Enemy Specification in the Alarm-Recruitment System of an Ant

Abstract. *Many kinds of ants use odor trails to recruit nestmates to food discoveries and new nest sites; minor workers of the myrmicine* Pheidole dentata *also use them to recruit major workers ("soldiers") to the vicinity of intruders. This newly discovered alarm-recruitment system has proved to be narrowly specific. Only fire ants and other members of the genus* Solenopsis, *which include some of the potentially most dangerous enemies of* Pheidole, *have been found to evoke the response when present as single scouts or small invading forces. The glandular source of the* Pheidole *trail pheromone and the cues by which* Pheidole *recognize* Solenopsis *have been experimentally determined.*

The employment of odor trails in the recruitment of nestmates to food discoveries and new nest sites has been thoroughly documented in many kinds of ants. This report describes one of the first cases of their use in defense against intruders, a process corresponding to the alarm-recruitment system of termites (*1*). Furthermore, it presents evidence of strong differences among the kinds of intruder in the intensity of stimulus required to activate the response, a variation which in effect constitutes specification of the enemy in colony defense.

Pheidole dentata is a medium-sized myrmicine ant which is abundant in woodland over most of the southern United States (*2*). Like other members of its genus, it is characterized by the sharp division of the worker caste into small-headed minor workers, which conduct the foraging and most of the other quotidian tasks of the colony, and large-headed major workers, or "soldiers" as they are often called, which function primarily in defense. *Pheidole dentata* is an especially favorable laboratory species because of the ease with which it can be collected and cultured (*3*). When workers of the native fire ant *Solenopsis geminata* are placed within 25 cm of a laboratory *Pheidole dentata* colony, they are soon encountered by the foraging *Pheidole* minor workers. Some of the foragers grapple with the intruders, while others flee momentarily and run in irregular circles through the surrounding area. Within several minutes, some of the minor workers run back to the nest, dragging the tips of their abdomens over the ground. The trail thus deposited attracts both minor and major workers from the nest in the direction of the invaders. The trail pheromone comes from the poison gland and is emitted through the sting. Extracts made from whole bodies of minor workers and from their poison glands caused typical following when laid in artificial trails, but extracts from other body parts were inactive. The major workers have never been seen to lay trails. Their poison glands are anatomically very different from those of the minors, and no part of their body contains a trail pheromone. Thus the communication is unilateral (*4*).

Upon arriving at the battle scene the major workers become highly excited, snapping at the fire ants with their powerful mandibles and soon chopping them to pieces. The recruited minor workers also join the fighting, but they are less persistent and remain in the area for much shorter periods of time. As a result the majors increase in proportion, and for all but the most transient invasions they eventually come to outnumber the minors, despite the fact that they constitute only 8 to 20 percent of the worker population in the great majority of nests. The majors remain in the battle area for an hour or more after the last *Solenopsis* has been dispatched, restlessly patrolling back and forth. Often a single *Solenopsis* worker is enough to evoke the full response, which brings ten or more *Pheidole* majors into the field. Under natural conditions such an "overreaction" can be construed as adaptive: it results in the elimination of scout workers before a serious invasion can be organized by an enemy colony.

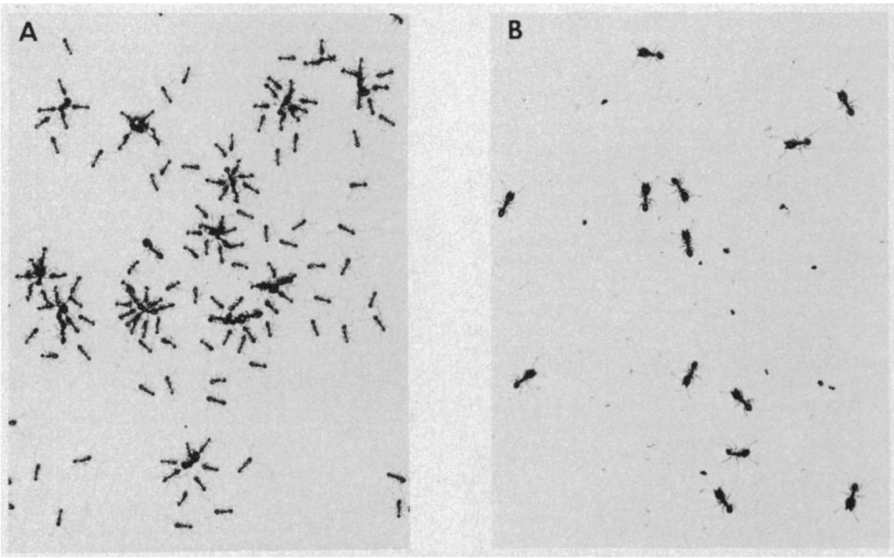

Fig. 1. Different modes of defense by the ant *Pheidole dentata* against two species of invading ants. (A) Workers of *Tetramorium caespitum* are pinioned and disabled mostly by *Pheidole* minor workers, which have not recruited major workers to their aid. The few majors present were stragglers already present in the area. (B) In response to the presence of the same number of workers of the fire ant *Solenopsis geminata*, the *Pheidole* minor workers have recruited large-headed major workers, which have already destroyed the invaders and are patrolling the battle area. The scene has been largely abandoned by the minor workers.

Five additional species of *Solenopsis* tested in the laboratory were all found to evoke alarm-recruitment in small numbers. These are the red imported fire ant *S. invicta*, the native fire ant *S. xyloni*, the European thief ant *S. fugax*, *S. molesta*, and *S. picta*; the last three forms are all members of the subgenus *Diplorhoptrum*, as distinct from the fire ants of the subgenus *Solenopsis*, and they are among the smallest of all ants. Their effectiveness in producing the response is thus even more impressive (*5*). All of the 16 other ant species tested thus far, representing 11 nonsolenopsidine genera and four of the eight living subfamilies, have not proved effective at comparable numbers (see Table 1 and Fig. 1). The one possible exception is *Pogonomyrmex badius*, which is ineffective when the number of invaders is three but evokes the response when the number is increased to ten. But compared to the *Solenopsis* and most of the other ant species tested, *P. badius* is gigantic; a single minor worker weighs about 6.88 mg, compared with 0.33 mg in the case of *Pheidole dentata*, 0.38 mg in *Solenopsis geminata*, and only 0.06 mg in *S. molesta*. There is possibly a level at which any ant species can cause the alarm-recruitment response. One hundred minor workers from alien colonies of *Pheidole dentata* were sufficient to cause it in two *P. dentata* colonies tested, as were 100 *Tetramorium caespitum*. However, 100 workers of *Crematogaster minutissima* still failed to evoke the response. Hence, in general, the number of nonsolenopsidine ants comparable in size to *Pheidole dentata* and *Solenopsis geminata* required to evoke the response is more than ten times that of *S. geminata*.

A more detailed comparison of the effects of two species is presented in Fig. 2. It can be seen that a single fire ant worker causes recruitment, and ten workers substantial recruitment, but 100 of these invaders reduces the magnitude of the response. The reason for the latter decline is the tendency of *Pheidole* minor workers to flee without laying odor trails when confronted by a large force of fire ants. When presented with truly overwhelming numbers, the *Pheidole* colonies suddenly abscond, abandoning the nest premises in a panicked retreat. Thus, colony defense consists of three phases initiated in sequence by an increasing magnitude of the challenge: (i) destruction of scouts and small enemy forces well away from the nest entrances; (ii) reduction of the defense perimeter so that fighting occurs closer to the nest until the enemy is eliminated by attrition; and (iii) absconding. Much greater numbers of alien *Pheidole* workers are required to induce even the first two phases of this defensive sequence.

In order to determine whether the response specificity is a widespread phenomenon, 15 colonies collected at four localities in Leon and Wakulla counties, northern Florida, were challenged at successive intervals with ten minor workers of *Solenopsis geminata* and ten minor workers from alien colonies of *Pheidole dentata*. All of the colonies responded positively to the *Solenopsis*, while only two were activated by the alien *Pheidole*. The latter two colonies recruited many more major workers to the *Solenopsis* than to the *Pheidole*.

An effort has been made to identify the cues by which *Pheidole* recognize *Solenopsis*. Using the same bioassay as before (*5*), it was found that freshly killed *S. geminata* are ineffective, even in large numbers, and whether intact or crushed. Nor do steel forceps and wooden dummies cause the response, even when violently agitated among the *Pheidole* foragers—even to the extent of crippling and killing some of them. But single, freshly killed *S. geminata* workers are effective when held in forceps and agitated. The abdomen alone is more effective than the head or the mesosoma alone. Wooden dummies treated with *S. geminata* venom and agitated cause the response, but so do live *S. geminata* workers whose abdomens (and hence venom) have been removed. Thus either contact with venom or the odor of the body surface is sufficient in itself, provided the chemical stimulus is associated with movement (*4*).

The following explanation of the specificity is suggested. The native fire ant *Solenopsis geminata* occurs in many of the same habitats and to some extent utilizes the same nest sites as *Pheidole dentata*. It

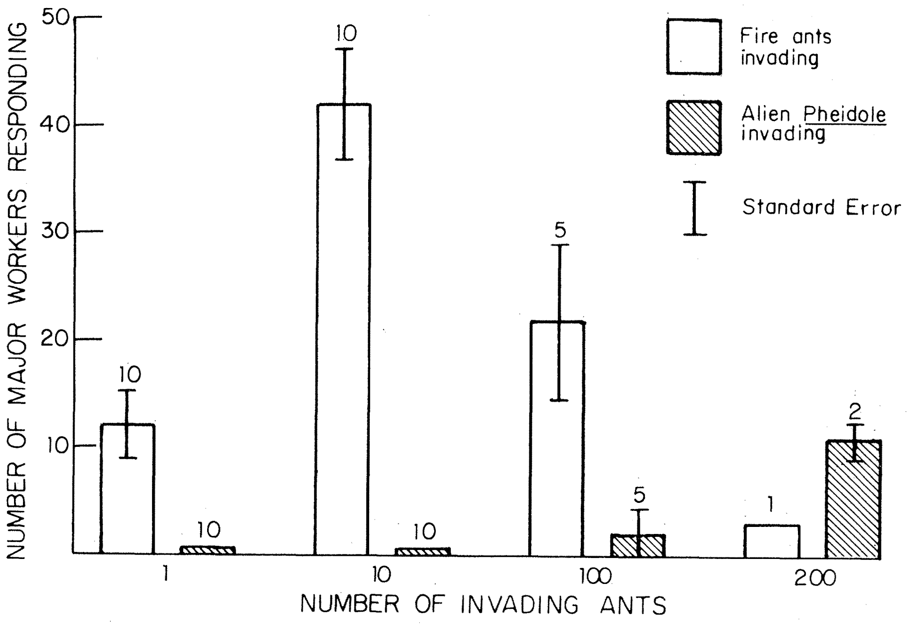

Fig. 2. Responses of a single *P. dentata* colony to increasing numbers of fire ants (*S. geminata*) and minor workers of *P. dentata* from another colony. The number of replications is given at the top of each bar. A second trial with 200 fire ant workers, not indicated here, resulted in an absconding by most of the *Pheidole* colony.

Table 1. Effectiveness of 20 ant species in evoking the alarm-recruitment response of *Pheidole dentata*. A positive response was recorded when major workers of *P. dentata* were recruited by the minor workers. Also shown is the change in the number of majors in the battle area (*5*).

Species	Invading workers (*N*)	Replications (*N*)	Positive responses (*N*)	Change in number of majors in battle area	
				Range	Mean
Subfamily Ponerinae					
Leptogenys manni	1 or 2	5	0	–2 to 0	–1
Subfamily Pseudomyrmecinae					
Pseudomyrmex elongatus	10	5	0	–1 to 1	0
Subfamily Myrmicinae					
Pogonomyrmex badius	3	5	0	–1 to 2	1
P. badius	10	1	1	41	
P. occidentalis	2	5	0	–1 to 3	1
Tetramorium caespitum	10	5	0	0 to 2	1
T. caespitum	100	1	1	15	
Atta sexdens, minor workers	10	5	0	0 to 1	0
Zacryptocerus varians, minors + majors	10	5	0	–2 to 2	0
Pheidole dentata	1	18	0	–2 to 5	0
P. dentata	10	26	4	–3 to 11	2
P. dentata	100	6	3	–3 to 63	17
P. dentata	200	4	4	10 to 57	32
P. desertorum	10	1	0	2	
P. floridana	10	3	0	0 to 1	1
Xenomyrmex floridanus	10	5	0	–1 to 3	1
Crematogaster atkinsoni	10	1	0	0	
C. minutissima	10	5	0	0	
C. minutissima	100	1	0	3	
Solenopsis fugax	1	5	2	–1 to 8	2
S. fugax	10	5	5	7 to 12	11
S. geminata	1	24	19	–1 to 37	9
S. geminata	10	33	33	3 to 72	27
S. geminata	100	6	6	4 to 31	20
S. geminata	200	2	2	3 to 10	6
S. molesta	10	5	4	1 to 32	11
S. picta	10	5	2	–1 to 10	3
S. xyloni	10	5	5	3 to 68	41
Subfamily Formicinae					
Brachymyrmex depilis	10	1	0	1	
Camponotus fraxinicola	10	3	0	0 to 2	1
C. planatus	5	3	0	–2 to 1	0

forms the largest, most aggressive colonies of any potential competing native ant species. It also employs a swift, precise trail system which is initiated by scouts when they discover food or new nest sites (*6*). It is of advantage to the *Pheidole* colony to strike hard and fast when a fire ant scout is discovered near the nest. The danger is sufficient to commit major workers to destroy the intruder and to search the surrounding area for the presence of additional scouts. Other ant species are less threatening, and evidently the *Pheidole* minor workers are able to subdue scouts and small parties of these insects without help. It will be of interest to learn whether alarm-recruitment systems occur in other ant species in addition to pure recruitment systems, as is the case in *P. dentata*, and whether they are specifically directed at principal enemies.

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References and Notes

1. For a review of general recruitment in ants and alarm-recruitment in termites, see E. O. Wilson, *The Insect Societies* (Harvard Univ. Press, Cambridge, Mass., 1971). M. C. Cammaerts-Tricot has independently found alarm-recruitment in the ant *Myrmica rubra* but does not report on enemy specification; see *Anim. Behav.* **23**, 124 (1975).
2. W. S. Creighton, *Bull. Mus. Comp. Zool. Harv. Coll.* **104**, 1 (1950).
3. Colonies collected in the field are placed in containers 28 by 45 cm and 16 cm deep, the sides of which are coated by Fluon (ICI America, Inc., Stamford, Conn.) to prevent escape. The ants are permitted to move into test tubes 148 mm long with 23-mm inner diameter, kept moist by tight cotton plugs that trap water at the bottoms of the tubes. The colonies are fed with synthetic c[illegible] [A. Bhatkar and W. H. Whitcomb, *Fla. Entomol.* **53**, 229 (1970)] and freshly killed insects.
4. A fuller account of the pheromone assay and other aspects of the alarm-recruitment response is being prepared for publication elsewhere.
5. A positive response is defined as the recruitment of major workers by minor workers following the introduction of alien ants 25 cm from the *Pheidole* nests. The number recruited is defined as the maximum number of majors seen in a 20 by 20 cm sector around the invaders for 30 minutes following the introduction minus the maximum number of majors seen in the same area during the 15 minutes immediately preceding the introduction.
6. E. O. Wilson, *Anim. Behav.* **10**, 134 (1962).
7. Supported by NSF grant GB 40247.

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Orientation of Water in Striated Frog Muscle

Abstract. *Proton and deuterium nuclear magnetic resonance spectra of striated frog gastrocnemius muscle exhibit angular dependence, indicating partial orientation of water in the muscle. Nonzero static dipolar and quadrupolar interactions resulting from the anisotropic motion of the water molecules modulate the spin echo decays, contributing to their nonexponential behavior.*

Partial orientation of water molecules in several biological systems has been observed using the technique of nuclear magnetic resonance (NMR) (*1*–*4*). The orientation produces a nonzero static dipolar interaction (*5*) for the protons in H_2O and a nonzero static quadrupolar interaction (*5*) for each deuterium in D_2O. These interactions may cause splittings in the NMR spectra (*6*). The splittings are determined by the average orientation of the water molecules. The orientation factor is related to the angle θ between the axis of the ordering matrix and the magnetic field by the relation $3\cos^2\theta - 1$ (*7*). Such splittings have been demonstrated in hydrated collagen (*1*, *2*), in oriented DNA (*3*), and in model membrane systems, such as lecithin multilayers (*4*). The orientation of water molecules on nerve fibers has been a subject of dispute (*8*, *9*). The nonzero interactions can also be detected by pulsed NMR experiments, because they would modulate the exponential decay of the spin echoes (*10*, *11*).

We have studied both the continuous wave (CW) and pulsed NMR of ^1H and ^2H in striated frog gastrocnemius muscle at various orientations in the magnetic field. The results show that the water molecules in muscle have partial orientation; they also enable us to gain new insight into the interpretation of spin echo data for biological systems.

Pulsed NMR experiments were performed using a home-built NMR spectrometer with a 12-inch high-resolution Bruker magnet. Spin echo trains were obtained by the Carr-Purcell method (*10*) with the Meiboom-Gill modification (*12*). The spin-lattice relaxation time, T_1, was measured by the standard 180°-τ-90° technique (*10*) with full magnetization plots. Continuous wave and Fourier transform spectra were obtained with a Varian XL-100 spectrometer equipped with pulse and Fourier transform accessories manufactured by Nicolet Technology Corporation.

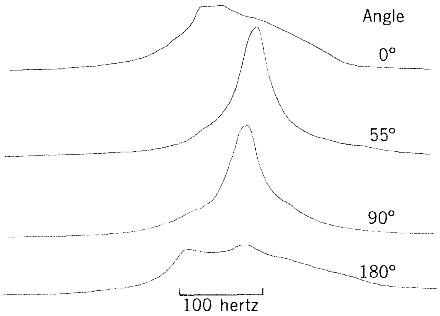

Fig. 1. Proton NMR spectra at 100 Mhz and 32°C for frog gastrocnemius muscle oriented in a magnetic field.

Frogs weighing about 20 g were used for the measurements. To introduce D_2O into the muscle, the frogs were kept in a container with 10 percent D_2O in H_2O for 3 days, then in 30 percent D_2O for 3 days, and finally in 50 percent D_2O for 1 to 3 weeks. Immediately after the frogs were killed, the gastrocnemius muscle was excised from the leg, and a striated piece was cut and carefully placed on a special Teflon plug. The plug was then fitted into the lower end of a sample tube which was cut open. Thus, the sample could be rotated about an axis perpendicular to the fiber axis so that the muscle fibers could form specific angles with respect to the magnetic field. Although small variations were observed for different frogs, the essential features of all results were reproducible for different samples.

The CW proton spectra for a normal frog muscle are shown in Fig. 1. The chemical shift of the signal is practically the same as that for liquid water. Since normal muscle contains over 70 percent water by weight, and the rest is mostly nonmobile macromolecules, one can safely assume that the CW proton signal observed is essentially due to H_2O in the muscle. When the muscle fibers were oriented parallel to the magnetic field (θ = 0° or 180°), the water signal was broad and split; when the sample was oriented at other angles, the line width was reduced, and it roughly obeyed the relation $3\cos^2\theta - 1$. The ^2H spectra for partly deuterated frog muscles (Fig. 2) did not show obvious splittings, but the line width had a similar angular dependence.

The features in the proton and deuteron NMR spectra of frog muscles indicate that the water molecules may be partly oriented in the muscle fibers. In addition to the change in line width, both the ^1H and the ^2H signals also show changes in chemical shift with the angle of orientation. Since water in different parts of a cell that are separated by physical boundaries may have different spin-lattice relaxation times (*13*), it is possible that the different fractions of water may have slightly different chemical shifts. The NMR peak may then be a composite signal of water molecules in separate parts of the cell, plus that due to extracellular water. Each part may change with the angle of orientation differently, resulting in an overall angular dependence for both the line width and the resonance position. Another possible cause of the change in chemical shift is the angular dependence of the magnetic susceptibility of the heterogeneous system.

There are two aspects in the spectra that deserve discussion. First, at physiological