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The chemistry of social regulation: Multicomponent signals in ant societies

BERT HÖLLDOBLER

Theodor-Boveri-Institut, Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Am Hubland, D-97074 Würzburg, Federal Republic of Germany

ABSTRACT Chemical signals mediating communication in ant societies are usually complex mixtures of substances with considerable variation in molecular composition and in relative proportions of components. Such multicomponent signals can be produced in single exocrine glands, but they can also be composed with secretions from several glands. This variation is often functional, identifying groups or specific actions on a variety of organizational levels. Chemical signals can be further combined with cues from other sensory modalities, such as vibrational or tactile stimuli. These kinds of accessory signals usually serve in modulatory communication, lowering the response threshold in the recipient for the actual releasing stimulus. Comparative studies suggest that modulatory signals evolved through ritualization from actions originally not related to the same behavioral context, and modulatory signals may further evolve to become independent releasing signals.

The impressive diversity and ecological dominance of ant societies are in large part due to their efficient social organization and the underlying communication system. The functional division into reproductive and sterile castes, the cooperation in rearing the young, gathering food, defending the nest, exploring new foraging grounds, establishing territorial borders, and discriminating and excluding foreigners from the society are regulated by the precise transmission of social signals in time and space.

Probably the best-studied communication behavior in ants is chemical communication, but other sensory modalities, such as mechanical cues, also play an important role in the formation of multicomponent signals in ant communication. Chemical releasers are produced in a variety of exocrine glands, and considerable progress has been made in chemically identifying many of these glandular secretions (for reviews see refs. 1 and 2). In this essay I will not emphasize, however, the natural product chemistry of ant pheromones, but rather concentrate on the proposition that communication in ant societies is often based on multicomponent signals, on nested levels of variation in chemical and other cues, which feature both anonymous and specific characteristics (3).

Pheromone Blends

Single exocrine glands usually produce mixtures of substances. The Dufour's gland secretions of the carpenter ant *Campono-tus ligniperda*, for example, include at least 41 compounds (4), and the mandibular glands of the weaver ant *Oecophylla longinoda* contain over 30 compounds, in colony-specific pro-

portions (5, 6). The identification of these components has mostly out-paced an understanding of their function, but in a few cases we begin to realize that the blends are part of the complex behavior-releasing key stimulus. In *Oecophylla*, for example, the mixture of mandibular gland secretions appears to regulate a temporal sequence of orientation and aggressive reactions, as different components diffuse outward from the point of origin (7). Vander Meer and his collaborators investigated the recruitment and trail following behavior of the fire ant (*Solenopsis* spp.), and they obtained experimental results which suggest that these behaviors are released by a complex blend of compounds derived from the Dufour's gland (8, 9). The principal components for trail orientation are two α-farnesenes, two homofarnesenes, and a still unidentified component which releases attraction behavior. Oddly, these substances remain inactive unless the ants have been induced by yet another, still-unidentified, component in the glandular secretions. The two substances responsible for attraction and inducing require about 250 times the relative concentration of the orientation pheromone. These recent findings can be conclusively related to previous observations that the more desirable the food find, the more intense is the trail laid by the recruiter ants (2). High quantities of discharged trail pheromone provide a sufficient amount of initial attraction and inducer pheromones to get the recruitment process started. Once "turned on" by these signals, the ants also follow trails consisting of relatively small amounts of orientation pheromones. A similar, although less complicated, effect of pheromone blends releasing a behavioral response was delineated by Attygalle and Morgan (10) in *Tetramorium caespitum*. This myrmicine lays trails composed of two pyrazines; workers respond maximally to a blend with a weight ratio of 3:7 of the two substances. However, such pheromone blending has not always arisen in evolution: in eight species of *Myrmica* trail following is released by the same single compound, 3-ethyl-2,5-dimethylpyrazine (11). The absence of species specificity in chemical recruitment trails has been reported in a number of ant species (2). However, cross-species trail-following does not necessarily mean identical trail pheromones. For example, in two closely related species of *Aphaenogaster*, *A. cockerelli* follows only its own trail, whereas *A. albisetosus* responds to the trails drawn with poison gland secretions of both species (12). A recent chemical analysis of poison gland contents of *Aphaenogaster* conducted by D. Morgan and his collaborators and subsequent behavioral tests of the identified compounds revealed that the main recruitment pheromone of *A. cockerelli* is (*R*)-(+)-1-phenylethanol, and that of *A. albisetosus* is 4-methyl-3-heptanone (41). But *A. cockerelli* poison gland secretions also contain 4-methyl-3-heptanone and 4-methyl-3-heptanol; *A. cockerelli* workers do not, however, respond to these latter secretions with trail-following behavior. On the other hand, *A. albisetosus* respond to the 4-methyl-3-heptanone in the *A. cockerelli* secretions but not to the phenylethanol. Incidentally,

the latter substance was not previously known to be an ant pheromone and is very unusual for poison gland contents. Thus, although we find cross-specific responses in *A. albisetosus*, this species reacts to a different component in the *A. cockerelli* trail than do *A. cockerelli* workers themselves.

In addition to pheromone blends in a single exocrine gland, multicomponent signals can derive from multisource systems. In such systems various compounds are released from multiple glandular sources. The substances may serve the same essential functions, but often the roles are different. In the harvester ant *Pogonomyrmex badius*, for example, the recruitment pheromone is voided from the poison gland, whereas the long-lasting homing pheromones originate at least in part in the Dufour's gland (13). Further investigations of this multisource system in the genus *Pogonomyrmex* have revealed that the recruitment signal is, as far as we know, invariant among several sympatric *Pogonomyrmex* species, whereas the Dufour's gland secretions contain species-specific mixtures of hydrocarbons (14, 15). Field and laboratory investigations suggest that in the partitioning of foraging areas among sympatric species of *Pogonomyrmex* both the short-lived anonymous recruitment signals and the more persistent species-specific Dufour's gland secretions are involved. The latter appear to mark the trunk routes, which also bear colony-specific markers, the origins of which are not yet known. A similar situation has been observed in ants of the genus *Myrmica*, which produce relatively anonymous recruitment signals originating in the poison gland and species-specific mixtures of hydrocarbons in the Dufour's glands that are used as home range markers (10, 11).

Many ponerine ant species conduct predatory raids on termites and other arthropods, and generally these are organized by powerful trail pheromones which are often composed of secretions from several glands. In group-raiding *Leptogenys* species one component originates from the poison gland, but a second recruitment pheromone, (3*R*,4*S*)-4-methyl-3-heptanol, derives from the pygidial gland (16, 17). We recently discovered an identical situation in *Megaponera foetens* (18). In both cases the poison gland secretions have a stronger orientation effect, while the pygidial gland secretions serve as the major recruitment signal. In several species of the legionary genus *Onychomyrmex*, both group raiding and colony emigrations are organized by trails laid with a sternal gland. However, chemical orientation appears to be supplemented by homing signals deposited from a basitarsal gland in the hindlegs (19).

Homing signals are often colony specific. A still finer level of specificity has recently been demonstrated among individual colony members, a surprising finding, given the prevailing view that individual differentiation among social insect workers is weak. Individual-specific orientation trails have been discovered in the ants *Pachycondyla tesserinoda* and *Leptothorax affinis* (20, 21), among others. The source of these highly specific markers and how they are chemically composed are not yet known.

In general, specificity in a multicomponent signal seems to be a form of modulation. Assuming that modulatory functions presuppose the existence of the behavior being modulated, a possible evolutionary route to signal specificity can be proposed (3).

The production of simple semiochemicals, releasing simple, anonymous reactions, is subject to the inevitable imprecision of all biosynthetic processes. The resulting degree of variation may well be perceptible to the receiver's sensory system, but it will ordinarily have no effect on the response to the signal. However, should an adaptive advantage happen to correlate with any of the available variants, selection will favor individuals which respond differently on the basis of these specific characteristics—i.e., modulation of the original response. Take as an example undecane, the anonymous alarm signal of many species of the subfamily Formicinae. It is usually the most abundant product in the formicine Dufour's glands. However,

other hydrocarbons are also present and the total mixture is often species specific (11). Thus, during alarm behavior undecane will be discharged together with a blend of other hydrocarbons. If, say, genetically similar colony members tend to produce similar hydrocarbon patterns, the signal may come to be modulated by this added specificity, informing workers whether nestmates or aliens are releasing the alarm. Once the presence and/or proportions of additional components significantly affect the response to the basic releaser in an adaptive manner, selection is expected to improve their distinctiveness and stereotyping.

Modulation and Ritualization of Multicomponent Signals

This evolutionary process by which a phenotypic trait is altered to serve more efficiently as a signal is called *ritualization*. Commonly, the process begins when some movement, anatomical feature, or physiological or biochemical trait that is functional in another context acquires a secondary value as a signal.

The evolution of specificity in a multicomponent signal described above can be interpreted as chemical ritualization, whereby increasingly functional specificity could derive from the biochemical "noise" in an ancestral anonymous signal and ritualization of specific variation is likewise possible with chemicals that initially were uninvolved in communication. For example, species-specific trail pheromones from the poison glands of myrmicine ants are generally the metabolic byproducts of venom synthesis (11), while the Dufour's gland hydrocarbons of formicines sprayed together with formic acid may enhance its spread and penetration. In fact, the evolutionary process of ritualization appears to have played an important role in the evolution of diverse modes of communication behavior in ant societies and is closely connected with the evolution of modulatory communication. Communication in complex social systems is not always characterized by a deterministic releasing process but sometimes plays a more subtle role. For example, in a group of ant workers certain communication signals suffice to adjust the behavior of group mates towards one another. These signals have the effect of shifting the probability for the performance of other behavioral acts, but they do not elicit particular behavioral responses. We have called this kind of communication system "modulatory communication" (22, 23). Modulatory signals are devices for shifting the threshold for the releasing effectiveness of other stimuli, thus enhancing the behavioral response to them. In this sense, the orientation-inducer pheromone in *Solenopsis* may also be called a modulatory signal (9).

In only a few cases has a statistical information analysis of modulatory communication been carried out; circumstantial evidence suggests, however, that it is widespread in insect societies. These more rigorously analyzed cases of modulatory communication concerned situations in which one signal modulates another of a different modality (22, 23). For example, in *A. cockerelli* or *A. albisetosus* a forager, after discovering a prey object too large to be carried or dragged by a single ant, releases poison gland secretion into the air. Nestmates as far away as 2 m are attracted and move toward the source. When a sufficient number of foragers have assembled around the prey, they gang-carry it swiftly to the nest. Time is of the essence, because *Aphaenogaster* must remove food from the scene before formidable mass-recruiting competitors, including fire ants and *Forelius pruinosus*, arrive in large numbers. *Aphaenogaster* workers, in addition to releasing the poison gland pheromone, also regularly stridulate at the prey object. Ants perceiving the substrate-borne signals start to encircle the prey sooner, and they are likely to release the attractive poison gland pheromone earlier. Overall, both the recruitment of workers and the retrieval of the food object are advanced by 1–2 min as a consequence of stridulation (22).

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It is conceivable that such rather unspecific modulatory signals obtain more specific significance in the communication process. A striking example is that of the leaf-cutter ant *Atta cephalotes* (24).

Atta workers stridulate when cutting an attractive leaf. Stridulatory vibrations migrate along the body of the leaf-cutting ant and are led into the substrate through the ant's head. We have evidence that the vibrations caused by the stridulation mechanically facilitate the cutting process. We could, in addition, demonstrate that the substrate-borne vibrations not only enhance the chemical recruitment signal, laid with poison gland secretions, but also suffice to attract nestmates to the cutting site. We therefore hypothesize that a motor pattern whose original function might have been to support the cutting process, secondarily became a modulatory signal, and subsequently has further evolved to function as an independent recruitment signal. In fact, Markl (25) has demonstrated that in another behavioral context the stridulatory substrate vibrations in *Atta* serve as stress and rescue signals.

Another striking example of the evolution of multicomponent signals in ant communication is found in the multiple recruitment system of the weaver ants (*Oecophylla*) (26). Workers of this genus utilize no fewer than five recruitment systems: for summoning nestmates to new food sources, to new terrain, for emigration, to territorial defense, and (short range) to territorial intruders. Although the messages differ from one another strongly, they are built out of pheromones from two or three organs—the rectal, sternal, and possibly also the mandibular gland—together with a modest array of stereotyped movements and tactile stimuli. The specificity of each of the recruitment systems comes principally from the combinations of chemical and tactile elements. For example, both recruitment to food and recruitment to territorial defense are guided by pheromones from the rectal gland. Territorial defense is further specified by forward jerking movements which closely resemble maneuvering during actual attack behavior. We have therefore interpreted the signals to be a ritualized version, "liberated" during evolution to serve as a signal when a nestmate is encountered rather than an enemy. When workers recruit nestmates to food, they use a wholly different set of movements. They wave their heads laterally while opening their mandibles. The movement resembles that of food offering and may have derived from that through a ritualization process. Other communicative motor patterns in ants—such as short runs or jerking or wagging motions employed during recruitment communication to summon nestmates to food sources, to nest sites, or to the defense of territories (26)—may in part have evolved from motor displays that originally served as general modulators. They have since been ritualized into specialized signals employed in specific contexts, usually in combination with other signals such as trail or alarm pheromones (27).

Nestmate Recognition and Exclusion of Foreigners

In most social insects, interactions between conspecific adults from different colonies are quite aggressive. Such behavior is considered to be adaptive, as workers obtain inclusive fitness benefits from aiding kin and discriminating against non-kin, and nestmates are usually more closely related to one another than to members of neighboring colonies. The semiochemicals involved in recognition at the colony level are simultaneously specific and anonymous. That is, workers are able to discriminate between nestmates and intruders, but they also tend to treat all nestmates as fellow colony members, irrespective of their true relatedness. This anonymity among genetically varied nestmates does not preclude specificity at the within-colony level. Generally, though, it appears that workers encountering one another in the context of territorial defense or nest guarding respond to chemical labels that indicate colony membership, rather than directly indicating kin. Especially species with larger colonies are characterized by a more or less homogeneous recognition signal or "colony odor," specific between colonies but anonymous throughout each colony. The sources of nestmate recognition signals in social insects have recently received a great deal of attention (for partial reviews see refs. 2, 3, 28, and 29), and several investigators paid special attention to possible colony-specific profiles of cuticular hydrocarbons (i.e., refs. 30–33). Obviously, to achieve such a high degree of specificity, such recognition labels must be rather complex, multicomponent signals. However, there exists no conclusive proof yet that the implied colony-specific hydrocarbon patterns serve as nestmate recognition labels, nor is it possible yet to develop a unified concept of the sociobiological foundations of nestmate recognition. The current results indicate that the behavioral mechanisms underlying nestmate recognition vary with the specific social organizations of the societies.

In monogynous (only one queen is present) carpenter ants (*Camponotus* spp.), for example, nestmates are distinguished by chemical labels acquired from a variety of sources, functioning in a hierarchical order of significance (2, 3, 34, 35). Workers removed as pupae from a colony and reared separately, in the absence of queens, are relatively tolerant of one another, but exhibit stronger aggressive behavior toward non-relatives. Diet differences slightly enhance aggression among separately reared kin. If a queen is present, however, workers attack both unfamiliar kin and non-kin with equal violence, a response which is unaffected by food odors. Cues derived from healthy queens with active ovaries are sufficient to label all workers in experimental colonies, while the workers' own discriminators become more important when their queen is infertile. As mentioned above, recent work suggests that the acquired recognition cues of *Camponotus* spp. workers may consist at least in part of colony-specific relative proportions of cuticular hydrocarbons (30, 31, 33). The queen is by no means the only source of shared extrinsic recognition cues. The discriminators produced by each worker may be transferred among them all, resulting in a "gestalt" or mixed label, as originally proposed by Crozier and Dix (36) and demonstrated, among others, in leptothoracine ants (37). In addition to heritable cues from other workers and/or queens, variation originating in the diet or other environmental differences external to the colony also contributes to nestmate recognition in several ant genera (for review see refs. 2, 3, and 38).

Conclusion

The early discovery of such extremely fine-tuned chemical sexual communication as that of the silkmoth *Bombyx mori* (39) encouraged the belief that, among insects, each behavioral response is released by a single chemical substance. By contrast, much greater population and individual variability was attributed to the chemical communication signals produced by vertebrates, particularly mammals, in which pheromones often mediate more interindividual interactions such as individual recognition, dominance ranking, and territorial marking. While the complex chemical composition of mammalian pheromones was examined for functional significance, the same degree of variation observed in an insect pheromone would be ascribed to contamination or biosynthetic "noise." It is now clear that such a double standard was, at best, an oversimplification. Most insect semiochemicals have proven to be complex mixtures, and single-compound pheromones are actually rare (40). In this respect at least, insects and vertebrates do not differ greatly in the sophistication of their chemical communication systems.

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