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The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) – interplay of colony odor uniformity and odor idiosyncrasy. A review

Abraham HEFETZ

Abstract

Hydrocarbons are the main lipid constituents on the insect cuticle, and generally provide the insect with a waterproof layer to prevent desiccation. In many insects this class of chemicals has been coopted to serve as pheromones. In social insects, in particular in ants cuticular hydrocarbons (CHCs) have at least two pheromonal functions. They act as recognition cues that facilitate colony insularity, protecting it from parasites or conspecific invasions. Supporting evidence for this function are their extreme complexity, their colony specific composition, and in a few cases also demonstrating elevated or reduced aggression between encountering ants as a function of the label (alien or nestmate) they were painted with. The second function of CHCs is in signaling fertility. In many ant species it was demonstrated that fertile individuals (queens, gamergates, or egg laying workers) have CHC profiles that are distinct from that of their sterile nestmates. This can be expressed as the augmentation of a single or a small subset of the blend components, or differences in the entire blend. The fact that these signals have an abundance of branched alkanes, which lower the break point of cuticular impermeability thus imposing cost on the individual, indicates that these may constitute honest signals.

This dual function seems contradictory since nestmate recognition necessitates a uniform colony odor, i.e., uniform CHC composition, whereas fertility signal requires idiosyncrasy since the fertile individual needs to be singled out among the colony members rather than conform to colony odor. A possible resolution is that species that use CHCs for nestmate recognition do not use them as fertility signals and vice versa. I propose an alternative solution whereby workers have variable discrimination thresholds and response to differences in the pheromone blend, large or small, in a context-dependent manner.

Key words: Cuticular hydrocarbons, nestmate recognition, fertility signals, pheromones, reproductive skew.

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Prof. Dr. Abraham Hefetz, Department of Zoology, George S. Wise Faculty of Life Science, Tel Aviv University, Ramat Aviv 69978, Israel. E-mail: hefetz@post.tau.ac.il

Introduction

Cuticular hydrocarbons (CHCs) have emerged in the past three decades as important sociochemicals (BLUM 1987). Research was first centered on their role as nestmate recognition cues, but recently accumulating evidence now points to their role also in the regulation of reproduction (two extensive reviews on the biological role of CHCs appear in (HOWARD & BLOMQUIST 1982, HOWARD & BLOMQUIST 2005). Here I discuss the multifaceted role of CHCs as sociochemicals, both as releaser (nestmate recognition) and primer (fertility signals) pheromones. I further argue that despite the apparent contradiction between their releaser and primer effects these need not be mutually exclusive, and propose a model that explains how the same chemical bouquets can serve multiple roles.

Before I discuss the various behavioral facets elicited by CHCs, it is worth noting that although hydrocarbons constitute the major lipid components on the cuticle, they are by no means the only lipids. The cuticle is fortified with both polar and non-polar constituents and includes a plethora of compounds (LOCKEY 1988, BUCKNER 1993). However, only a few studies have explicitly contrasted the role of hydrocarbon and non-hydrocarbon lipids as behavior modifiers (THOMAS & al. 1999, LAHAV & al. 1999, WAGNER & al. 2001, DIETEMANN & al. 2003, OZAKI & al. 2005). Most studies used non-polar solvents and assumed that these contain only hydrocarbons, but although these solvents extract hydrocarbons preferentially, they also extract more polar compounds that have a long alkane chain such as aliphatic alcohols long-chain fatty acids and wax-type esters (VANDER MEER & MOREL 1998).

Hydrocarbons as cuticular protective agents and co-option for communication

Cuticular hydrocarbons probably evolved primarily as a water-impermeable layer to protect the insect from desiccation (HADLEY 1994). Impermeability of hydrocarbons is due to their semi-solid state and liquid-crystal-like structure (ROURKE & GIBBS 1999), and is temperature-sensitive. Impermeability breaks down at a critical temperature, termed the breakpoint, that is species specific. Since the breakpoint is linearly related to the melting temperature of hydrocarbons, it clearly depends on the hydrocarbon composition, e.g., on their molecular weight on the one hand and their degree of branching on the other hand (GIBBS & MARKOW 2001). Straight-chain alkanes have higher melting temperatures than branched alkanes of comparable carbon chain length, and the greater the branching the lower is the melting temperatures. Thus an optimal waterproof system should be composed of a homogenous layer of only a few straight-chain alkanes. However, CHCs of many insects are rich in a large variety of branched alkanes, which suggests that branched alkanes have additional functions, the benefit of which offset their disadvantage in waterproofing. For example, their mixing with the straight-chain alkanes increases the complex fluidity, which assists in their transport through the pore canals as well as facilitates their even spread over the cuticular surface (BUCKNER 1993). Another function, unlinked to cuticular permeability, may be as antimicrobial agents. Branched fatty acids, in particular the iso- and anteiso-methyl branched fatty acids (e.g., branching at the penultimate and antepenultimate carbon, respectively) are very effective antimicrobial agents (LARSSON & al. 1975). Since fatty acids are the direct precursors of hydrocarbons (HOWARD & BLOMQUIST 2005), it is but a small leap from possessing branched fatty acids to branched hydrocarbons on the epicuticle. Moreover, branched alkanes possess higher molecular diversity than straight-chain alkanes, and are therefore assumed to possess higher informational content. This, coupled with the fact that they are spread over the body surface, may have paved the way to their cooption as semiochemicals, in particular in the social insects (HOWARD 1993). Their highly diversified composition, which enables the creation of highly specific blends, corroborates this hypothesis. The abundance of internally-branched monomethyl alkanes and that of multiply-branched alkanes (di- and trimethylalkanes) is another intriguing feature of CHCs of social insects. Such abundance imposes difficulties upon the insect because internally-branched hydrocarbons lower the temperature breakpoint of the epicuticle even further, which increases the vulnerability of the insect to desiccation even at lower ambient temperatures. I thus suggest that the abundance of these hydrocarbons evolved not only to increase the informational content of the signal, but also, by incurring an indirect cost to the insect, to make them a likely candidate for honest signaling (ZAHAVI & ZAHAVI 1999).

Studies on CHC composition in the ant *Pogonomyrmex barbatus* (SMITH, 1858) revealed their importance both as providing an impermeable cuticular surface and in communication. Workers use CHCs primarily as nestmate recognition cues (WAGNER & al. 2000), but a more extensive study of individual profiles among nestmates revealed a task-related compositional variation. Guards and foragers that are often exposed to high temperatures and low humidity have a larger proportion of straight-chain alkanes as compared to in-nest workers, in line with the individual's need for better cuticular impermeability. This was confirmed by artificially exposing in-nest workers to such conditions and showing that they developed higher proportions of straight-chain hydrocarbons as opposed to the control, laboratory-kept workers (WAGNER & al. 2001). This task-related shift seems to have been further coopted by this species for regulating task allocation, in that patrollers that return to the nest are recognized as such, thus stimulating workers to begin foraging (GREENE & GORDON 2003). Similar subcaste differences in CHC profiles were also reported in *Camponotus vagus* (SCOPOLI, 1763), where brood-tender workers removed from the nest to the foraging arena were transported back to the nest, whereas foragers were not. It was postulated that this differential treatment is due to the brood-tenders specific hydrocarbon composition (BONAVITA-COUGOURDAN & al. 1993).

These studies exemplify how through evolution the role of CHCs may have been coopted for various communicative functions. It should be emphasized that although this review is limited to ants, these phenomena are by no mean unique to ants. Similar examples can be found in other social insects as well as in non-social insects.

The role of hydrocarbons in nestmate recognition

Nestmate recognition is a fundamental characteristic of social insects that protects the colony from alien exploitation, in particular by conspecific members of other colonies. Recognition of nestmates, or the ability to discriminate between nestmates and alien individuals, is based on a label (the chemical blend used to identify the colonial origin of the individual) that each individual possesses, and a template (a neural representation of the colonial odor) to which the encountering ants compare the label. A mismatch generally results in aggression and rejection of the alien individual (LACY & SHERMAN 1983). The fact that the response to recognition cues requires antennal contact with the body surface, irrespective of which part of the body is contacted, indicates that the label is spread over the body and is embedded in the epicuticle. The nature of the template is less understood, but is believed to be neural. A recent study has indicated that discrimination may already be achieved at the peripheral neural system, i.e., the antennae (OZAKI & al. 2005), although the involvement of the antennal lobes or higher neural centers can not be excluded.

Several recent reviews on nestmate recognition have been published (VANDER MEER & MOREL 1998, LENOIR & al. 1999, HOWARD & BLOMQUIST 2005). Here I will present some of the key issues pertaining to the role of hydrocarbons in nestmate recognition, rather than provide an extensive review of the literature on the subject.

The role of hydrocarbons as recognition pheromones

Hydrocarbons, being the major lipid class on the epicuticle, are likely candidates to serve as recognition pheromones. This hypothesis is largely supported by multiple indirect correlative evidences, and in a few species by direct causative evidences.

Complexity and specificity of CHCs: The first indirect evidence is that of the extreme complexity of CHC profiles. In most species investigated, e.g., *Camponotus fellah* DALLA TORRE, 1893 (BOULAY & al. 2004), many *Cataglyphis* species (DAHBI & al. 1996), *Aphaenogaster senilis* MAYR, 1853 (LENOIR & al. 2001a), *Pogonomyrmex barbatus* (SMITH, 1858) (WAGNER & al. 2000), *Linepithema humile* (MAYR, 1868) (DE BISEAU & al. 2004), *Harpegnathos saltator* JERDON, 1851 (LIEBIG & al. 2000), the CHC profiles are composed of tens of components including alkanes, alkenes and various classes of branched alkanes. This strongly indicates that these profiles, as a single bouquet or as subsets of compounds, are communicative in nature. It is argued that if the sole role of CHCs was to provide impermeability, a single or just a few components (preferably high molecular weight straight-chain alkanes), would have been sufficient and more effective. As will be discussed later, such complexity and in particular the abundance of mono-, di- and trimethylalkanes, are supportive of the communicative function hypothesis.

One premise of nestmate recognition is that each colony bears a unique odor composition. There are multiple studies that have shown both species and colony specificity in CHCs, in accordance with their role in nestmate recognition. While species specificity generally relies on qualitative differences in the composition, between-colony differences are based on quantitative differences, i.e., the relative amounts of the blend components. Colony specificity is generally assessed by multivariate statistics such as principle components of discriminant analysis (BONAVITA-COUGOURDAN & al. 1987, HENDERSON & al. 1990, PROVOST & al. 1993, LAHAV & al. 2001, TENTSCHERT & al. 2002, LAVINE & al. 2003). Colony odor however is not constant but may change with respect to time and season (VANDER MEER & al. 1989, DAHBI & LENOIR 1998, NIELSEN & al. 1999, LAHAV & al. 2001, KATZERKE & al. 2006).

Behavioral evidence for the role of CHCs in nestmate recognition: Nestmate recognition is based on label / template matching between two encountering ants; a mismatch generally results in aggression. The bioassay therefore should include two tests: 1). Augmentation of aggression between nestmates if one is applied with alien recognition cues. 2). Diminishing aggression between alien ants if one is applied with nestmate recognition cues (LAHAV & al. 1999). Aggression is generally measured by indexing various aggressive behaviors (e.g., mandibular opening, biting, curling the abdomen in a stinging or poison ejection posture). Scoring of the aggression can range from registering the highest aggressive act in an encounter (OBIN & VANDER MEER 1988), or by formulating an aggression index taking into account both the aggressive act and its duration (HEFETZ & al. 1996, ERRARD & al. 2006). The type of encounter also varies, from encounters using two live ants to encounters of a live and a freshly-killed ant (ROULSTON & al. 2003). Only a few reports have critically tested the role of hydrocarbons as recognition cues (THOMAS & al. 1999, LAHAV & al. 1999, WAGNER & al. 2000, OZAKI & al. 2005), but all have shown that nestmate recognition resides in the hydrocarbon fraction of the extractable cuticular lipid. Additional evidence for the role of hydrocarbons in nestmate recognition comes from experiments in which the natural bouquet was supplemented by external non-native (MESKALI & al. 1995) or native (DANI & al. 2001) hydrocarbons. In the latter case it was further demonstrated that the branched alkanes rather than the straight-chain are the active component.

Hydrocarbon molecular diversity and informational content: CHCs are a variable blend of straight-chain alkanes, alkenes, and branched alkanes. From the molecular structure point of view each class is postulated to have different informational content potential. The *n* alkanes can vary only in chain length whereas alkenes can vary with respect to the numbers and positions of the double bonds, thus theoretically possessing greater potential for conveying information. Clearly, the branched alkanes can exhibit the highest variation in terms of the number of branches (mostly mono-, di- or trimethylalkanes), their position in the molecule (i.e., the branching point), and their optical configuration, thus having the greatest informational content possibilities. A study with *Polistes dominulus* (CHRIST, 1791) tested this hypothesis by applying various hydrocarbons on live wasps and observing the reactions of their nestmates upon their return to their nest. While the *n* alkanes did not have an effect, application of either alkenes or branched alkanes elicited aggression towards these wasps, with a slightly stronger reaction towards wasps applied with the branched alkanes (DANI & al. 2001). This suggested that it is the branched alkanes that are mostly responsible for encoding colonial identity, while the straight-chain alkanes may function to provide a waterproof epicuticle. However, straight-chain alkanes can still play a role in providing additional complexity to the hydrocarbon blend, and thereby increase its informational content. A recent study in the ants *Linepithema humile* and *Apaenogaster cockerelli* ANDRÉ, 1893 demonstrated that at least two structural classes of hydrocarbons are necessary for species and nestmate recognition, but without preference of specific hydrocarbon classes (GREENE & GORDON 2007). Another interesting point in the selection of branched alkanes for increasing the informational content of a CHC blend is that, as mentioned above, their abundance is correlated with a lower melting point of the epicuticle. This increase in vulnerability to desiccation may indicate that these hydrocarbons constitute an honest signal.

An indirect confirmation of the importance of how much information CHCs might transmit comes from a study with *Formica truncorum* FABRICIUS, 1804 (BOOMSMA & al. 2003). Nests headed by multiply inseminated queens show a clear split sex ratio and the bias towards male correlates with the differences in CHC profiles. The authors argue that the differences in patriline profiles contain sufficient information to gain inclusive fitness through biasing the sex ratio, but not for exploiting and gaining individual fitness by nepotistic behavior. This is supported by the poor representation of CHCs in the epicuticle of *F. truncorum*, consisting primarily of only a few *n* alkanes with two minor alkene peaks and no branched alkanes. However, these unusually simple CHC blends may have been the result of incomplete chemical analysis. A recent study analyzed the CHCs of this species from Japan and Finland using a GC column that permitted analyses at much higher oven temperature, revealing a complex mixture of about 53 components, most of which was in a higher range of molecular (>C31) weight than described before. Furthermore, the profile was rich in monomethyl- and dimethylalkanes (AKINO 2006). Unfortunately, neither study tested directly whether workers actually perceive these hydrocarbons.

Both the behavioral and chemical evidence suggesting that CHCs are involved in nestmate recognition point to the fact that the differences between colonies is quantitative rather than qualitative, i.e., different colonies present different chemical profiles based on the relative abundance of the same components. This raises an important sensory question: are these profiles perceived as individual components and profiling is performed in the brain; or are specific profiles already perceived as a unit by the peripheral neural system? A recent study with *Camponotus japonicus* has shown that in ants the latter seems to be the selected option (OZAKI & al. 2005). It was demonstrated that workers reacted aggressively to nestmates that were painted with alien CHCs, and that this recognition was based on perception of the entire blend by a single sensillum. The electrophysiological study that showed that this sensillum reacted to alien but not nestmate profiles, was supplemented by an ultrastructure study that demonstrated that the sensillum has multiple receptors, presumably for each of the reactive components in the blend.

Colony odor uniformity and specificity

One of the premises of nestmate recognition is that members of a particular nest possess specific recognition cues that differ at least from those of ants from neighboring nests. Several hypotheses have been suggested as to the mechanism to obtain nest specificity (CROZIER & DIX 1979). The "individual blend hypothesis" suggests that each nest member has unique recognition cues that are recognized by all other nestmates. This requires an extremely acute sensory discriminatory ability coupled with a large memory capacity (HEFETZ & GRAUR 1988), and it is generally accepted that if this model exists it must be limited to very small colonies. The "colony gestalt hypothesis" suggests that all members of a colony bear a uniform and unique odor blend. This can be created either by possessing a specific blend of "odor alleles" or by blending all individual odors into a uniform colony odor. This model is applicable both to small and large colonies and requires limited blend variation as well as limited sensory acuteness.

There are also two alternative models for attaining a uniform colony odor. The "queen-centered hypothesis" suggests that the queen produces specific recognition cues that are disseminated to all nest members. This model solves the problem of genetic vs. phenotypic heterogeneity since in most species the probability of neighboring queens being related are generally low. Several requirements have to be met in the queen-centered model. First, since recognition cues are spread over the individual body, the queen needs to produce copious amounts of the pheromone so that sufficient secretion can be applied on each member of the colony. Second, there should be a mechanism by which the substances disseminate within the nest: workers may acquire the recognition pheromones either directly from the queen or secondarily from workers that have already acquired it. Lastly, although the acquired substances can evenly spread over the entire body surface by diffusion, this will be very inefficient, necessitating the use of a more active mechanism such as self- and allogrooming. Most important to the present discussion, is that if CHCs act both as queen-born recognition and queen fertility signals (see below) there should be a mechanism that compartmentalizes the two sets of hydrocarbons according to function.

The alternative hypothesis is the "worker-centered hypothesis", which suggests that nest odor uniformity is obtained by admixing individual blends to create a colony gestalt. This requires a constant exchange of recognition cues between nestmates. It also best accommodates findings that recognition cues may also be affected by environmental factors such as diet and nest materials (OBIN & VANDER MEER 1988, CROSLAND 1989, HEINZE & al. 1996).

Supporting evidence for the "queen centered hypothesis" includes elegant studies with *Camponotus* spp., in which by using heterospecific and homospecific cross-fostering it was demonstrated that the queen has a decisive role in the formation of colony odor and that her own label masks any innate label that workers may possess (CARLIN & HÖLLDOBLER 1983, 1986, 1987). In *Temnothorax lichtensteini* (BONDROIT, 1918), it was shown that two queenless groups of workers were able to maintain their common colony odor if the queen was placed alternately in these groups (PROVOST 1989). Chemical evidence for queen effect comes from comparative chemical analysis of cuticular hydrocarbon profiles of artificially-created colonies of *Messor barbarus* (LINNAEUS, 1767) with one, two, or three queens, revealing divergences that were interpreted as queen contribution (PROVOST & al. 1994).

Studies supporting the "worker-centered hypothesis" include experiments with *Rhytidoponera confusa* WARD, 1980, where workers housed with an alien queen were still able to maintain their original recognition label and were treated as nestmates upon return to their natal nest (CROSLAND 1990). Likewise, *Temnothorax curvispinosus* (MAYR, 1866) workers kept in isolation from the pupal stage were readily accepted by their parent colonies when reintroduced as adults (STUART 1987). Finally, chemical studies with *Cataglyphis niger* (ANDRÉ, 1881) have shown that colony label is contributed by workers and not the queen (LAHAV & al. 1998), and that constant and efficient exchanges of reconition cues occurs either through body contact, allogrooming or trophallaxis (SOROKER & al. 1995). Experimental evidence has further singled out the postpharyngeal gland as a prime exocrine gland implicated in gestalt formation, whereby acquired recognition cues are admixed and from which they are further disseminated (HEFETZ & al. 1992, SOROKER & al. 1994, VIENNE & al. 1995). The position of the gland and its opening to the buccal cavity is optimal for such a function. Secretion can be acquired as well as disseminated rapidly through allogrooming and / or trophallaxis, and spread evenly over the individual's body by self-grooming. Moreover, any exogenous substances such as food or nest odors can be sequestered by the gland and through that blend into the recognition bouquet. Uniform colony odor is achieved most rapidly in species that perform trophallaxis, with less rapidity in species that perform only allogrooming and the slowest in species that perform neither (SOROKER & al. 1998, LENOIR & al. 2001b, SOROKER & al. 2003).

The role of CHCs in signaling fertility

A prominent phenomenon in social insects is the marked reproductive skew whereby reproduction is limited to one or a few individuals in the colony while most of the other nest members are sterile and help in rearing the offspring of the fertile individuals. The evolution of worker sterility is enigmatic and the prevailing theory explaining it is kin selection (HAMILTON 1964, HAMILTON 1972). Two contrasting, but not necessarily mutually exclusive, hypotheses have been proposed for achieving and maintaining such reproductive skew. The "queen-control hypothesis" suggests that the queen coercively inhibits worker reproduction, and that we expect a sequential arms race between the queen and workers. The alternative "worker control hypothesis" suggests that workers will refrain from reproduction as long as the cost in selfish reproduction surpasses the inclusive fitness gained in helping the reproductive individuals (KELLER & NONACS 1993, HAMMOND & KELLER 2004). Both hypotheses predict that the queens possess caste-specific pheromones that either suppress worker ovarian development (queen primer pheromone), or signal workers of the queen's presence and her reproductive potential (queen fertility signal). Fertility signals have been the focus of many studies in the past decade, most of which have singled out CHCs as such signals.

Evidence for fertility-specific CHC profiles

The first line of evidence that CHCs function as fertility signals comes from correlation studies in which the CHCs were shown to be fertility-specific. In ponerine ants, workers can mate (and thus are called gamergates) and in the absence of the queen (or when she becomes senescent) they take over reproduction while forming dominance hierarchies. Other species lack queens completely and reproduction is done solely by gamergates. Gamergates are typified by distinct profiles as judged by their separation from infertile workers in discriminant analyses (MONNIN & al. 1998, LIEBIG & al. 2000, CUVILLIER-HOT & al. 2001, CUVILLIER-HOT & al. 2004a). It is important to note that in most of the studies hydrocarbon analysis was based on SPME (solid phase microextraction) and therefore they lack absolute quantitative data, but rely on the relative abundance of one or more components. Furthermore, since the sample size (ants) is generally small compared to the variables investigated (individual hydrocarbons in the blend) the discriminant analysis is performed only on partial blends, generally considering the components that contribute most to the inter-individual variation. While these may be the important components in the blend, this is not necessarily so, and further behavioral experimentation with natural or synthetic blends is needed.

There is evidence that CHCs also act as fertility signals among other ant subfamilies. In the bulldog ant *Myrmecia gulosa* (FABRICIUS, 1775) queens and fertile workers have specific hydrocarbon profiles both on the epicuticle and the postpharyngeal gland that are typically rich in 9-pentacosene and 3-methylpentacosane. It appears that the presence of the queen profile is sufficient to induce worker policing, and thus results in worker reproductive self-restraint (DIETEMANN & al. 2003). In the carpenter ant *Camponotus floridanus* (BUCKLEY, 1866) queen CHC profile is distinct from that of the workers in having an additional set of hydrocarbons in the C-25 to C-27 range that is absent or exists only as traces in workers. As in the case of *Dinoponera quadriceps* KEMPF, 1971, queen and worker eggs have profiles that are characteristic to the caste that laid them and are congruent with that individual's CHCs (ENDLER & al. 2004). Fertility signals also characterize fecundity of the different queens in polygynous species. In *Formica fusca* LINNAEUS, 1758, queens with higher fertility received more attention from workers. Since fertility is correlated with the queen CHCs profile, with 5,13-dimethylpentacosane and 5-methylpentacosane being the most important components for queen discrimination, it is assumed to be the basis for worker recognition (HANNONEN & al. 2002). Similar preferential treatment of more fecund queens was also demonstrated to be chemically mediated in *Leptothorax* sp. A, but a chemical analysis was not provided (ORTIUS & HEINZE 1999). In the Argentine ant *Linepithema humile* fecund queens possess a distinctive CHC profile. Since workers in this invasive species are permanently sterile, these profile differences are assumed to be important in regulating the production of new sexuals in the colony (DE BISEAU & al. 2004).

Indirect evidence – changes in profiles with changes in status

Additional evidence of the role of CHCs as fertility signals comes from experiments where dominance hierarchy was manipulatively altered. In *Dinoponera quadriceps* for example, gamergates possess significantly greater relative amounts of 9-hentriacontane than the beta workers. However, if we allow betas to take over reproduction by removing the gamergates they start to produce large amounts of this compound concomitant with ovarian development (PEETERS & al. 1999). Moreover, 9-hentriacontane is also present only on the eggs of the gamergates, protecting them from cannibalism by nestmate workers (MONNIN & PEETERS 1997). A more causative correlation was shown in *Streblognathus peetersi* ROBERTSON, 2002, in which the differences between fertile and infertile workers are expressed as shifts in the complete CHC blend (CUVILLIER-HOT & al. 2004a). A close look at these differences reveals that infertile workers possess several compounds that are practically absent in egg-laying alpha workers (figure 3 in the above study), which raises the possibility that the disappearance, rather than the elevation, of a subset of compounds may mark the fertile individuals. In this species juvenile hormone (JH) is associated with low ranking and sterility (BRENT & al. 2006); thus application of pyriproxyfen (a JH analog) on the alpha worker lowered her reproductive capacity. Such downgrading of the alpha worker was accompanied by a switch in hydrocarbon profile from gamergate-like to infertile-worker-like, a change that was recognized by nestmate workers, eliciting their aggression towards the downgraded gamergate (CUVILLIER-HOT & al. 2004b). In *Pachycondyla inversa* SMITH, 1858 queens are typified by an abundance of 3,11-dimethylheptacosane, which also accumulates in egg-laying workers concomitant with their ovarian development (HEINZE & al. 2002). Coupled GC / EAD studies revealed that workers are able to perceive 3,11-dimethylheptacosane, which is consistent with its presumed pheromonal activity (D'ETTORRE & al. 2004), but direct behavioral assays using the synthetic hydrocarbon are still lacking.

Direct evidence – manipulating worker behavior with caste specific CHCs

More direct evidence for the role of CHCs comes from a recent study with the carpenter ant *Camponotus floridanus* (BUCKLEY, 1866) (ENDLER & al. 2004). This species forms large monogyne colonies with a clear reproduction bias towards the queen. Although workers can lay viable male eggs, they do not reproduce in the presence of the queen even if located in satellite nests away from direct contact with the queen. Since brood is transferred between the satellite nests it was assumed that the presence of the brood signals the fact that the colony has a fecund queen. Indeed, replacing the queen with her eggs – but not larvae and pupae – inhibited worker reproduction. Moreover, worker eggs did not have such an effect, and in fact were rapidly destroyed in the presence of a queen. The basis of this discrimination seems to be differences in hydrocarbon profiles, which match the cuticular hydrocarbon of the individual that produced the eggs. Moreover, painting worker eggs with queen CHCs not only protected them from destruction, but also effectively inhibited worker ovarian development. CHCs in *C. floridanus* seem to fulfill another premise of fertility signals – that their profiles are correlated with queen fecundity (ENDLER & al. 2006).

Chemical basis of CHC specificity in fertility signaling – from a single compound to complete profiles

The effective use of CHCs as fertility signals requires that these will be specific to the reproductive individuals, will be perceived continuously by workers and, at least in large colonies, the existence of an effective mode of information transfer, i.e., signal dissemination, within the nest. An overview of analyses of fertility signal chemistry in ants reveals several patterns. The most simple model and easiest to explain is that fertility is signaled by a fertility-specific single component or a small subset of compounds out of the complex CHC blend. This may be the case of 9-hentriacontene in *Dinoponera quadriceps* or 3,11-dimethylheptacosane in *Pachycondyla inversa* that are specific to gamergates / queens, respectively, and whose production is boosted in dominant workers to coincide with the advancement of their ovarian development (MONNIN & al. 1998, PEETERS & al. 1999, HEINZE & al. 2002). These cases are similar to that found in the honeybee *Apis mellifera* LINNAEUS, 1758, in which the mandibular and Dufour's gland secretion have a specific queen composition but that also occurs in egg-laying workers (SLESSOR & al. 1988, KATZAV-GOZANSKY & al. 1997). The acquisition of these compounds in the ants may be via direct contact with the fertile individual, since their volatility is very low, or with other workers that may act as messenger ants, similar to the queen mandibular gland pheromone of honeybees (NAUMANN & al. 1991). In the ants, small amounts of the putative fertility signals are also found on sterile workers, but it is unclear whether these originate from endogenous synthesis or are acquired from the reproductive ant through physical contact. If worker ants act as pheromone dissemination units, it has to be assumed that the half-life of the pheromones on workers is limited, as found in honeybees (WINSTON & SLESSOR 1992).

The second system that emerges is the use of a larger CHC subset of the profile (about 50 % of the composition) to delineate fertility, as exemplified in the differences between queens and sterile workers in *Camponotus floridanus* (ENDLER & al. 2004), or differences between fertile and infertile queens in *Linepithema humile* (DE BISEAU & al. 2004). In these cases, too, the sterile workers possess minute amounts of the queen-specific components, whose origin in the workers is also elusive.

The most commonly described system for signaling fertility via CHCs is through differences in the complete profile. These are quantitative differences in the relative intensities of the blend components that are not readily apparent, but can be analyzed by multivariate statistics such as principle component or discriminant analyses (LIEBIG & al. 2000, CUVILLIER-HOT & al. 2002, DIETEMANN & al. 2005, HARTMANN & al. 2005, CUVILLIER-HOT & LENOIR 2006, LOMMELEN & al. 2006). Such a quantitative basis for recognition of the fertile individuals raises the question of signal dissemination particularly in populated colonies. For such a signal to be disseminated within the nest by messenger ants, the fertile individual must produce copious amounts of the secretion for sufficient dispersal, and both the queen and worker profiles must be extremely dynamic, e.g., rapidly metabolized, as otherwise the nest will be rapidly saturated with the queen specific profile to the point of habituation and ineffectiveness. None of these has been demonstrated experimentally to date. One way to overcome these problems is to mark the eggs with the queen profile, as described for *Camponotus floridanus* (ENDLER & al. 2004).

Resolving the duality of CHC role in ant sociality

The use of CHCs as both a fertility signal and a nestmate recognition pheromone may seem contradictory since the first function requires within-nest idiosyncrasy, i.e., for discriminating fertile from sterile individuals, while the second function requires within-colony odor uniformity. How can we reconcile these two seemingly opposite requirements? Fertility signals comprising a single hydrocarbon or a small subset of compounds do not pose a real problem, since they are unique to the queen and can be readily catabolized by workers upon contamination. We can expect the evolution of such a process because any non-fertile workers that keep the signal for long will be recognized as superfluous reproductives and thus attacked by their nestmate. In contrast, a system that relies on differences in a large subset of components or even the entire profile is more problematic. Maintaining a fertility-specific hydrocarbon profile means either that these compounds do not serve as nestmate recognition cues, or that the fertile individual is outside the colony gestalt. Except for one report (FRANKS & al. 1990) all other studies on nestmate recognition in ants point to CHCs as the signal. However, the number of species investigated so far is rather small, prohibiting any generalization regarding the chemistry of nestmate recognition. Unfortunately, none of the species studied with respect to nestmate recognition were also studied with respect to fertility signaling and vice versa. If CHCs do function in nestmate recognition, and if the gestalt model prevails for this species, it is hard to conceive how the queen is not contaminated by the worker hydrocarbons during in-nest interactions. One way to overcome such contamination would be to have a more dynamic hydrocarbon metabolism that will rapidly mask any adsorption of worker-born hydrocarbons. This awaits explicit studies of queen vs. worker hydrocarbon turnover. Another possibility is that CHCs can be used for both function, but the distinction is made at the perception level (LE CONTE & HEFETZ in press). In this model we suggest that workers have different thresholds for discrimination in different contexts. For example, discriminating between nestmates and alien ants is rather simple, since we expect high quantitative differences between different nests, and therefore the perception threshold for discrimination may be high. Task differences in profile may require a slightly lower threshold, with smaller differences being recognized but the response – instead of aggression – being the regulation of task allocation. A special case of such discriminatory threshold may be the queen (or any fertile individual). Finally, the lowest threshold of discrimination may be that of different patrilines, which enable for example the adjustment of sex ratio (BOOMSMA & al. 2003) or even nepotism (HANNONEN & SUNDSTROM 2003). The advantage of this proposed discriminatory mechanism lies in its high plasticity, and in that the degree of threshold sensitivity could be shaped by natural selection. Each species is selected to stop at a certain level of specificity, without the more elaborate need to alter its pheromone chemistry.

In summary, CHCs provide an excellent example of the cooption of chemicals that originally functioned to regulate physiology, to function also in communication. One of the problems in studying hydrocarbons, despite the relative ease of their identification, is that their synthesis is not simple, in particular those that may have one or more optical isomers. Their extreme complexity also renders any bioassay laborious to the point of impossibility. This may be the reason why there are no published studies that have attempted to test the multiple hypotheses presented in this review in causative rather than correlative experiments.

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Zusammenfassung

Kohlenwasserstoffe sind die wichtigsten Lipidkomponenten der Kutikula von Insekten und haben bei diesen generell die Funktion einer wasserdichten Schichte zum Schutz vor Austrocknung. Bei vielen Insekten ist für diese chemische Stoffklasse aber eine Rolle als Pheromone hinzugekommen. Bei sozialen Insekten, insbesondere bei Ameisen, haben kutikuläre Kohlenwasserstoffe (CHC) zumindest zwei verschiedene Funktionen als Pheromone. Sie fungieren als Erkennungssignale, die den Zusammenhalt der Kolonie ermöglichen und sie vor der Infiltration durch Parasiten oder auch Artgenossen bewahren. Diese Rolle von CHC wird untermauert durch ihre extreme Komplexität, ihre koloniespezifische Zusammensetzung sowie in einigen Fällen auch durch Versuchsergebnisse, die eine gesteigerte oder verringerte Aggression zwischen aufeinandertreffenden Ameisen als Funktion des experimentell aufgetragenen Geruchs (Nestgenosse oder Nicht-Nestgenosse) ergeben haben. Die zweite Funktion von CHC ist jene von Fertilitätssignalen. Es wurde für viele Ameisenarten gezeigt, dass sich die CHC Profile von fertilen Individuen (Königinnen, Gamergaten oder Eier legende Arbeiterinnen) deutlich von jenen steriler Nestgenossen unterscheiden. Diese Information kann in der verstärkten Intensität einer einzelnen Substanz oder einer kleinen Untermenge der gesamten Mischung kodiert sein, oder auch in Unterschieden der gesamten Mischung. Die Tatsache, dass diese Signale häufig auf verzweigten Alkanen beruhen, welche die Undurchlässigkeit der Kutikula verringern und somit Kosten für das Individuum darstellen, deutet darauf hin, dass es sich bei ihnen um "ehrliche Signale" handelt.

Diese duale Funktion erscheint insofern widersprüchlich, als zur Erkennung von Nestgenossen ein einheitlicher Koloniegeruch, also einheitliche CHC Zusammensetzung, erforderlich ist, zum Signalisieren von Fertilität aber das Vorhandensein individueller Eigenheiten, da ja das fertile Individuum aus der Menge der restlichen Kolonieangehörigen heraus erkennbar sein muss und nicht Teil eines Einheitsgeruchs sein darf. Eine mögliche Lösung dafür ist, dass Arten, die CHC zur Erkennung von Nestgenossen verwenden, diese nicht als Fertilitätssignal nutzen und umgekehrt. Ich schlage aber eine alternative Lösung vor, wonach Arbeiterinnen sich eines variablen Schwellenwerts zur Unterscheidung bedienen können und auf deutliche und geringfügige Unterschiede in der Pheromonmischung kontextabhängig unterschiedlich reagieren.

References

AKINO, T. 2006: Cuticular hydrocarbons of *Formica truncorum* (Hymenoptera: Formicidae): description of new very long chained hydrocarbon components. – Applied Entomology and Zoology 41: 667-677.

BLUM, M.S. 1987: The basis and evolutionary significance of recognitive olfactory acuity in insect societies. In: PASTEELS, J. & DENEUBOURG, J.-L. (Eds.): From individual to collective behavior in social insects (Experientia supplement). – Birkhäuser Verlag, Basel, pp. 277-293.

BONAVITA-COUGOURDAN, A., CLÉMENT, J.L. & LANGE, C. 1987: Nestmate recognition: The role of cuticular hydrocarbons in the ant *Camponotus vagus* SCOP. – Journal of Entomological Science 22: 1-10.

BONAVITA-COUGOURDAN, A., CLÉMENT, J.L. & LANGE, C. 1993: Functional subcaste discrimination (foragers and brood-tenders) in the ant *Camponotus vagus* SCOP.: Polymorphism of cuticular hydrocarbon patterns. – Journal of Chemical Ecology 19: 1461-1477.

BOOMSMA, J.J., NIELSEN, J., SUNDSTROM, L., OLDHAM, N.J., TENTSCHERT, J., PETERSEN, H.C. & MORGAN, E.D. 2003: Informational constraints on optimal sex allocation in ants. – Proceedings of the National Academy of Sciences of the United States of America 100: 8799-8804.

BOULAY, R., KATZAV-GOZANSKY, T., HEFETZ, A. & LENOIR, A. 2004: Odour convergence and tolerance between nestmates through trophallaxis and trooming in the ant *Camponotus fellah* (Dalla Torre). – Insectes Sociaux 51: 55-61.

BRENT, C., PEETERS, C., DIETEMANN, V., CREWE, R. & VARGO, E. 2006: Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*. – Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology 192: 315-320.

BUCKNER, J.S. 1993: Cuticular polar lipids of insects. In: STANLEY-SAMUELSON, D.W. & NELSON, D.R. (Eds.): Insect lipids: chemistry, biochemistry and biology. – University of Nebraska Press, Lincoln and London, pp. 227-270.

CARLIN, N.F. & HÖLLDOBLER, B. 1983: Nestmate and kin recognition in interspecific mixed colonies of ants. – Science 222: 1027-1029.

CARLIN, N.F. & HÖLLDOBLER, B. 1986: The kin recognition system of carpenter ants (*Camponotus* spp.) I. Hierarchical cues in small colonies. – Behavioral Ecology and Sociobiology 19: 123-134.

CARLIN, N.F. & HÖLLDOBLER, B. 1987: The kin recognition system of carpenter ants (*Camponotus* spp.) II. Larger colonies. – Behavioral Ecology and Sociobiology 20: 209-217.

CROSLAND, M.W.J. 1989: Kin recognition in the ant *Rhytidoponera confusa* I. Environmental odour. – Animal Behaviour 37: 912-917.

CROSLAND, M.W.J. 1990: The influence of the queen, colony size and worker ovarian development on nestmate recognition in the ant *Rhytidoponera confusa*. – Animal Behaviour 39: 413-425.

CROZIER, R.H. & DIX, M.W. 1979: Analysis of two genetic models for the innate components of colony odour in social Hymenoptera. – Behavioral Ecology and Sociobiology 4: 217-224.

CUVILLIER-HOT, V., COBB, M., MALOSSE, C. & PEETERS, C. 2001: Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. – Journal of Insect Physiology 47: 485-493.

CUVILLIER-HOT, V., GADAGKAR, R., PEETERS, C. & COBB, M. 2002: Regulation of reproduction in a queenless ant: aggression, pheromones and reduction in conflict. – Proceedings of the Royal Society of London Series B-Biological Sciences 269: 1295-1300.

CUVILLIER-HOT, V. & LENOIR, A. 2006: Biogenic amine levels, reproduction and social dominance in the queenless ant *Streblognathus peetersi*. – Naturwissenschaften 93: 149-153.

CUVILLIER-HOT, V., LENOIR, A., CREWE, R., MALOSSE, C. & PEETERS, C. 2004a: Fertility signalling and reproductive skew in queenless ants. – Animal Behaviour 68: 1209-1219.

CUVILLIER-HOT, V., LENOIR, A. & PEETERS, C. 2004b: Reproductive monopoly enforced by sterile police workers in a queenless ant. – Behavioral Ecology 15: 970-975.

D'ETTORRE, P., HEINZE, E., SCHULZ, C., FRANCKE, W. & AYASSE, M. 2004: Does she smell like a queen? Chemoreception of a cuticular hydrocarbon signal in the ant *Pachycondyla inversa*. – Journal of Experimental Biology 207: 1085-1091.

DAHBI, A. & LENOIR, A. 1998: Nest separation and dynamics of the Gestalt odor in the polydomous ant *Cataglyphis iberica* (Hymenoptera, Formicidae). – Behavioral Ecology and Sociobiology 42: 349-355.

DAHBI, A., LENOIR, A., TINAUT, A., TAGHIZADEH, T., FRANCKE, W. & HEFETZ, A. 1996: Chemistry of the postpharyngeal gland secretion and its implication for the phylogeny of Iberian *Cataglyphis* species (Hymenoptera: Formicidae). – Chemoecology 7: 163-171.

DANI, F.R., JONES, G.R., DESTRI, S., SPENCER, S.H. & TURILLAZZI, S. 2001: Deciphering the recognition signature within the cuticular chemical profile of paper wasps. – Animal Behaviour 62: 165-171.

DE BISEAU, J.C., PASSERA, L., DALOZE, D. & ARON, S. 2004: Ovarian activity correlates with extreme changes in cuticular hydrocarbon profile in the highly polygynous ant, *Linepithema humile*. – Journal of Insect Physiology 50: 585-593.

DIETEMANN, V., LIEBIG, J., HÖLLDOBLER, B. & PEETERS, C. 2005: Changes in the cuticular hydrocarbons of incipient reproductives correlate with triggering of worker policing in the bulldog ant *Myrmecia gulosa*. – Behavioral Ecology and Sociobiology 58: 486-496.

DIETEMANN, V., PEETERS, C., LIEBIG, J., THIVET, V. & HÖLLDOBLER, B. 2003: Cuticular hydrocarbons mediate discrimination of reproductives and nonreproductives in the ant *Myrmecia gulosa*. – Proceedings of the National Academy of Sciences of the United States of America 100: 10341-10346.

ENDLER, A., LIEBIG, J. & HÖLLDOBLER, B. 2006: Queen fertility, egg marking and colony size in the ant *Camponotus floridanus*. – Behavioral Ecology and Sociobiology 59: 490-499.

ENDLER, A., LIEBIG, J., SCHMITT, T., PARKER, J.E., JONES, G.R., SCHREIER, P. & HÖLLDOBLER, B. 2004: Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. – Proceedings of the National Academy of Sciences of the United States of America 101: 2945-2950.

ERRARD, C., HEFETZ, A. & JAISSON, P. 2006: Social discrimination tuning in ants: Template formation and chemical similarity. – Behavioral Ecology and Sociobiology 59: 353-363.

FRANKS, N.R., BLUM, M.S., SMITH, R. & ALLIES, A.B. 1990: Behaviour and chemical disguise of cuckoo ant *Leptothorax kutteri* in relation to its host *Leptothorax acervorum*. – Journal of Chemical Ecology 16: 1431-1444.

GIBBS, A.G. & MARKOW, T.A. 2001: Effects of age on water balance in *Drosophila* species. – Physiological and Biochemical Zoology 74: 520-530.

GREENE, M.J. & GORDON, D.M. 2003: Cuticular hydrocarbons inform task decisions. – Nature 423: 32.

GREENE, M.J. & GORDON, D.M. 2007: Structural complexity of chemical recognition cues affects the perception of group membership in the ants *Linephithema humile* and *Aphaenogaster cockerelli*. – Journal of Experimental Biology 210: 897-905.

HADLEY, N.F. 1994: Water relations of terrestrial arthropods. – Academic Press, San Diego, CA, 356 pp.

HAMILTON, W.D. 1964: The genetical evolution of social behaviour. I, II. – Journal of Theoretical Biology 7: 1-52.

HAMILTON, W.D. 1972: Altruism and related phenomena mainly in the social insects. – Annual Review of Ecology and Systematics 2: 193-232.

HAMMOND, R.L. & KELLER, L. 2004: Conflict over male parentage in social insects. – PLoS Biology 2: 1472-1482.

HANNONEN, M., SLEDGE, M.F., TURILLAZZI, S. & SUNDSTRÖM, L. 2002: Queen reproduction, chemical signalling and worker behaviour in polygyne colonies of the ant *Formica fusca*. – Animal Behaviour 64: 477-485.

HANNONEN, M. & SUNDSTRÖM, L. 2003: Worker nepotism among polygynous ants. – Nature 421: 910.

HARTMANN, A., D'ETTORRE, P., JONES, G.R. & HEINZE, J. 2005: Fertility signaling – the proximate mechanism of worker policing in a clonal ant. – Naturwissenschaften 92: 282-286.

HEFETZ, A., ERRARD, C., CHAMBRIS, A. & LE NÉGRATE, A. 1996: Postpharyngeal gland secretion as a modifier of aggressive behavior in the myrmicine ant *Manica rubida*. – Journal of Insect Behavior 9: 709-717.

HEFETZ, A., ERRARD, C. & COJOCARU, M. 1992: Heterospecific substances in the postpharyngeal gland of ants reared in mixed groups. – Naturwissenschaften 79: 417-420.

HEFETZ, A. & GRAUR, D. 1988: The significance of multicomponent pheromones in denoting specific compositions. – Biochemical Systematics and Ecology 16: 557-566.

HEINZE, J., FOITZIK, S., HIPPERT, A. & HÖLLDOBLER, B. 1996: Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. – Ethology 102: 510-522.

HEINZE, J., STENGL, B. & SLEDGE, M.F. 2002: Worker rank, reproductive status and cuticular hydrocarbon signature in the ant, *Pachycondyla* cf. *inversa*. – Behavioral Ecology and Sociobiology 52: 59-65.

HENDERSON, G., ANDERSEN, J.F., PHILIPS, J.K. & JEANNE, R.L. 1990: Internest aggression and identification of possible nestmate discrimination pheromones in polygynous ant *Formica montana*. – Journal of Chemical Ecology 16: 2217-2228.

HOWARD, R.W. 1993: Cuticular hydrocarbons and chemical communication. In: STANLEY-SAMUELSON, D.W. & NELSON, D.R. (Eds): Insect lipids: chemistry, biochemistry and biology. – University of Nebraska Press, Lincoln and London, pp. 179-226.

HOWARD, R.W. & BLOMQUIST, G.J. 1982: Chemical ecology and biochemistry of insect hydrocarbons. – Annual Review of Entomology 27: 149-172.

HOWARD, R.W. & BLOMQUIST, G.J. 2005: Ecological, behavioral, and biochemical aspects of insect hydrocarbons. – Annual Review of Entomology 50: 371-393.

KATZAV-GOZANSKY, T., SOROKER, V., HEFETZ, A., COJOCARU, M., ERDMANN, D.H. & FRANCKE, W. 1997: Plasticity of caste-specific Dufour's gland secretion in the honey bee (*Apis mellifera* L.). – Naturwissenschaften 84: 238-241.

KATZERKE, A., NEUMANN, P., PIRK, C.W.W., BLISS, P. & MORITZ, R.F.A. 2006: Seasonal nestmate recognition in the ant *Formica exsecta*. – Behavioral Ecology and Sociobiology 61: 143-150.

KELLER, L. & NONACS, P. 1993: The role of queen pheromones in social insects: Queen control or queen signal? – Animal Behaviour 45: 787-794.

LACY, R.C. & SHERMAN, P.W. 1983: Kin recognition by phenotype matching. – American Naturalist 121: 489-512.

Lahav, S., Soroker, V., Hefetz, A. & Vander Meer, R.K. 1999: Direct behavioral evidence for hydrocarbons as ant recognition discriminators. – Naturwissenschaften 86: 246-249.

Lahav, S., Soroker, V., Vander Meer, R.K. & Hefetz, A. 1998: Nestmate recognition in the ant *Cataglyphis niger*: Do queens matter? – Behavioral Ecology and Sociobiology 43: 203-212.

Lahav, S., Soroker, V., Vander Meer, R.K. & Hefetz, A. 2001: Segregation of colony odor in the desert ant *Cataglyphis niger*. – Journal of Chemical Ecology 27: 927-943.

Larsson, K., Noren, B. & Odham, G. 1975: Antimicrobial effect of simple lipids with different branches at the methyl end group. – Antimicrobial Agents and Chemotherapy 8: 742-750.

Lavine, B.K., Davidson, C., Vander Meer, R.K., Lahav, S., Soroker, V. & Hefetz, A. 2003: Genetic algorithms for deciphering the complex chemosensory code of social insects. – Chemometrics and Intelligent Laboratory Systems 66: 51-62.

Le Conte, Y. & Hefetz, A. in press: Primer pheromones in social Hymenoptera. – Annual Review of Entomology 53.

Lenoir, A., Cuisset, D. & Hefetz, A. 2001a: Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae). – Insectes Sociaux 48: 101-109.

Lenoir, A., Fresneau, D., Errard, C. & Hefetz, A. 1999: Individuality and colonial identity in ants: the emergence of the social representation concept. In: Detrain, C., Deneubourg, J.L. & Pasteels, J.M. (Eds): Information processing in social insects. – Birkhäuser Verlag, Basel, pp. 219-237.

Lenoir, A., Hefetz, A., Simon, T. & Soroker, V. 2001b: Comparative dynamics of gestalt odour formation in two ant species *Camponotus fellah* and *Aphaenogaster senilis* (Hymenoptera: Formicidae). – Physiological Entomology 26: 275-283.

Liebig, J., Peeters, C., Oldham, N.J., Markstadter, C. & Hölldobler, B. 2000: Are variations in cuticular hydrocarbons of queens and workers a reliable signal of fertility in the ant *Harpegnathos saltator*? – Proceedings of the National Academy of Sciences of the United States of America 97: 4124-4131.

Lockey, K.H. 1988: Lipids of the insect cuticle: origin, composition and function. – Comparative Biochemistry and Physiology 89B: 595-645.

Lommelen, E., Johnson, C.A., Drijfhout, F.P., Billen, J., Wenseleers, T. & Gobin, B. 2006: Cuticular hydrocarbons provide reliable cues of fertility in the ant *Gnamptogenys striatula*. – Journal of Chemical Ecology 32: 2023-2034.

Meskali, M., Provost, E., Bonavita-Cougourdan, A. & Clément, J.-L. 1995: Behavioural effects of an experimental change in the chemical signature of the ant *Camponotus vagus* (Scop.). – Insectes Sociaux 42: 347-358.

Monnin, T., Malosse, C. & Peeters, C. 1998: Solid-phase microextraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. – Journal of Chemical Ecology 24: 473-490.

Monnin, T. & Peeters, C. 1997: Cannibalism of subordinates' eggs in the monogynous queenless ant *Dinoponera quadriceps*. – Naturwissenschaften 84: 499-502.

Naumann, K., Winston, M.L., Slessor, K.N., Prestwich, G.D. & Webster, F.X. 1991: Production and transmission of honey bee queen (*Apis mellifera* L.) mandibular gland pheromone. – Behavioral Ecology and Sociobiology 29: 321-332.

Nielsen, J., Boomsma, J.J., Oldham, N.J., Petersen, H.C. & Morgan, E.D. 1999: Colony-level and season-specific variation in cuticular hydrocarbon profiles of individual workers in the ant *Formica truncorum*. – Insectes Sociaux 46: 58-65.

Obin, M.S. & Vander Meer, R.K. 1988: Sources of nestmate recognition cues in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). – Animal Behaviour 36: 1361-1370.

Ortius, D. & Heinze, J. 1999: Fertility signaling in queens of a North American ant. – Behavioral Ecology and Sociobiology 45: 151-159.

Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T. & Yamaoka, R. 2005: Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. – Science 309: 311-314.

Peeters, C., Monnin, T. & Malosse, C. 1999: Cuticular hydrocarbons correlated with reproductive status in a queenless ant. – Proceedings of the Royal Society of London, Series B - Biological Sciences 266: 1323-1327.

Provost, E. 1989: Social environmental-factors influencing mutual recognition of individuals in the ant *Leptothorax lichtensteini* Bondr. (Hymenoptera, Formicidae). – Behavioural Processes 18: 35-59.

Provost, E., Riviere, G., Roux, M., Bagneres, A.G. & Clement, J.L. 1994: Cuticular hydrocarbons whereby *Messor barbarus* ant workers putatively discriminate between monogynous and polygynous colonies. Are workers labeled by queens? – Journal of Chemical Ecology 20: 2985-3003.

Provost, E., Riviere, G., Roux, M., Morgan, E.D. & Bagneres, A.G 1993: Change in the chemical signature of the ant *Leptothorax lichtensteini* Bondroit with time. – Insect Biochemistry and Molecular Biology 23: 945-957.

Roulston, T.H., Buczkowski, G. & Silverman, J. 2003: Nestmate discrimination in ants: effect of bioassay on aggressive behavior. – Insectes Sociaux 50: 151-159.

Rourke, B.C. & Gibbs, A.G. 1999: Effects of lipid phase transitions on cuticular permeability: Model membrane and in situ studies. – Journal of Experimental Biology 202: 3255-3262.

Slessor, K.N., Kaminski, L.-A., King, G.G.S., Borden, J.H. & Winston, M.L. 1988: Semiochemical basis of the retinue response to queen honey bees. – Nature 332: 354-356.

Soroker, V., Fresneau, D. & Hefetz, A. 1998: Formation of colony odor in ponerine ant *Pachycondyla apicalis*. – Journal of Chemical Ecology 24: 1077-1090.

Soroker, V., Lucas, C., Simon, T., Fresneau, D., Durand, J.L. & Hefetz, A. 2003: Hydrocarbon distribution and colony odour homogenisation in *Pachycondyla apicalis*. – Insectes Sociaux 50: 212-217.

Soroker, V., Vienne, C. & Hefetz, A. 1995: Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera, Formicidae). – Journal of Chemical Ecology 21: 365-378.

Soroker, V., Vienne, C., Hefetz, A. & Nowbahari, E. 1994: The postpharyngeal gland as a "gestalt" organ for nestmate recognition in the ant *Cataglyphis niger*. – Naturwissenschaften 81: 510-513.

Stuart, R.J. 1987: Individual workers produce colony-specific nestmate recognition cues in the ant, *Leptothorax curvispinosus*. – Animal Behaviour 35: 1062-1069.

Tentschert, J., Bestmann, H.J. & Heinze, J. 2002: Cuticular compounds of workers and queens in two *Leptothorax* ant species – a comparison of results obtained by solvent extraction, solid sampling, and SPME. – Chemoecology 12: 15-21.

Thomas, M.L., Parry, L.J., Allan, R.A. & Elgar, M.A. 1999: Geographic affinity, cuticular hydrocarbons and colony recognition in the Australian meat ant *Iridomyrmex purpureus*. – Naturwissenschaften 86: 87-92.

Vander Meer, R., Saliwanchik, D. & Lavine, B. 1989: Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*. Implications for nestmate recognition. – Journal of Chemical Ecology 15: 2115-2125.

Vander Meer, R.K. & Morel, L. 1998: Nestmate recognition in ants. In: Vander Meer, R., Breed, M., Winston, M. & Espelie, K. (Eds): Pheromone communication in social insects: Ants, wasps, bees and termites. – Westview Press, Boulder, CO, pp. 79-103.

Vienne, C., Soroker, V. & Hefetz, A. 1995: Congruency of hydrocarbon patterns in heterospecific groups of ants: transfer and/or biosynthesis? – Insectes Sociaux 42: 267-277.

Wagner, D., Tissot, M., Cuevas, W. & Gordon, D.M. 2000: Harvester ants utilize cuticular hydrocarbons in nestmate recognition. – Journal of Chemical Ecology 26: 2245-2257.

Wagner, D., Tissot, M. & Gordon, D. 2001: Task-related environment alters the cuticular hydrocarbon composition of harvester ants. – Journal of Chemical Ecology 27: 1805-1819.

Winston, M.L. & Slessor, K.N. 1992: The essence of royalty: Honey bee queen pheromone. – American Scientist 80: 374-385.

Zahavi, A. & Zahavi, A. 1999: The handicap principle: A missing piece of Darwin's puzzle. – Oxford University Press, Oxford, UK, 304 pp.