984), as well as the Dolichoderinae Azteca forelji (Alvarez 1984) and the Myrmicinæ Crematogaster sumichrasti (Fino 1984). An abdominal gland is suspected as the source of a territorial marker in a Conomyrma sp. (Colmenares 1982) of the subfamily Dolichoderinae.

Studies of Territorial Behaviour

Two approaches have been used to demonstrate the presence of territorial behaviour. In the first, petri dishes lined with 9-cm diameter filter papers were placed for 48 hours on the wooden foraging table of the colonies. For the test, the dish and filter paper were removed from the foraging table and a test was conducted by placing two workers, chosen at random from two different colonies, on the contaminated or a clean piece of filter paper. Observations were made as to which ant initiated an attack and the time each ant took in leaving the dish. Any statistically significant colony-specific differences were attributed to the existence of a colony-specific mark(s) on the paper. If the same results were obtained with a specific extract of workers, it was taken as evidence of territorial marking.

In case no evidence of territorial recognition was noted, a second bioessay was performed. In this case, every effort was made to keep topographical features of the test areas identical. For example, objects, such as glass nests and petri dishes with water or food, as well as the placement of the wooden tables in the laboratory, were the same for the different test colonies (at least from the experimenter's point of view). Two fixed visual designs were employed. Workers were taken at random from colonies with each design and were released in their colony of origin (control) or in conspecific colonies with either design (experimental). The number of ants going to the nest, and the time taken for it was measured. If no statistically significant difference could be detected between the control and the experimental colonies with the same design, but differences were detected when the control and the experimental had different designs, then visual signals were suspected as having an influence on territorial recognition.

The data (Table 3) show that Atta and Acromyrmex mark their territory with a pheromone, but other Attini do not. S. geminata also marks its territory. The source of the territorial pheromone in Atta is the valves gland (Bazire-Benazet and Zylberberg 1979; Jaffe et al. 1979); whereas in Solenopsis it is produced by the metathoracic gland (Jaffe and Puche 1984). T. urichi recognizes its
territory only through visual cues.

TABLE 3. Results of bioassays for detection of territorial marking and recognition.

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony specific</th>
<th>Recognition</th>
<th>Marking</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atta cephalotes</td>
<td>territorial</td>
<td>++</td>
<td>x</td>
<td>Jaffe et al. 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Naccarata 1983;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vilela 1983</td>
</tr>
<tr>
<td>Acromyrmex</td>
<td>octospinosus</td>
<td>++</td>
<td>x</td>
<td>Jaffe 1980</td>
</tr>
<tr>
<td></td>
<td>landolti</td>
<td>++</td>
<td>x</td>
<td>Navarro 1983</td>
</tr>
<tr>
<td>Trachymyrmex urichi</td>
<td></td>
<td>#</td>
<td>-</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Myrmicocrypta sp.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Mycocepurus sp.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Mycetophylax sp.</td>
<td></td>
<td>-</td>
<td>-</td>
<td>Villegas 1982</td>
</tr>
<tr>
<td>Solenopsis geminata</td>
<td></td>
<td>++</td>
<td>x</td>
<td>Jaffe and Puche 1984</td>
</tr>
</tbody>
</table>

Symbols under recognition column indicate that either odour (++) or visual (#) cues are the main sources of territorial recognition; x indicates the presence of marking behaviour.

CONCLUSION

Nestmate recognition systems and territorial behaviour seem to be very similar among the different Formicidae. In the advanced Attini (Atta and Acromyrmex) and in S. geminata, nestmate recognition is based on alarm pheromones (abdominal and cephalic in the case of S. geminata and cephalic only in the case of the higher Attini). All of these species mark their territory chemically. Species not marking their territory, such as T. urichi, have a nestmate recognition system based on non-specific odours and territorial behaviour based on visual cues. The very primitive species of Attini, Mycetophylax, Myrmicocrypta, and Mycocepurus, lack both.
Evolution of nestmate recognition seems to be linked to territorial behaviour (Jaffe and Marcuse 1983; Jaffe and Sanchez 1984). Three distinct steps are recognized in this evolution:

1. Non-specific odours, intrinsic and environmental, are used for nestmate recognition and no territorial recognition through odours exists.

2. Non-specific intrinsic ant odours are used as nestmate recognition signals; and, in some cases, the same odours, secreted on the substrate, are used as territorial recognition signals (Fresneau 1980).

3. Alarm pheromones serve as nestmate recognition signals. Territorial pheromones are secreted onto the substrate.

Environmental odours present on the cuticle and used for nestmate recognition are possible in any of the above steps.

Thus, evolution tends to simplify the recognition signals; i.e., reduce the number of signals involved and at the same time give them a more specific meaning or function. This would indicate that the more evolved species, such as Atta and Solenopsis must have simple nestmate recognition signals. The relative concentrations of a few compounds of the alarm pheromone complex should be enough to give a colony specific signal (Crozier and Dix 1979), such as described for A. cephalotes (Jaffe 1983).

The territorial mark is time dependent. This means that areas which are not intensively used lose the chemical mark on it (Jaffe et al. 1979; Jaffe and Puche 1984). This suggests that the territories of these ants are variable in space and time, depending on the exploitation of the resources. A permanent partition of space can occur in stable habitats, such as those reported for S. saevissima (Wilson et al. 1971), but are not likely to occur for the Attini, as the resources vary in space and time (Cherrett 1980).

REFERENCES CITED


