Chemicals on the ant cuticle: nestmate recognition, territorial marking and pollution indicators

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The insect cuticle is generally considered to be a barrier against the environment and protects against desiccation (Gibbs, 1998). It is mainly formed by a lipid layer. In social insects, cuticular lipids also play a communication role, which is generally attributed to hydrocarbons. We will first review the role of hydrocarbons in nestmate recognition and the formation of colony odor in ants. In a second section, we will present new data on the exploitation of the common nest odor by various parasites and guests. In a third section, we will describe how the cuticle is also a barrier against potential parasites. Finally, we will present data showing that the cuticle is contaminated with various pollutants.

1) Nestmate recognition and colony odor

Many studies have shown that cuticular hydrocarbons are colony-specific and play a role in nestmate recognition, a process by which social insects discriminate between members of their own colony and conspecific aliens. Hydrocarbons are stored in the postpharyngeal gland and permanently exchanged by trophallaxis and allogrooming among individuals of the colony to form a common "gestalt" odor (see recent reviews: Hefetz, 2007; d'Ettorre and Lenoir, 2009). Colony area marking is a widespread phenomenon (Hölldobler and Wilson, 1990) and it is generally thought that marking is due to colony-specific hydrocarbons deposited passively on the foraging arena (Yamaoka and Akino, 1994).

We recently studied *Lasius niger*, a monogynous and multicolonial species. It was confirmed that they have a colony-specific hydrocarbon profile, characteristic of colonies closed to non-nestmates. For the first time, we demonstrated that the inner walls of the ant nest are coated with the same hydrocarbons as those found on the cuticle, but in different proportions. The high amount of inner-nest marking and its lack of colony-specificity may explain why alien ants are not rejected once they succeed in entering the nest. The cuticular hydrocarbons also are deposited in front of the nest entrance and on the foraging arena, with a progressive increase in the proportion of n-alkanes (Fig. 1) (Lenoir et al., 2009). Chemical signatures laid over the substrate are colony specific only when we consider methyl-branched alkanes. Our data confirm that these "footprint hydrocarbons" are probably deposited passively by the contact of ant tarsae with the substrate and that nest surroundings are home range marked rather than a true territorial marking (Devigne and Detrain, 2006). These results suggest that the CHC chemical profiles used by ants in colony recognition are much more complex than a single template: ants have to learn and memorize odors that vary according to their context of perception (Lenoir *et al*, 2009).

It has been shown that *Cataglyphis niger* or *C. iberica* callows have very small quantities of cuticular hydrocarbons, which increase in a few days to attain the mature level (Soroker et al., 1995; Dahbi et al., 1998). This increase in hydrocarbon quantities from an initial low level was also observed in *Aphaenogaster senilis*. In this species, individual isolation induces a deficit in the development of the postpharyngeal gland, whereas the cuticle quantity is not modified. This effect of social isolation on the PPG hydrocarbon level indicates the importance of hydrocarbon transfer between nestmates through the PPG and lends support to the gestalt model of nestmate recognition (Ichinose and Lenoir, 2009). This low quantity of hydrocarbons has been called "chemical insignificance" followed by "chemical integration" (Lenoir et al., 1999) and has revealed to be very general in social insects (see for example recent papers on wasps: Cini et al., 2009)). Chemical insignificance can be a strategy used by parasites (for example *Acromyrmex insinuator*) and some myrmecophiles to integrate the host society (Lambardi *et al.*, 2007; Witte *et al.*, 2008). It is also a putative explanation of the tolerance of some guests like snakes in *Atta* nests (Baer et al., 2009).

The problem of nestmate recognition in social insects is not completely solved, as volatiles can be involved (see (d'Ettorre and Lenoir, 2009)), as can long chain hydrocarbons (up to C47) (Lucas et al., 2005; Akino, 2006; Lambardi *et al*, 2007). These hydrocarbons can be associated with unusual substances like undetermined steroids (Menzel et al., 2008). Moreover some peptides and venom are used by wasps to mark their hibernation sites (Turillazzi et al., 2006) and these substances may be used by ants also.

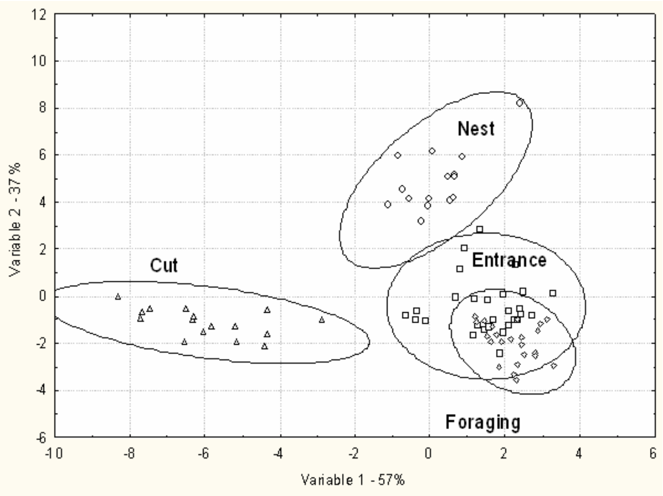

Fig. 1- Discriminant analysis of hydrocarbons obtained by SPME from cuticle (Cut), inner walls of the nest (Nest), nest entrance (Entrance) and foraging arena (Foraging), of *Lasius niger*, independently of the colonies. Ellipses are 95% confidence interval. After (Lenoir *et al*, 2009).

2) Exploitation by parasites and guests

The common nest odor can be exploited by different parasites and guests who mimic the host odor. Mimicry can be passive, for example by host licking and rubbing, or active by synthesis of the host hydrocarbons (see review by (Lenoir et al., 2001; Akino, 2008). We studied *Myrmica karavajevi*, a social parasite of the ant *Myrmica scabrinodis* and found that it mimics the host odor as predicted (Fig. 2).

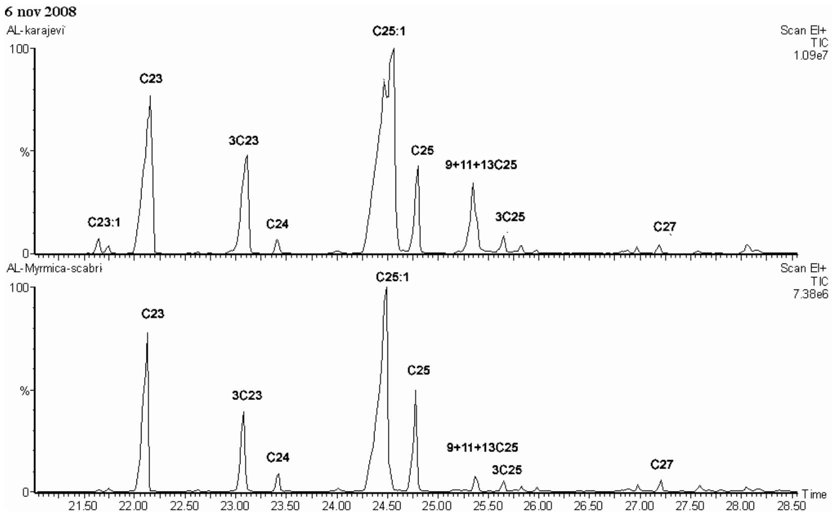

Fig. 2- Gas chromatogram of cuticular hydrocarbons of *Myrmica karavajevi* (top) and *M. scabrinodis* (bottom) showing chemical mimicry between the parasite and the host. Note that

Myrmica which live in humid places have only short length hydrocarbons with a high proportion of alkenes (Alain Lenoir, Unpublished data)

Some myrmecophiles have co-evolved with their hosts and synthesize the same hydrocarbons. We studied two myrmecophilous beetles: *Thorictus martinezi* associated with the ants *Cataglyphis sp.* from Burkina Faso (Háva and Lenoir, 2008) and *Sternocoelis hispanus* associated with *Aphaenogaster senilis* in Andalusia. *Thorictus* beetles are known to clutch at one antenna of the host with their mandibles and remain on the same worker for long periods. We do not know what exactly they eat. *Sternocoelis* can also fix on a leg of ant to be transported, but they are never on the antennae. They probably eat some detritus, mainly on larvae. These beetles can be adopted in a conspecific colony, but adoption requires a longer time than in the original colony. They are never adopted in an allospecific species. They mimic the host cuticular hydrocarbon profile but keep a distinct quantitative profile like slave ants and their hosts (Fig. 3). We measured the hydrocarbon quantities of *Sternocoelis* beetles and it appeared that after 30 days in total separation with the ant host, they kept the same quantities of hydrocarbons, indicating that probably they are able to synthesize them. The hydrocarbon profiles of different species of *Aphaenogaster* and *Cataglyphis* are sufficiently different for chemical mimicry to explain the host specificity as it has been hypothesized for the caterpillars of *Maculinea* and their hosts *Myrmica* (Elmes et al., 2002).

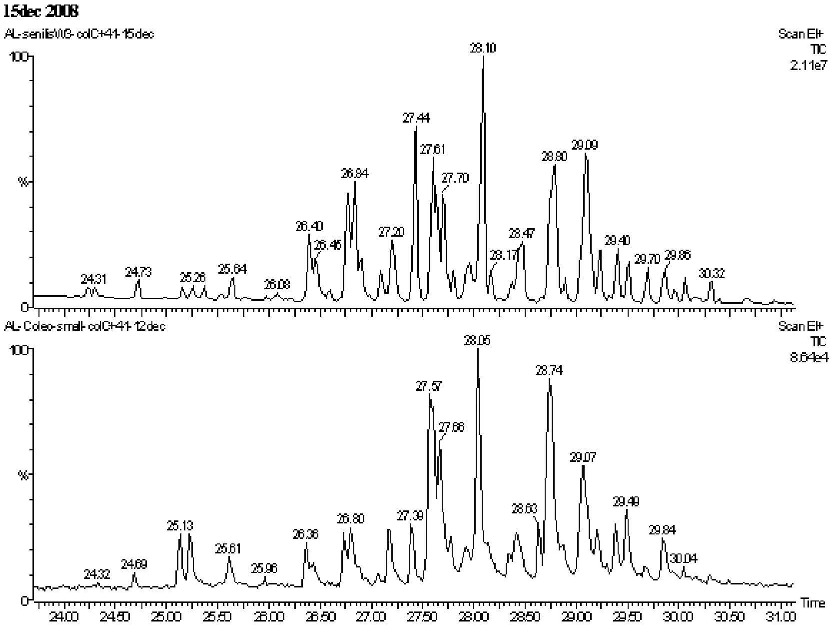

Fig. 3- Gas chromatograms of *Aphaenogaster senilis* (top) and *Sternocoelis hispanus* (bottom) showing qualitative mimicry but some quantitative differences for the substances indicated in bold (Quentin Chalon & Alain Lenoir, unpublished data)

3) Cuticle as a barrier against potential parasites.

Carpenter ants, *Camponotus spp.*, have the endosymbiotic bacteria Blochmannia which have mainly an alimentary contribution to their hosts (Feldhaar et al., 2007). It was recently demonstrated that they also improve immune functions: bacteria number in the gut is positively correlated to encapsulation degree (Souza et al., 2009). After antibiotic treatment with rifampicin, the ants may have both a nutritive and immunological deficit, inducing a stress which provokes an increase in the melanization of the cuticle and in the cuticular hydrocarbons quantities (Danival J. de Souza, Séverine Devers and Alain Lenoir, unpublished data). We suggest that the bacteria / ant symbiosis is part of a trade-off in the life-history traits of the ants. The increase of hydrocarbon quantities and melanization will enhance the cuticular protection to desiccation, but also enhance protection against invasions of pathogens and parasites. The nestmate recognition system due to the cuticular hydrocarbon profile is not modified by the antibiotic treatment, contrarily to what was observed in termites (Matsuura, 2001).

4) Cuticle and environmental contaminants.

The cuticle is a trap that fixes hydrocarbons from neighboring ants (for example in artificial mixed species colonies or social parasites), but also all sorts of substances and particularly some pollutants which are generally neglected in the analyses.

Phthalate esters are used industrially in many products, including cosmetics, shampoos, soaps, lubricants, pesticides and paints. They are also used to make plastics more flexible in a variety of products like polyvinyl chloride. Several phthalates have been identified and classified as endocrine disruptors (EDCs) possibly due to their estrogenic and anti-androgenic activity in animal models (see review by (Colborn, 1998)). Because phthalate esters are not chemically bound to the plastics, they can be easily released from products and migrate into food or water that comes into direct contact (Jen and Liu, 2006). As they are hydrophobic they are generally extracted by organic solvents.

We found that the cuticle of *Lasius niger* is contaminated with three phthalates: DEHP (Di Ethyl Hexyl Pht = DOP), DBP (Di Butyl Pht) and its isomer DIBP (Di IsoButyl Pht). The quantities were significant: 2 to 10 ng per ant which represents 1 / 1 000 of the ant weight (mainly DBP) and 1 to 3% of the cuticular chemicals. This proportion is higher (10%) on legs which are in direct contact with the plastic of the nests. Phthalates were found on all ant species studied, in the glands like Dufour and postpharyngeal gland. We found them also on honeybees. Ants collected in the field without any contact with plastic were also contaminated (mountains like Alps, Morocco Atlas, Andalusia, Greek islands) confirming that phthalates are dispersed as aerosols everywhere in the world (Alain Lenoir, Séverine Devers, Jean-Philippe Christidès and Frédéric Montigny, unpublished results).

The effects of these phthalates on insects are mostly unknown. They have deleterious effects on all freshwater animals (Oehlmann et al., 2009). On insects like *Drosophila* they have an ecdysteroid antagonist effect but at very high levels; they have toxic effects on *Chironomus* probably by influencing ecdysone receptor (Oehlmann *et al*, 2009). Apparently, they do not have any effect on the ants, which may have a detoxifying system for metals as has been shown in *Formica polyctena* (Jeantet et al., 1974) and *Lasius niger* (for cadmium see Grzes, 2009)). In *Formica* ants, however, the accumulation of heavy metals disturbs immune response (Sorvari et al., 2007).

Some other contaminants from plasticware are found in laboratories, for example oleamide (= 9-octadecenamide) (McDonald et al., 2008). We found this substance in chromatograms, therefore, it is recommended to use only glass for preparation of extracts.

Conclusions

The chemistry of the ant cuticle is a very interesting field for evolutionary biologists. Cuticular chemicals are fundamental in nestmate recognition, one of the major aspects of sociality. The common odor is frequently exploited by many guests and parasites that mimic it. The cuticle is also a barrier against parasites, which can be increased in case of “stress.” It also reflects environmental pollution, as it adsorbs many contaminants. More research is necessary to evaluate the effects of these pollutants.

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