Genomic and brain expansion provide ants with refined sense of smell

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Chemical communication is the most ancient way of information transfer among organisms, and chemical senses, although universal, are particularly developed in social species. Many animals use odors in circumstances that are critical for survival and reproduction: to detect suitable food sources and nest sites, to avoid predators, and to find mates. Social animals use chemical communication also to regulate interactions within a social group and to distinguish group members from strangers. Chemical communication is exquisitely developed and multifaceted in some insects, usually described with the idiosyncratic term of "eusocial" insects (from Greek eu- meaning "well" or "good"). These include all known species of ants and termites, and some bees and wasps, and represent the most advanced and evolutionarily successful social organisms. A new study (1) by a team of researchers from The Rockefeller University reveals that ants evolved a remarkably large clade of odorant receptor (OR) genes expressed in the antennae that allow detecting complex social chemical cues. This genomic expansion went hand-in-hand with the expansion of a particular group of glomeruli (functional units) in the antennal lobes, the insect primary olfactory brain centers. These results shed novel light on the molecular basis of social life.

Ant societies evolved more than 100 million years ago (2) and became the most ecologically successful among insect societies. Due to their absolute numbers and extraordinary diversity, ants dominate many terrestrial ecosystems (3). Some resemblances between human and ant societies have long fascinated biologists and even philosophers (4). Ants build cities, maintain infant nurseries, use sophisticated techniques of waste management, practice agriculture and husbandry, and conduct wars with their neighbors. A fundamental difference between human and ant societies concerns the magnitude of division of labor, which in ants reached an evolutionary peak involving individuals specialized for reproduction (so-called queens) and individuals giving up their reproduction (workers) to conduct the tasks necessary for colony maintenance and growth. This social organization has been defined as "obligate eusociality" because caste is determined at the larval stage and individuals belonging to the worker caste are typically not able to disperse and found their own colonies (5). To maintain and optimize such extreme division of labor among society members, an efficient transfer of information is of critical importance. This is usually achieved in ants and other social animals by means of chemical messengers.

Pheromones are chemical signals transmitted between individuals of the same species that produce a behavioral and/or physiological response in the receiver (6). They can be single molecules or a precise combination of molecules, and they represent species-wide signals, which do not require learning because the response is typically hardwired (7). Many pheromones are volatile, such as alarm pheromones and trail pheromones, and are produced by specific exocrine glands, which have been well studied in ants (8). Pheromones, however, can also be relatively low-volatile substances that adhere to the body surface and are perceived at small distance. This is the case of some recently discovered ant queen pheromones, represented by one or few long-chain hydrocarbons present in large amount on the cuticle of the queen (9, 10). Queen pheromones inform the workers that the queen is present, healthy, and fertile, thus contributing to the optimal regulation of reproductive division of labor. The cuticular profile of ants is characterized by many different hydrocarbons (from about 20 up to more than a hundred), only few of which have been identified as pheromones. This complex blend of cuticular hydrocarbons is also acting as "signature mixture," defined as a set of molecules of the ant chemical profile, which is variable among colonies and therefore has to be learned by the receiver (7). Each ant species typically shows a species-specific cuticular chemical profile, but the relative amount of the various hydrocarbons composing the profile is different in different colonies of the same species, which therefore have a distinct "colony odor" (11). Qualitative and quantitative variation among cuticular chemical profiles allows ants to discriminate between group members (nestmates), which bear the colony odor, and strangers (nonnestmates) belonging to different

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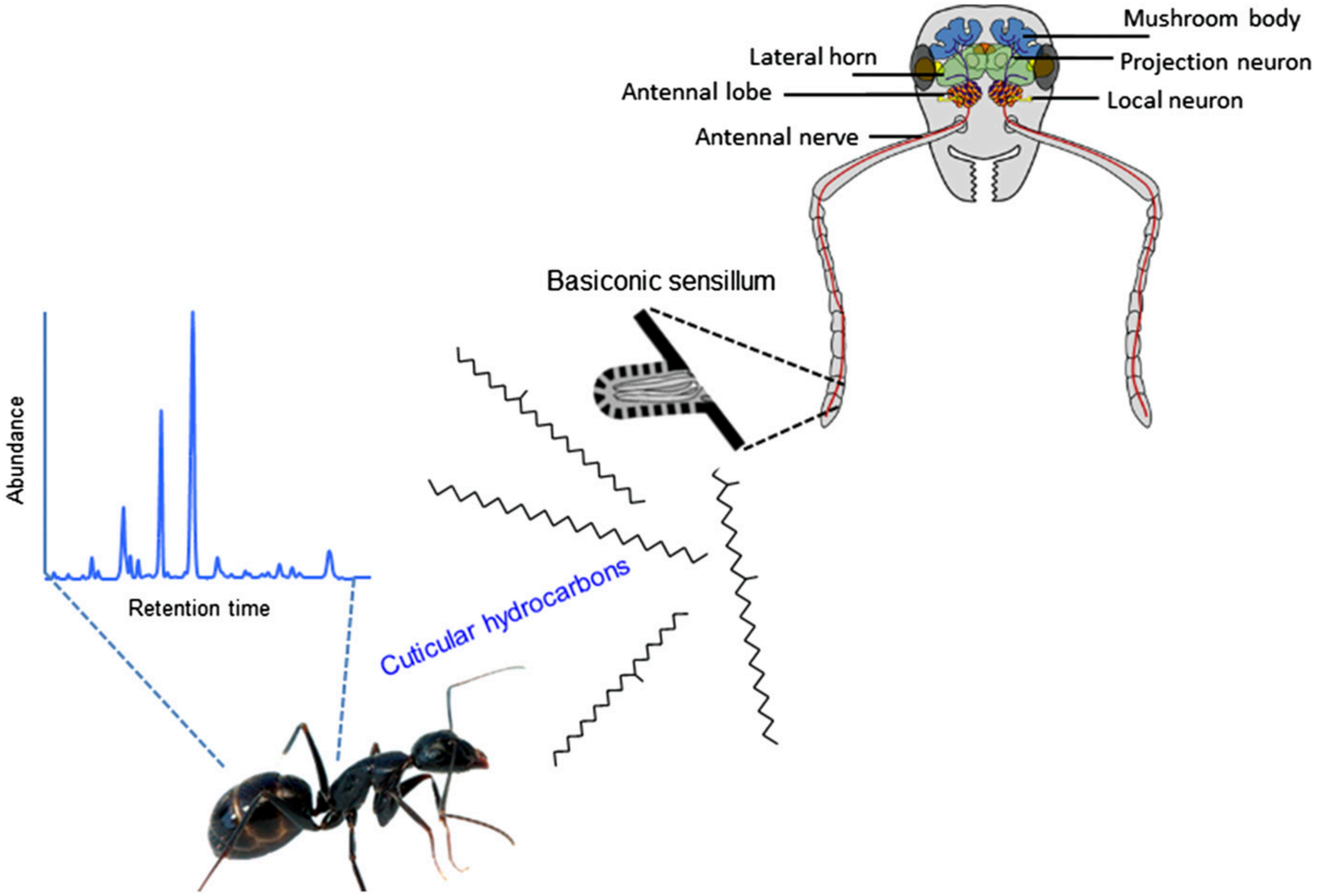

Fig. 1. The ant cuticle possesses a complex blend of long-chain hydrocarbons, which can be characterized by gas chromatography and mass spectrometry (an example of gas chromatogram is showed on the *Left*). Hydrocarbons are detected by ORNs with dendrites located in basiconic sensilla on the antenna. These neurons project to the antennal lobes, made of many functional units (glomeruli). The processed information is then passed to the higher brain centers (mushrooms bodies, lateral horn). Drawing of ant head by Margot Perez.

colonies of the same or different species. Effective nestmate recognition prevents exploitation of colony resources by competitors and parasites, thus contributing to the evolutionary success of ant societies. The existence of a nest smell has been postulated since the beginning of 1900 (12), although the identity of the chemical compounds involved in the colony odor was revealed almost a century later (reviewed in ref. 13).

Cuticular hydrocarbons are detected at small distance (14) by olfactory receptors expressed in olfactory receptor neurons (ORNs) housed in special sensory organs (basiconic sensilla) located in the antennae. From each antenna, ORNs converge into the homolateral antennal lobe (the insect equivalent of the vertebrate olfactory bulb), which is organized in globular neuropil elements, the glomeruli. Projection neurons convey information from the antennal lobes to the higher brain centers, such as the mushrooms bodies (Fig. 1). Electroantennography (15, 16) and calcium imaging (16), measuring activity patterns in the antennal lobes, revealed that cuticular hydrocarbons are indeed detected by ORNs. More recently, a multidisciplinary study characterized the response of the ant basiconic sensilla (which contains multiple ORNs) to a large number of hydrocarbons and found that ant antennae can detect a broad spectrum of these low-volatile chemicals, a result that was supported by behavioral assays (17). Ants can also discriminate between different concentrations of the same hydrocarbon (18). Nevertheless, we are far from knowing how exactly the social signals are encoded in this complex cuticular bouquet, and how the information is processed by the brain.

The new findings of McKenzie et al. (1) in the ant species *Ooceraea biroi* significantly contribute to a deeper understanding of the ant chemical communication system. The study advances previous work because its relevance is threefold. First, with electron microscopy, they found that basiconic sensilla are present exclusively in female ants (males are produced very rarely in this clonal species, and in general males do not take active part in the organization of ant societies because they are only involved in seasonal reproduction). These sensilla are organized in an array on the anterior–ventral surface of the antennal club, and this limitation to a specific surface of the antenna was not yet described in other ant species. Second, with transcriptomics, McKenzie et al. (1) find that a large clade of ORs (180 ORs belonging to the nine-exon subfamily; see also ref. 19) are expressed specifically in the female antennae, particularly within the ventral half of antennal club. Neuroanatomy of the antennal lobe of males and workers of *Ooceraea biroi* allowed characterizing the glomeruli. The female ants possess nearly 500 glomeruli against only about 100 found in males. A particular group of glomeruli, the T6 cluster, which receives input from the ORNs, is extremely developed in female workers (288 glomeruli) but not in males (15 glomeruli). Remarkably, the number of T6 glomeruli positively correlates with the number of nine-exon OR genes expressed in the antennae. Third, evolutionary analyses revealed that this clade went through an extraordinary gene expansion in the ant ancestors (and continued at a slower rate in subsequent lineages) and that gene diversification occurred at the same time as the evolution of eusociality in ants.

Cuticular hydrocarbons in insects are originally important for protection against desiccation (13), and they have assumed an important communication role in social interactions during the evolution of social lifestyle. Further research is required to better understand the odor-coding system. In insects, some odorants are detected by very specific ORs that activate dedicated neuronal circuits (dedicated channels). By contrast, in "combinatorial coding," each OR is broadly tuned and responds to different odorants. Therefore, single odorants activate several ORs and consequently several glomeruli in the antennal lobe. This system increases the number of odorants that can be detected and improves overall flexibility and robustness. It has been hypothesized that combinatorial coding would evolve in highly social species with large OR repertoires (20); this can now be tested in ants.

We have gone a long way since 1874, when A. Forel (21), a pioneer in the study of ant chemical communication, discovered that the antennae, and in particular the club-shaped sensilla, are their principal olfactory organs. Wheeler (4) reports Forel's description of the ants' sense of odor shape: "To make this clear he suggests that we fancy ourselves to be blind or in total darkness and in possession of delicate olfactory organs in our finger-tips. Then, if we moved about, touching objects to the right and left along our path, our environment would appear to us to be made up of shaped odors, and we should speak of smells that are spherical, triangular, pointed, etc." (p. 510). The new study by McKenzie et al. (1) helps us in deciphering the odor-shaped language of ants, whose genius is definitely in their antennae.

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