Chemical Communication in Honeybees

Gene E. Robinson

In animal societies, communication provides the ties that bind, and chemical communication is especially important for social insects and mammals. Because chemical communication is best understood in insects, it is no surprise that this subject also is advanced for social insects relative to other animals that live in complex societies. Dozens of pheromones have been identified that mediate nearly all aspects of colonial life, including orientation, social foraging, social defense, brood care, kin recognition, mating, and the rigid reproductive hierarchy that is the hallmark of an advanced insect society. But no single social insect pheromone has been studied more intensively than the one produced by the mandibular glands of the queen honeybee, *Apis mellifera*. Plettner *et al.* in this issue of *Science* (*1*) describe the biosynthesis of honeybee queen mandibular pheromone, a significant development because of the pivotal role this substance plays in the regulation of social life.

The story of research on honeybee queen mandibular pheromone (*2*) highlights many important themes in chemical ecology. A honeybee queen lays millions of eggs during her life, while her older daughters, the adult workers, by and large rear their little sisters (and a few brothers) rather than reproduce directly. Research in the late 1950s implicated the queen's mandibular glands as the source of chemicals responsible for maintaining this stark social order. A mandibular gland fatty acid, 9-keto-(*E*)2-decenoic acid (9-ODA), was identified by Callow and Johnston and Barbier and Lederer in 1960, just the second pheromone ever to be identified. 9-ODA, and to a lesser extent 9-hydroxy-(*E*)2-decenoic acid (9-HDA), another mandibular gland compound identified shortly thereafter, was reported to inhibit the rearing of new queens by workers and the development of worker ovaries. Such complex and long-lasting pheromonal effects on physiology and behavior have come to be called "primer" effects, in contrast to the more rapid and short-term "releaser" effects of sex or alarm pheromones. That these influences were attributable to both 9-ODA and 9-HDA foreshadowed the emergence of the important concept of a pheromone as a blend rather than a single compound. And pheromone-based reproductive dominance has proven widespread in both insect and vertebrate societies (*3*).

Queen honeybee surrounded by workers who both feed and groom the queen as they receive her mandibular pheromone. The "retinue" response is one of several important social behaviors mediated by this pheromone. [Photo by S. Camazine/Penn State University]

Queen mandibular pheromone was soon found to lead a double life; while its effects inside the beehive on workers are mostly oppressive, its effect outside, on males, is positively liberating—it acts as a sex pheromone! Just as the "one pheromone, one chemical" idea became passé, so did "one pheromone, one message," especially for social insects. This pheromone has since been shown to influence other worker behavior, including foraging and orientation. Moreover, although drones will ardently pursue even a dead queen or a lure dabbed with the pheromone outside the hive, they are completely refractory toward queen pheromone inside, graphically illustrating the importance of context in chemical communication.

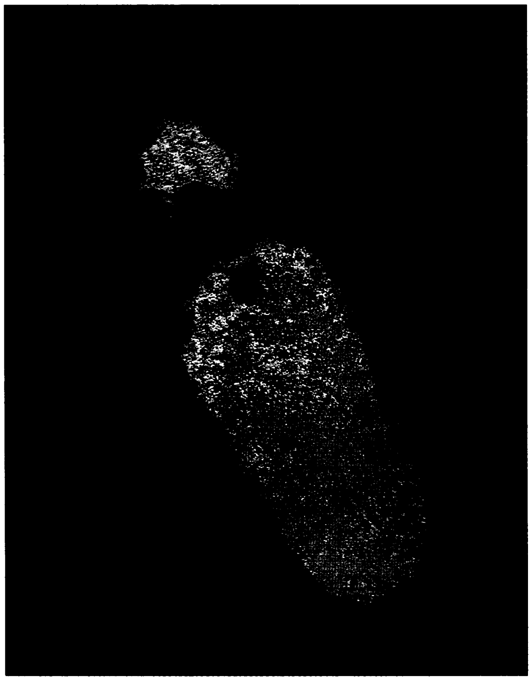
Hundreds of releaser pheromones have been identified throughout the animal kingdom. Not so for primer pheromones—only two are known with certainty: queen mandibular pheromone and a goldfish secretion that also regulates reproductive development (*4*). Why the terrible imbalance? Because the key to pheromone identification is a good behavioral assay, and it is much easier to measure the rapid, all-or-nothing behavior that is associated with, for example, mating (unless it only occurs on the fly at 20 kilometers per hour, as it does for honeybees) than the slower developing, graded, often endocrine-mediated responses that are under the influence of primer pheromones. This also explains why research on queen mandibular pheromone lagged after a promising beginning. 9-ODA and 9-HDA were quickly found to be poor substitutes for the real thing, but it was not until almost 30 years after the identification of 9-ODA that a team of researchers headed by Keith Slessor and Mark Winston reported (*5*) that the complete queen mandibular pheromone is composed of five compounds: 9-ODA, *R*-9-HDA, *S*-9-HDA, and two aromatics, methyl *p*-hydroxybenzoate and 4-hydroxy-3-methoxyphenylethanol. Sure enough, the breakthrough was the development of an effective bioassay based on still another (releaser) effect of this pheromone on workers: the urge to surround, feed, and groom a queen (see the figure). Complete chemical identification has not only led to a fuller picture of this intricate communication system, but has also sparked the development of lucrative pheromone-enhanced crop pollination systems (*2*). This reflects still another noteworthy, and politically correct, aspect of chemical ecology: generating new products from basic research (*6*).

Plettner *et al.*'s report on the biosynthesis of mandibular gland fatty acids produced by queens and workers opens an important new chapter of this story. It had been known that worker bees produce substances that are similar to those of queens and apparently act as larval nutrients and food preservatives. The new results suggest that the queen acids are not just an elaboration of what the workers have. The predominant worker mandibular acid, 10-hydroxy-(*E*)2-decenoic acid (10-HDA), also is synthesized from stearic acid as is queen 9-HDA, both through hydroxylation and chain-shortening steps. It thus appears that worker mandibular gland compounds also have been shaped by so-

Before 1 July 1996, the author will be in the Department of Biological Chemistry and the Charles E. Smith Family Laboratory for Collaborative Research in Psychobiology, Hebrew University, Jerusalem, Israel 91904. E-mail: generobi@pop.life.uiuc.edu After 1 July, he will be in the Department of Entomology, University of Illinois, Urbana, IL 61801, USA.

Enter *Listeria*, Unruffled

For many pathogens, the inside of mammalian cells represents an ideal protected niche, away from circulating antibodies and other host defenses. Understanding how microbes trigger their own uptake into nonphagocytic cells, a process known as induced phagocytosis, is at the top of the agenda of the emerging field of cellular microbiology (*1*). Work on a trio of Gram-negative bacteria—*Yersinia*, *Salmonella*, and *Shigella*—has blazed the trail, but a recent paper from the Pasteur Institute in *Cell* (*2*) provides the first details of how a Gram-positive bacterium, *Listeria monocytogenes*, is taken up.



Listeria monocytogenes. Electron micrograph of a *Listeria* bacteria with daughter cell at top left; 54,000× [Photo by K. Lounatmaa, Photo Researchers Inc.]

The protein on *Listeria*'s surface that interacts with mammalian cells was described some years ago (*3*): It is internalin, an 80-kilodalton protein that is a member of the growing family of LRRs (leucine-rich repeats) proteins. The new work shows that the receptor for internalin on the surface of epithelial cells is E-cadherin. That the two proteins interact was revealed by affinity chromatography: Amino-terminal sequencing identified the two epithelial cell products retained on an internalin column as E-cadherin and its proteolytic fragment. The authors emphasize the significance of the interaction by testing the ability of *Listeria* strains (with and without internalin) to invade panels of fibroblastic cell lines expressing different cadherins. Invasion was prevented when the fibroblasts expressed N-cadherin instead of E-cadherin. Thus, the internalin–E-cadherin interaction mediates both specific binding and entry of the bacterium, a finding supported by the blockade of invasion by antibodies to E-cadherin.

E-cadherin is a member of the cadherin family of cell adhesion molecules, which mediate cell-cell adhesion through homotypic interactions of their extracellular domains. E-cadherin is expressed mainly on epithelial cells and participates in cell sorting during development and in the maintenance of adult tissue architecture. Cadherins are connected to the actin cytoskeleton by their cytoplasmic domains through interactions with catenins.

The new work from Mengaud *et al.* (*2*) does not prove that signaling through E-cadherin mediates the induced-phagocytosis of *Listeria*, but the actin polymerization that occurs at the site of entry is a tantalizing hint in this direction. The amount of F-actin accumulation is modest, considerably lower than that found with the entry of *Salmonella* and *Shigella*, which is accompanied by dramatic localized membrane ruffling. For *Listeria* there is no membrane ruffling, and the process is similar, morphologically, to the entry of *Yersinia* mediated by the cell-surface protein invasin (*4*). Clearly, the haven of the mammalian cell is so desirable that bacteria have developed multiple strategies of entry.

Richard B. Gallagher

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cial evolution, perhaps for as yet undiscovered communicative functions. Is the biosynthetic machinery of the queen more intricate? Plettner *et al.* show that 9-ODA, the principal queen mandibular acid, is only produced after a third reaction in which 9-HDA is oxidized; in workers the oxidation of 10-HDA yields diacid (C10:1DA), a minor component, at least quantitatively (*7*). Queens also have about 10 times more mandibular acid than do workers. Despite these distinctions, the biosynthesis of mandibular acids in the two castes reveals intriguing overlap. Plettner and colleagues show that very young virgin queens can oxidize 10-HDA to the diacid like workers, and previously they found 9-HDA in workers (*7*). But neither workers nor young virgin queens oxidize 9-HDA to 9-ODA, whereas older virgins and mated queens can. "False queens" are an exception; these workers, which sometimes develop in queenless, broodless, colonies and lay male eggs, have queenlike mandibular gland secretions, complete with a lot of 9-ODA (*7, 8*). Caste differences in mandibular acid biosynthesis thus appear to be sensitive to both intrinsic and extrinsic factors. Further exploration of how these factors influence mandibular gland biosynthesis may lead to new insights into the evolution of the worker and queen castes, a central question in sociobiology. Another fertile line of investigation now possible is to identify the enzymes involved in mandibular gland pheromone biosynthesis, and their genes, and study their regulation as a function of age, mating status, and social condition. No one has yet reported the cloning of a pheromone biosynthesis gene. There is no doubt that honeybee queen mandibular pheromone, already an exemplar, will continue to be used as a chemical beacon in both sociobiology and chemical ecology.

References

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