Colony Specificity in the Trail Pheromone of an Ant

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Chemical cues which permit individuals in an ant colony to identify nestmates and aliens are common [1], but these colony odors are not only perceived by direct contact between workers. For example, harvester ants (*Pogonomyrmex*) from different nests are able to distinguish the scent of their own colony in their nest material [2], and also detect colony-specific signals in homing trails laid from secretions of the Dufour's gland [3]. In addition, African weaver ants, *Oecophylla longinoda*, use colony-specific pheromones present in rectal fluid to advertise territories [4]. Only in the latter example has the source of the specificity been identified. Here I report on the colony specificity of the trail pheromone of the formicine ant *Lasius neoniger*, and its ecological significance.

L. neoniger, the most abundant ant species in North America, commonly occurs in open habitats where it mounts incredibly dense populations [5]. As many as five colonies have been recorded in a sqaure meter, and nest entrances of neighboring colonies are often as close as 4 cm. Each nest is composed of several nest craters, and associated with each crater is a group of foragers that exhibit strong site fidelity. In addition to this structural and behavioral subdivision of the foraging area of a colony is a more subtle partitioning. Workers leaving a nest entrance travel along persistent trunk trails and depart at various distances to forage individually [6]. Field and laboratory studies have shown that these trail systems arise from recruitment trails composed of hindgut material which contains an ephemeral stimulatory component and a more durable substance which functions as an orientation cue. In a binary choice test, workers were capable of discriminating between artificial trails composed of hindgut extracts prepared from nestmates from those of workers from conspecific neighboring colonies.

The trail pheromone was extracted by crushing five hindguts in 50 µl 100% ethanol and solutions were prepared from hindguts of workers from two different colonies. Next, a Y-shaped pattern was drawn in pencil on a sheet of chromatography paper. The stem of the Y pattern was 3 cm long and the two branches, each 25 cm long, diverged at a 90° angle. 1.5 µl of hindgut solution prepared from workers from the colony to be assayed was deposited on the 3-cm stem of the Y with a microsyringe. This trail continued on one branch of the Y at a volume of 5 µl extract/25 cm. The other branch was composed of an equal volume of foreign extract. Workers from the test colony were then slowly baited to the base of the stem of the Y at which point the bait was removed. Recruited workers then followed the 3-cm stem to the junction of the two trails. Counts were taken in a 10-min period only of those ants that tropotactically contacted both trails and then continued on one for at least one-half its length. The results of three series of experiments are presented in Table 1.

Clearly, workers of *L. neoniger* are able to discriminate their own trail pheromone from that of conspecifics. As a control, artificial trails prepared from the same extracts were offered to a third colony. There was no difference between the number of ants following either trail, therefore, concentration differences or conditioning effects were not responsible for the preferences of the ants. Although many studies have demonstrated various degrees of species-specificity [7], this is the first reported case of trail pheromone specificity at the colony level.

Table 1. Results of three series of experiments in which workers were given a choice between following artificial trails prepared from nestmates (A) or from workers of a neighboring colony (B). The mean and standard deviations are given, followed by the range, in parentheses, of the number of workers responding to either trail for each series. *N* number of replicates. Series III was carried out in the field. The statistical evaluation was based on Student's t-test

Series	*N*	A	B	*P*
I	6	15.3± 7.7 (7–28)	5.3±2.6 (2–9)	$0.02>p>0.01$
II	4	46.3±23.4 (13–69)	19.3±9.7 (5–27)	$0.05>p>0.02$
III	6	12.0± 6.8 (4–21)	1.8±1.6 (0–4)	$0.01>p>0.005$

The specificity of the trail pheromone appears to lie in the persistent component of the rectal material. Artificial hindgut trails have a recruitment effect independent of their origin. The excitation inducing recruitment decays rapidly, but aged trails still can orient previously stimulated workers. Laboratory studies strongly suggest that the persistent component of the trail substance increases in concentration as pheromone is deposited on trails by foragers. Colonies fed on the indigestible dye Azorubin S visibly accumulated red-colored hindgut material on trunk trails. In the field, workers developed strong directionality in their foraging if baited to the same area for 2–3 h a day. Although the initial direction of the trail depends on the location of resources, the ultimate direction and duration of use are influenced by aggressive interactions with neighboring colonies. Field experiments illustrate that adjustments are made in the use of foraging area depending upon the location and activity of competing colonies. In the dense *L. neoniger* populations, trail systems appear to be an important spacing adaptation which reduces the occurrence of large, mutually detrimental confrontations between adjacent nests.

The colony-specific trail pheromone of *L. neoniger* may play a role in territorial defense. Confrontations between workers from different colonies are settled quickly if contact occurs close to a nest entrance. In this circumstance, the intruding individual is quickly dragged into the crater, whereas hostilities are more prolonged or result in both workers fleeing if they meet away from their nests. It is possible that the enduring component of the trail pheromone provide an intrinsic scent cue that allows individuals to recognize that they are "fighting on their own territory". However, it does not appear to be a true territorial pheromone as is known to exist in *Oecophylla* [4]. Workers of *L. neoniger* do not show aggressive or aversive behavior when confronted with the hindgut material of an alien colony.

The identification of the trail pheromone of *Lasius fuliginosus* as a series of six fatty acids [8] may provide an explanation of the nature of the specificity of the trail substance of *L. neoniger*. If this rectal fluid

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is of a similar composition, then colony specificity could be based on internidal differences in the relative amounts of the constituent acids. To delineate the ultimate parameters responsible for colony specificity, the genetic variance between queens, dietary differences between nests, or a combination of both factors must be considered.

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Accuracy of Sound Localization in a Miniature Dendrobatid Frog

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The relatively few quantitative studies of the accuracy of sound localization in animals have been mostly confined to laboratory situations [1, 2]. The advantages of such approaches are that potential disturbances from multiple sound sources, sound reflections and visual, olfactory and tactile stimuli can be reduced or eliminated. Nevertheless, it is also extremely important to learn how well animals localize sounds under natural conditions, where the acoustic environment is complex and where the ability evolved.

We have exploited the territorial behavior of a small dendrobatid frog *Colostethus nubicola* to quantify the accuracy of phonotaxis in nature. Males of this species call during the day in lowland forests of Central America. Unlike many others these frogs do not form dense choruses, and individual males tend to be spatially separated. We were thus able to introduce an artificial sound source in such a way that we could study phonotaxis without capturing the male or removing other calling males in the vicinity.

After locating a calling male, we slowly placed a plywood grid board within 30 cm of the frog (Fig. 1). In most cases it stopped calling but did not move very far. We next placed a small horn tweeter (Radio Shack 40-12228, being hidden by leaves) about 30 cm from the opposite end (long axis) of the grid board and mounted a 16-mm movie camera (Beaulieu R16 with 16–80 mm lense; 16 fps) on a tripod above the grid. By the time the equipment was in place and we began broadcasting the conspecific calls (Nagra IV recorder and DH amplifier) at a sound pressure level of 75 to 80 dB (*re* $2 \cdot 10^{-5}$ N/m^2 at 1 m), the male had often resumed calling. Whether or not it was calling, the playback usually elicited a rapid approach toward the speaker. We began filming (Tri-X film) as soon as it approached the grid board and continued until it hopped on or near the speaker.

An example of an approach by a male of *C. nubicola* is presented in Fig. 1a. Notice that the long axis of the body (indicated by arrows) rarely coincided with the direction of the subsequent jump, which, in general, was towards or across the target axis. The accuracy of body orientation was usually less than that of the jumps (see below). This pattern of approach has been labeled "zig-zag hopping" [3] and its possible significance for sound localization is discussed elsewhere [4]. We measured the

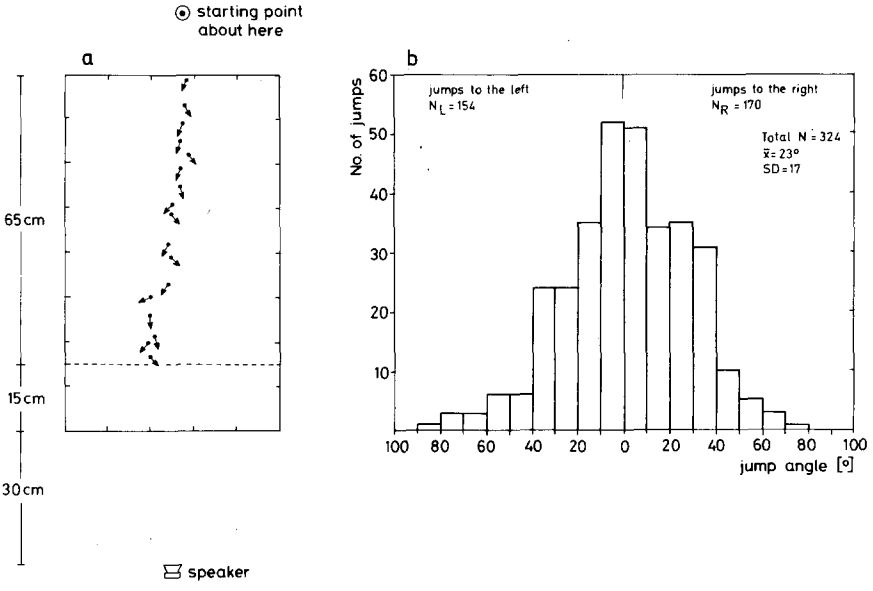

Fig. 1. (a) Diagram of a phonotactic approach of a male *Colostethus nubicola*. The dots indicate the positions of the frog on a plywood grid board (50 × 80 cm with 10 cm-square grid lines), and the arrows indicate the body orientation prior to the next hop. Within this approach the mean jump angle (between the target axis and jump direction) was 17° (SD = 6) and the mean body orientation angle (relative to the target axis) was 32° (SD = 20). We did not make measurements of jump angles or body orientation before the animal had entered the grid and at distances closer than about 45 cm from the speaker (see the dashed line; thus we can be confident that far-field conditions predominated). (b) Distribution of jump angles (of a total of 22 phonotactic approaches to the right and left of the speaker (target) axis. Since the distribution is approximately symmetrical, mean jump angles were calculated without regard to the direction (right or left). Jump angle is the angle between the target axis – a line connecting the frog's position and the speaker position – and its jump direction (we estimate the accuracy of our single frame analysis of the film records to be ± 2°)

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