Nestmate recognition in social insects and the role of hydrocarbons

Jelle S. van Zweden and Patrizia d'Ettorre

One of the conditions favoring the evolution and maintenance of sociality is the ability to discriminate between kin and non-kin, because it allows altruistic acts to be directed to recipients of high relatedness (Hamilton, 1987). Nestmate recognition is the process whereby social insects recognize individuals belonging to their own colony or an alien colony, and accordingly allow or prohibit entry to their nest. Nestmate recognition often results in the discrimination of kin from non-kin, but in social insects there is a distinction to be made between nestmate and kin recognition. Where kin recognition is the assessment of the degree of relatedness towards another individual, nestmate recognition is the binary recognition of group membership (Arnold *et al.*, 1996; Lenoir *et al.*, 1999). In ants, wasps and termites, the blend of hydrocarbons present on the cuticle appears to comprise the essential compounds that serve as nestmate recognition cues (Howard and Blomquist, 2005), although in honeybees fatty acids and esters may also play an important role (Breed, 1998a; but see Dani *et al.*, 2005; Châline *et al.*, 2005). At the end of the 1990s, the notion that cuticular hydrocarbons act as recognition cues was supported mainly by correlative evidence (Singer, 1998; Vander Meer and Morel, 1998; Lenoir *et al.*, 1999), but significant progress has been made over the last decade. In recent years, not only have some studies provided the direct evidence needed, but they have also shown the differential importance of structural groups of hydrocarbons (i.e. linear and methyl-branched alkanes and alkenes) in the discrimination between nestmate and non-nestmate. On the other hand, it is still largely unknown which physiological, perceptual and cognitive processes are responsible for nestmate recognition, from encoding of cues in the hydrocarbon profile to the detection and processing of these cues in the antennae and further up in the neural pathway (see Chapter 10). Only a few studies have started to experimentally address these proximate mechanisms underlying behavioral decision rules. In this essay we will first summarize the evidence that cuticular hydrocarbons serve as recognition cues, with a particular focus on which hydrocarbons are most informative. We will then explore the mechanism behind nestmate recognition, i.e. how the perception of these hydrocarbons leads to the rejection of non-nestmates and the acceptance of nestmates.

Nestmate recognition is believed to involve the matching of a label, the so-called colony odor (i.e. the chemical profile containing the nestmate cues), with a template (the neural representation of the colony odor stored in the memory), and depending on the

similarity (or dissimilarity) between the two, a conspecific will be accepted or rejected (Hölldobler and Michener, 1980; Vander Meer and Morel, 1998). The expression of the label can be influenced by multiple sources, namely heritable cues of the individuals in a nest, cues derived from the environment (e.g. food, nest material) or cues from the queen (which could also include heritable and environmental factors). For example, *Temnothorax* (*Leptothorax*) *ambiguus* and *T.(L.) longispinosus* ant workers that had eclosed in isolation were attacked significantly less by nestmate adults than by non-nestmate adults, suggesting that the recognition cues produced by the callow worker were similar to those of nestmates, and hence a genetic source is supported (Stuart, 1988). When, however, workers were allowed to eclose in a non-nestmate colony and were subsequently introduced to their parental colony, they were attacked just as often as control non-nestmates, suggesting that the genetic odor is transferred among individuals in the colony (Stuart, 1988). Similarly, honeybee workers reared and kept under laboratory conditions use genetic cues to distinguish between nestmates and non-nestmates (Breed, 1983). However, under more natural conditions, the exposure to comb wax overrides these genetic cues, so that the bees come to rely on environmental recognition cues (Breed *et al.*, 1988; Downs and Ratnieks, 1999). Evidence for queen-derived recognition cues comes from the ant *Camponotus floridanus*, where intercolonial aggression between unrelated workers whose adopted queens are sisters is lower than between sister workers whose adopted queens are unrelated, suggesting that the odors of the queen are the dominant recognition cues (Carlin and Hölldobler, 1988). The relative influences of genetic, environmental and queen odors may differ per species, and may thus depend on general ecology of the species, colony kin-structure and (social) parasite pressure. In ants, recognition cues are mixed throughout the colony by means of trophallaxis (transfer of liquid food between individuals) and allogrooming, in combination with the use of the postpharyngeal gland (PPG), thereby establishing and continuously updating the colony odor (Soroker *et al.*, 1994, 1995). In wasps and bees, the nest paper and comb wax, respectively, seem to have this role as colony odor homogenizers (Espelie *et al.*, 1990; Singer and Espelie, 1996; Breed *et al.*, 1988). For termites, the dynamics of homogenization of recognition cues are less well understood. Here caste odor profiles generally appear to be more distinct than colony-specific odor profiles, but differences in colony odor profiles correlate with aggression and genetic differences, indicating nestmate recognition based on these cues (Kaib *et al.*, 2004; Dronnet *et al.*, 2006; Liebig *et al.*, 2009; Weil *et al.*, 2009).

The manifestation of the template is less well understood. The observation that ant brood and callow workers are less aggressive towards non-nestmates than are adults suggests that there is an early learning period for template acquisition (Jaisson, 1991; Errard, 1994a). There is, nevertheless, evidence that individuals constantly need to reinforce and fine-tune their template with nestmate odors (e.g. Errard and Hefetz, 1997; Lenoir *et al.*, 2001a; Breed *et al.*, 2004; Leonhardt *et al.*, 2007). The degree of mismatch between label and template generally translates into a graded response, from complete acceptance to rejection, i.e. from investigation to threat to overt aggression (e.g. Carlin and Hölldobler, 1986; Obin and Vander Meer, 1988; Errard and Hefetz, 1997).

Cuticular hydrocarbon profiles of the same species typically have the same qualitative make-up, i.e. the same set of compounds (e.g. Nowbahari *et al.*, 1990; see Chapter 7), and as a result social insects should rely on the quantitative variation of hydrocarbons within the profile (different concentrations and/or ratios) for nestmate recognition (e.g. Bonavita-Cougourdan *et al.*, 1987; Espelie *et al.*, 1990; Vander Meer *et al.*, 1989; Martin *et al.*, 2008a). Consistent with this observation, cuticular hydrocarbon profiles have a significant heritable component (e.g. Ross *et al.*, 1987), although environmental components have been implicated as well (e.g. Liang and Silverman, 2000) and the profiles may change over time (Vander Meer *et al.*, 1989; Provost *et al.*, 1993; Lenoir *et al.*, 2001a). Hence, both label and template are not fixed, but are continuously changing. This naturally leads to the question of what kind of template can be both restrictive and flexible, something we will address in the second part of this essay. First, we will go through the evidence that leads us to believe that hydrocarbons are indeed the key nestmate recognition cues.

Evidence for hydrocarbons as nestmate recognition cues

Social parasites

Some of the indirect evidence for the use of hydrocarbons in nestmate recognition comes from social parasites. Since these are able to break the code and integrate into the normally closed colony of a social insect, the recognition cues responsible for their success can be found by studying their chemical profiles. We will give a few examples that serve the purpose of illustration; a full review of social parasitism and hydrocarbons is given elsewhere (Lenoir *et al.*, 2001b; Nash and Boomsma, 2008; see Chapter 14).

Social parasite species can use either chemical camouflage (recognition cues are acquired from its host) or chemical mimicry (recognition cues are synthesized by the parasite), or a combination of both (Lenoir *et al.*, 2001b). However, the result is the same: they overcome detection as non-nestmates. The staphylinid termitophile beetles *Trichopsenius frosti*, *T. depressus*, *Xenistusa hexagonalis*, and *Philoterme howardi* are guests in termite nests and were found to have hydrocarbon profiles similar to those of their respective hosts (Howard *et al.*, 1980, 1982). Similarly, the paper wasp *Polistes atrimandibularis* is an obligate social parasite of another paper wasp, *P. biglumis bimaculatus*. It was shown that, at the point of the colony life cycle that the parasite needs to integrate into its host colony, the hydrocarbon profile of the parasite changes from one that is characterized by unsaturated hydrocarbons into one that matches the host's profile: characterized by the same saturated methyl-branched hydrocarbons, without any unsaturated hydrocarbons (Bagnères *et al.*, 1996). Another paper wasp, *P. sulcifer*, was found to adopt a colony-specific hydrocarbon profile of its host, *P. dominulus*. When presented to a non-nestmate parasitized colony, or when treated with the hydrocarbons of the parasite of a non-nestmate parasitized colony, the *P. sulcifer* individuals were aggressively rejected (Sledge *et al.*, 2001). Also, it was shown that the ant *Polyergus rufescens* can achieve social integration in nests of multiple hosts of the genus *Formica* (subgenus *Serviformica*) by adopting a hydrocarbon profile

that is similar to that of their host, even if the experimental host is not a natural host, like *Formica selysi* (d'Ettorre *et al.*, 2002).

Box 11.1. Diagnostic power (DP)

One of the problems in investigating the evolutionary chemical ecology of nestmate recognition is that researchers are somewhat impaired in manipulating the chemical profile, due to the lack of synthetic hydrocarbons. There is no commercial availability of methyl-branched alkanes at the present moment (although linear alkanes are and some alkenes may be available) and synthesizing these is a costly and time-consuming process. The search for likely nestmate recognition cues therefore often relies on correlative evidence, i.e. stepwise discriminant analysis (DA) between colonies based on cuticular hydrocarbons. The downside to this analysis is that it requires large sample sizes, unless preceded by principal component analysis (PCA). However, this procedure can complicate things, as this often results in one or two of the principal components giving good separation between colonies, with the putative recognition cues associated to these principal components to some degree.

We propose that the diagnostic power for each separate hydrocarbon can be calculated, by dividing the standard deviation among sampled individuals by the pooled standard deviation within nests (Christensen *et al.*, 2005). The rationale behind this is that likely nestmate recognition cues should be most variable between colonies, but should be consistent within colonies due to mixing of odors and/or high relatedness). Therefore, the diagnostic power (DP) of a compound is calculated as:

$$DP_i = \frac{SD_i}{\sqrt{\frac{\sum_{j=1}^N SD_{ij}^2 \times (n_j - 1)}{\sum_{j=1}^N (n_j - 1)}}$$

in which SD_i is the standard deviation of the normalized peak area of the i 'th compound over all individuals of N colonies, SD_{ij} is the standard deviation of the normalized peak area of the i 'th compound in the j 'th colony, and n_j is the number of individuals in the j 'th colony. Compounds can then be ranked according to their DP and the most likely candidates for nestmate recognition cues can be found.

In *Formica rufibarbis* and *Camponotus aethiops*, for example, we found the linear alkanes to be almost as variable within colonies as between colonies, whereas some methyl-branched hydrocarbons were more than six times as variable between colonies as within colonies (J.S. van Zweden *et al.*, in preparation; van Zweden *et al.*, 2009). This is a similar approach as used by Martin and others (2008a) in their search for

colony-specific hydrocarbons within the profiles of *F. exsecta* and *F. fusca*. They measured the correlation (r^2) between the relative abundance of compounds at the species and at the colony levels and subsequently excluded those compounds that had either low or high correlation at both levels, but included those that had low correlation at the species level and high at the colony level, which would be those with diagnostic power (*DP*) a high.

Correlative evidence and removal-and-replacement experiments

Evidence for identification of nestmate recognition cues can constitute either correlative evidence, i.e. when cues are shown to be colony specific, or can come from removal-and-replacement experiments, i.e. extracting the putative recognition cues from an individual and supplementing with the extract from another individual, followed by behavioral experiments (Breed, 1998b). Although the latter may appear to be a rather direct approach, the problem is that the extract may also contain other compounds that remain undetectable, even by gas-chromatography with mass-spectrometry (GC–MS) (Howard and Blomquist, 2005). These types of evidence for the use of hydrocarbons in nestmate recognition in ants and wasps come from a number of studies (e.g. Vander Meer *et al.*, 1989; Espelie and Hermann, 1990; Butts *et al.*, 1991; Gamboa *et al.*, 1996; Lorenzi *et al.*, 1997; Lenoir *et al.*, 1999; Hefetz, 2007), but we shall restrict ourselves to a few examples.

Workers of the ant *Camponotus vagus* showed significantly more aggression towards freshly killed nestmates when their chemical profiles had been removed and their bodies covered by an extract of a non-nestmate worker. Only hydrocarbons were found in a GC–MS analysis of these extracts, giving a good indication that these are the compounds responsible for the reaction (Bonavita-Cougourdan *et al.*, 1987). Adults of the paper wasp *Polistes metricus* were found to be unable to recognize their own nest next to an alien nest when these had been washed in hexane, but the recognition was restored when the extracts were reapplied to the nests (Espelie *et al.*, 1990). Using multivariate statistics (e.g. factor analysis), it was also shown that the hydrocarbon profiles of adult workers and nest paper were colony-specific. Again, hydrocarbons were found to be the major compounds in the cuticular washes, which suggests their use in recognition. For the ant *Cataglyphis cursor* it was shown that there is a correlation between the adoption of workers into alien colonies and the similarity of the hydrocarbon profiles of the original and the adopting colony, as calculated by the Nei index (Nowbahari *et al.*, 1990). These authors observed equally low aggression towards freshly killed nestmates and non-nestmates when these had been washed in the solvent, but aggression could be restored by applying the extract of a non-nestmate.

More recently, some evidence for the use of hydrocarbons in recognition in semi-social insects has been found. In the burying beetles *Nicrophorus vespilloides* and *N. orbicollis* a male and female bury a small vertebrate carcass together and defend it against intruders, in order to utilize it for their reproduction (Müller *et al.*, 2003). They will attack any conspecific

adult, unless it is in the same breeding stage as themselves and their partner. This progressed breeding stage is associated with a change in their hydrocarbon profiles, which are likely to be the cues used in recognition (Steiger *et al.*, 2007, 2008; Scott *et al.*, 2008). Even though this is not nestmate recognition *sensu stricto* and in *N. vespilloides* a more volatile substance seems to be involved in the recognition system as well (S. Steiger, personal communication), this example does illustrate the more widespread use of hydrocarbons in nest defense.

Volatile chemicals have also been proposed as nestmate recognition cues in highly eusocial insects. For several species of ants, volatile components of alarm pheromones were suggested, because the freeze-drying of ants (during which volatiles can evaporate) removed differential behavior towards nestmates and non-nestmates (Jaffe, 1987). However, to our knowledge, no compelling evidence has been presented that the actual variation (quantitative or qualitative) in these secretions, instead of the mere presence, is the cause of differential aggression. On the other hand, volatile chemicals may also be involved in the nestmate recognition system of the ant *Camponotus fellah*. Workers that were isolated with a double mesh within the nest showed significantly different hydrocarbon profiles from non-isolated workers after 21 days, but aggression was not significantly elevated, suggesting the involvement of volatile nest odors (Katzav-Gozansky *et al.*, 2004).

Direct evidence for cuticular hydrocarbons

Direct evidence for the use of hydrocarbons in nestmate recognition in social insects has been accumulating over the last fifteen years. When workers of the subterranean termites *Reticulitermes speratus* and *Coptotermes formosanus* were topically supplemented with the purified hydrocarbon profile of the other species, this was followed by an increase of aggression by nestmate soldiers (Takahashi and Gassa, 1995). Similarly, the topical application of (Z)-9-C_{23:1} onto the cuticle of workers of the ant *Camponotus vagus* resulted in increased antennation and threat in the form of mandible opening (Meskali *et al.*, 1995b). These examples, however, concern qualitatively different hydrocarbons, and thus not variation in abundance that is typical for colonies of the same species.

The ant *Cataglyphis niger* was among the first for which conspecific cuticular hydrocarbons were tested (Lahav *et al.*, 1999). The postpharyngeal gland (PPG) contains the same mixture of hydrocarbons as is found on the cuticle of this species (Soroker *et al.*, 1994; see Chapter 5). Extracts obtained from the PPG were applied onto ant workers and the aggression these workers elicited in nestmates in a neutral arena was observed. When the purified hydrocarbon fraction of the PPG extract (or the entire PPG extract) of a non-nestmate was applied, significantly more aggression was observed than in the sham control, whereas this was not the case for the non-hydrocarbon fraction (Figure 11.1). This shows that it is, indeed, the hydrocarbons that are the key to the nestmate recognition code. Similarly, when the hydrocarbon fraction of a nestmate was applied onto a non-nestmate, the aggression was reduced, even though the effect was not as strong.

The differential importance of alkenes, and linear and methyl-branched alkanes in the nestmate recognition system of the paper wasp *Polistes dominulus* was tested using

Figure 11.1 *Cataglyphis niger* workers were supplemented with extracts of the postpharyngeal gland (PPG), the hydrocarbon fraction of this extract (HC) or the non-hydrocarbon lipid fraction of this extract (Non-HC). Subsequently, untreated workers of a colony A were tested against untreated nestmates (A) or untreated non-nestmates (B) (Control), against nestmates supplemented with non-nestmate extract (A^B), or against non-nestmates supplemented with nestmate extract (B^A), and their aggression was observed. Asterisks depict significant statistical differences. Modified from Lahav *et al.* (1999).

synthetic hydrocarbons (Dani *et al.*, 2001). Synthetic hydrocarbons (n-C₂₁, n-C₂₃, n-C₂₅, n-C₂₇, n-C₂₉, n-C₃₁, 11-MeC₂₉, 7-MeC₂₉, (Z)-11-C_{29:1}, (Z)-9-C_{29:1}, and (Z)-11-C_{31:1}) were topically applied onto anesthetized workers one at a time, after which these were reintroduced to their nest and the reaction of their nestmates was observed. Linear alkanes were never found to have any aggression-eliciting effect on nestmates, whereas alkenes and methyl-branched alkanes did have this effect. Especially when the authors applied an unnaturally high quantity (200µg), the addition of a single hydrocarbon (but not linear alkanes) disrupted the nestmate signal and elicited aggressive reactions. These were all hydrocarbons that naturally occur on the cuticles of *P. dominulus* (although the alkenes are only found in traces), so it is the *increase in relative abundance* of a single hydrocarbon that elicits the aggression.

Similarly, in honeybees, *Apis mellifera*, alkenes were found to be more important in disrupting the nestmate signal than alkanes (Dani *et al.*, 2005). On the other hand, hydrocarbons may not be the only nestmate recognition cues in honeybees. Comb wax, used in nest construction, seems to be the crucial medium for the acquisition of nestmate recognition cues among nestmates (Breed *et al.*, 1988; d'Ettorre *et al.*, 2006; Couvillon *et al.*, 2007). The major compounds found in this wax are free fatty acids (12% of the total wax content) and hydrocarbons (14%) (Breed, 1998a). In supplementation experiments, the increase in relative abundance of several hydrocarbons, free fatty acids and esters was found to lower the acceptability of worker bees to nestmates (Breed and Stiller, 1992; summarized in Buchwald and Breed, 2005). The honeybee is, therefore, one of the few examples where the evidence suggests that hydrocarbons are only part of the nestmate recognition cues.

It may be that this applies to more bees, since other species in the families Halictidae and Apidae have been found to have fatty acids and esters on their cuticles (*Lasioglossum zephyrum*: Smith *et al.*, 1985; *Trigona fulviventris*: Buchwald and Breed, 2005; *Frieseomellita varia*, *Lestrimelitta limao*: Nunes *et al.*, 2008; *Megalopta genalis*: J. S. van Zweden and W. T. Wcislo, unpublished results).

A recent study on *Formica japonica* (Akino *et al.*, 2004) showed that both alkenes and linear alkanes are necessary for nestmate recognition in this species. When the entire CHC-profile (consisting of (Z)-9-alkenes and linear alkanes from 25 to 33 carbon atoms) of a non-nestmate was imitated on an oval-shaped glass dummy, it was attacked. On the other hand, when only the alkene or only the alkane fraction of the profile was applied there was no significant difference between the reactions to nestmate and non-nestmate profiles. Hence, the presence of linear alkanes was necessary to elicit aggressive reactions between non-nestmates. These results are consistent with the results reported by Dani *et al.* (2001, 2005), because linear alkanes were present on the cuticle of the *Polistes* wasps. A similar study has been performed with *Formica exsecta* (Martin *et al.*, 2008b), but here linear alkanes do not seem to be necessary for nestmate recognition. The hydrocarbon profile of these ants also consists of (Z)-9-alkenes and linear alkanes, ranging from 23 to 31 carbon atoms. When workers were presented with glass beads coated with the nestmate profile, they exhibited no aggression. When the alkene fraction was missing or was altered, they attacked the glass beads significantly more, whereas the alkane fraction did not show this effect, either when it was missing, or when it was altered.

Unlike *P. dominulus*, *A. mellifera* and *F. exsecta*, the European hornet, *Vespa crabro*, and the Argentine ant, *Linepithema humile*, show some support for linear alkanes as nestmate recognition cues. Workers of *V. crabro*, when leaving their nest, were significantly more aggressive to dead nestmates when n-C₂₁, n-C₂₃ or (Z)-9-C_{23:1} had been increased in the hydrocarbon profile of these nestmates (Ruther *et al.*, 2002). Workers of *L. humile* also responded aggressively to an increase in multiple linear alkanes (n-C₂₃ to n-C₃₀ and n-C₃₃) in the profile of nestmates, but when the mixture of linear alkanes or the nestmate profile was presented alone, no significant increase in aggression was observed compared with a blank control. Hence, alteration of the relative concentrations of the linear alkanes only elicited any effect when methyl-branched alkanes and alkenes were present as well, which shows that multiple structural classes of hydrocarbons are necessary for the nestmate signal in this species (Greene and Gordon, 2007).

Altogether these studies show that hydrocarbons are essential for nestmate recognition in ant and wasp species, but they are, perhaps, not the only cues in bees. For termites, direct evidence for hydrocarbons as nestmate recognition cues is still lacking, although correlative data suggest its use (Kaib *et al.*, 2004; Dronnet *et al.*, 2006). The differential importance of structural classes – linear alkanes, methyl-branched alkanes and alkenes – may also differ among species, although linear alkanes have so far received the least support for a role in nestmate recognition. This may be attributed partly to the fact that linear alkanes have only the length of the chain of carbon atoms as a discriminative feature, whereas methyl-branched and unsaturated hydrocarbons also have the position of the methyl group

or the double bond (cf. Châline *et al.*, 2005). A non-exclusive alternative hypothesis is that the relative abundance of linear alkanes is mostly related to (recognition of) castes, tasks, and social status within the colony (Wagner *et al.*, 2001). The variation in linear alkanes is then equally high within colonies and between colonies and, therefore, the supplementation of one or a few of these compounds will not alter nestmate-recognition-related aggression (see Chapter 12).

Models of decision rules in nestmate recognition

The early models

Crozier and Dix (1979) quantitatively analyzed two genetic models formalizing how nestmate recognition could work (Figure 11.2A). Under their “individualistic” model, odors are not transferred between individuals within the nest and individuals only recognize each other if they share at least one recognition allele on the nest odor loci. Under the “Gestalt” model, odors mix within the nest and individuals recognize each other only if they share all recognition alleles. In a similar analysis, Getz (1982) extended the possible models to include a “genotype recognition” model, in which individuals only accept each other as nestmates when their exact recognition allele genotypes are present in the nest (Figure 11.2A). Furthermore, he considers a “foreign-label rejection” model, in which an individual is rejected when it carries a foreign recognition allele. Lastly, he proposed an “habituated-label acceptance” model, which assumes the acceptance of nestmates when they share at least one recognition allele regardless of the other recognition allele and is, therefore, even less restrictive than the Crozier–Dix “individualistic” model (Figure 11.2A).

The acceptance threshold

In the models of Crozier and Dix (1979) and Getz (1982), nestmate recognition is based on the matching of alleles at recognition loci. However, directly comparing genotypes is impossible, and the matching of alleles would thus have to be done at the phenotypic level, e.g. the hydrocarbon pattern (Waldman, 1987; Liebert and Starks, 2004). And, as mentioned before, nestmate recognition in social insects most probably relies on quantitative differences rather than qualitative differences (Hölldobler and Michener, 1980).

Sherman *et al.* (1997) mention two models regarding how the detection of cues leads to acceptance of nestmates. These are similar to the earlier models proposed by Crozier and Dix (1979) and Getz (1982), yet explicitly on the phenotypic level. Under the desirable-present model (D-present), individuals are accepted when they possess desirable cues, i.e. cues that are possessed by nearly all desirable individuals (nestmates), but also by some undesirable individuals (non-nestmates), due to overlapping distribution of cues (Figure 11.2B). Under the undesirable-absent (U-absent) model, individuals are accepted when they lack undesirable cues, i.e. cues that are possessed by undesirable individuals, but also by some desirable individuals. These two fundamentally different models are implicitly also different in their

Figure 11.2 Schemes of nestmate recognition models. (A) Models based on matching of alleles. Haplodiploidy is assumed, as in Hymenopteran societies. (B) Models based on phenotype matching. Arrows indicate which phenotypes will be accepted under the respective model. Acceptance errors will be made more frequently when the desirable-present model applies, whereas more rejection errors will be made under the undesirable-absent model (Sherman *et al.*, 1997).

restrictiveness: the D-present model allows for some undesirable (additional) cues to be present and still be accepted, thus leading to more acceptance errors, whereas the U-absent model does not allow this deviation and thus may lead to more rejection errors.

Some support for the U-absent model comes from a study that was designed to test if hydrocarbons are the essential nestmate recognition cues in the ant *Cataglyphis niger* (Lahav *et al.*, 1999; Figure 11.1). When nestmates were treated with the hydrocarbon fraction of a non-nestmate (A versus A^B), aggression towards them increased dramatically to the level of an untreated non-nestmate, whereas when non-nestmates were treated with nestmate hydrocarbon extract (A versus B^A), aggression was only lowered slightly (though

significantly), and not down to the level of an untreated nestmate. This pattern indicates that it is easier for *C. niger* workers to detect differences than similarities (Lenoir *et al.*, 1999; see also Gamboa *et al.*, 1986b).

More recently, Couvillon and Ratnieks (2008) performed a study using the stingless bee *Frieseomelitta varia*. Using a simple protocol with unwashed collecting vials, workers were treated with non-nestmate odors and subsequently introduced either into their own colony or into the non-nestmate colony from which the odor had come. It was found that nestmates were significantly less accepted when “contaminated” with non-nestmate odor, whereas non-nestmates were not better accepted when they were “contaminated” with nestmate odor. This shows that the presence of non-nestmate odor on nestmates led to rejection, whereas the presence of nestmate odor on non-nestmates did not lead to better acceptance, which favors the U-absent model over the D-present model for *F. varia*. Similar experiments in the honeybee, *Apis mellifera*, yield the same outcome (F. L. W. Ratnieks, personal communication; see Couvillon *et al.*, 2007), which together with experiments showing equally high rejection of a number of harmless insects (Kärcher and Ratnieks, in preparation), support a more widespread occurrence of a U-absent recognition system.

Plasticity of the acceptance threshold

Reeve (1989) proposed an adaptively shifting acceptance threshold that minimizes the chance of making an acceptance or rejection error. His mathematical model showed that an acceptance threshold that maximizes fitness depends on (1) the fitness consequences of accepting or rejecting desirable (nestmates) and undesirable individuals (non-nestmates), and (2) the frequency of interaction with these classes of individuals. A critical test of this adaptive acceptance threshold in *Polistes dominulus* confirmed that discrimination depended on the presence of nest material or a nestmate that may indicate vicinity to the nest and therefore increased fitness loss of accepting non-nestmates (Starks *et al.*, 1998). In *Apis mellifera* an increasing acceptance of non-nestmates was found to follow an increase in nectar availability or extra feeding. Since higher nectar availability results in reduced robbing of food by non-nestmates, and therefore reduced fitness loss upon acceptance of non-nestmates, this fits the predictions of the adaptive threshold model (Downs and Ratnieks, 2000; Downs *et al.*, 2001). Evidence for the frequency of interaction comes from a study using the ant *Formica rufibarbis* and its social parasite, *Polyergus rufescens*. In an unparasitized population, the aggression exhibited by host workers towards parasite workers was always high, whereas the parasitized population (which had encountered the social parasite before) only showed high aggression in the raiding season of the social parasite when the frequency of encounters increases (d’Ettorre *et al.*, 2004).

The referents and gestalt odor

The accuracy and flexibility of the nestmate recognition system may depend on the referents used for the template against which incoming individuals will be compared (Lacy and

Figure 11.3 The effect of mixing odors within colonies. The distribution of recognition cues is narrowed, which reduces the probability of acceptance and rejection errors (shaded area).

Sherman, 1983). Self-referent phenotype matching would likely result in the most accurate assessment of kin, but, to our knowledge, there is no evidence for this mechanism in social insects. Nestmate-referent phenotype matching, on the other hand, has been proposed in a number of species (e.g. Buckle and Greenberg, 1981; Gamboa *et al.*, 1986a; Errard, 1994a). The idea of nestmates as referents is that the average quantity of each recognition cue among individuals in the nest is learned, and individuals are accepted as long as they do not deviate more than a certain threshold from this mean (“mean template” model; Breed and Bennett, 1987). The efficiency of learning this mean odor is significantly increased when all individuals in the colony indeed also carry the mean odor, i.e. by creating a Gestalt. Evidence for a Gestalt comes from the mixing of odors (Soroker *et al.*, 1994; Meskali *et al.*, 1995a; Lenoir *et al.*, 2001a), cross-fostering within species (Stuart, 1988; Downs and Ratnieks, 1999; Harano and Sasaki, 2006; J. S. van Zweden *et al.*, unpublished results) and cross-fostering between species (Errard, 1994b; Errard and Hefetz, 1997).

An additional feature of creating a Gestalt is that, while reducing the variation in cues, it also results in nestmates and non-nestmates becoming more dissimilar (less cue overlapping). This, in turn, reduces the possibility of rejection and acceptance errors, compared to unmixed odors (Figure 11.3). This can thus be viewed as an example of an evolutionary shift in recognition cues to optimize the balance between rejection and acceptance errors that can operate next to a shifting rejection threshold (Sherman *et al.*, 1997).

The neural mechanism and a new model

The traditionally accepted model for nestmate recognition of label-template matching involving long-term memory and thus higher integration centers in the brain, has recently

been challenged by the hypothesis that more peripheral neuronal structures might be used (Ozaki *et al.*, 2005). A sensillum in the antennae of the ant *Camponotus japonicus* gave spikes when it perceived the hydrocarbon profile of a non-nestmate, but not when a nestmate profile was presented. This implies that the receptor should be adapted (desensitized) to the multi-component nestmate odor, and hence some integration of the concentrations and the ratios between the different components would occur already at the level of the antenna (see Chapter 10).

Evidence against a template at the antennal level comes from a related ant, *Camponotus floridanus*. By treating the antennae of restrained workers (so that these could not clean their antennae) with the PPG extract of either nestmates or non-nestmates, it was shown that a constant exposure to a non-nestmate odor can induce a change in the neural template leading to reduced aggression towards these non-nestmates. However, the effect was only observed after 15h of exposure, and not after 2h, so the authors argue that receptor adaptation is unlikely to play a role since this process works on a much shorter time scale (Leonhardt *et al.*, 2007).

The integration of information from different receptor neurons takes place in the antennal lobes, the first synaptic relay of the central nervous system. Hence, habituation (the most basic form of learning) to nestmate odor could occur at the level of the antennal lobes and therefore the nestmate odor signal would not be passed on to higher brain centers. In this habituation state, a reaction would follow only the perception of non-nestmate odor (Dalton, 2000). In the experiments with *C. floridanus* (Leonhardt *et al.*, 2007), associative learning can likely be excluded, since treated workers were isolated and the perception of hydrocarbons was therefore not reinforced by any stimulus. Also, the hydrocarbons were permanently present on the antennae, not having the predictive value needed for associative learning, thereby arguing against a template being stored in the long-term memory. Habituation is more parsimonious, however, since it also involves neuronal reformation in the central nervous system (but does not involve long-term memory) and operates on the right time scale to explain the results obtained in *C. floridanus* (Leonhardt *et al.*, 2007).

Since individuals within the colony are almost constantly encountering their nestmates, it would not be adaptive to continuously have to compare their profiles with an internal template in the long-term memory. The alternative would be to filter out all this input early in the neural structures and only react to hydrocarbon profiles that are dissimilar to the nestmate ones. With either desensitized antennae or habituated antennal lobes, individuals in the social insect colony would not detect or respond to the hydrocarbon profiles of nestmates (D-present), nor would they actively detect that non-nestmate hydrocarbon patterns are absent (U-absent) when accepting an encountered individual, as proposed by Sherman *et al.* (1997). Instead, they would only detect cues present on non-nestmates, since only these trigger the antennae or the antennal lobe to respond, and on this basis reject the encountered individual. Social insects would thus use the following as a simple rule of thumb: if the receptor neurons are not spiking and/or the pattern of activation in the antennal lobes is not altered (no differences in nestmate recognition cues are detected), the encountered individual is accepted as a nestmate. This results in the same acceptance and rejection outcome

Figure 11.4 The Gestalt non-nestmate recognition model. Individuals are habituated (or desensitized) to the referent phenotype and will thus only detect dissimilarities from this phenotype. On the right-hand side of the rejection threshold (dashed line) the dissimilarity is large enough, so that the detection of undesirable phenotypes leads to rejection. The shaded area depicts rejection errors.

as under the long-term-memory model, but the neural structures involved are different. We thus propose not a new *nestmate* recognition model, but a *non-nestmate* recognition model: the “U-present” model (Figure 11.4).

If nestmate recognition occurs early in the olfactory system, other more sophisticated levels of recognition are likely to require higher integration structures in the brain. This is the case for within-colony recognition of worker castes (Greene and Gordon, 2003; see Chapter 12), queen fertility signal (Monnin, 2006; Hefetz, 2007; Heinze and d’Ettorre, 2009; see Chapter 13), genetic lineages (Hannonen and Sundström, 2003; but see Holzer *et al.*, 2006), cheating workers (Ratnieks *et al.*, 2006, and references therein) and recognition of individual identity (d’Ettorre and Heinze, 2005; Dreier *et al.*, 2007).

Synthesis

In order to synthesize the ideas that have been proposed and supported regarding hydrocarbons and nestmate recognition, we suggest that most evidence is pointing towards a model for nestmate recognition which is (1) based on quantitative variation among hydrocarbons that are homogeneous among nestmates, (2) based on a graded response to increasingly different concentrations and ratios, but perhaps not a linear response, so that we could still refer to the existences of a “soft” response threshold, (3) taking a context-dependent rejection threshold into account, (4) using nestmates as referents for comparison of phenotypic

characters (at whatever level of the nervous system this matching occurs) and (5) working according to an “undesirable-present” principle (Figure 11.4). Below, we will briefly summarize why we believe these points are supported.

The increase in the abundance of specific hydrocarbons (or also fatty acids, in the case of the honeybee) on the cuticle of a social insect worker can increase the aggression that it receives, which implies that the worker is now perceived as a non-nestmate (Lahav *et al.*, 1999; Dani *et al.*, 2001; Breed *et al.*, 2004; Dani *et al.*, 2005; Guerrieri *et al.*, 2009). Also, the mere observation that workers can perceive each other as non-nestmates, even though they have the same set of hydrocarbons on their cuticles, adds to the evidence in support of the first point of this model. A social insect worker will thus (at the level of the antennae or antennal lobes) compare the dissimilarity of the cuticular hydrocarbon profile of an encountered individual with its nestmate profile, which will give a graded function and hence a graded rejection response. Graded responses, from acceptance to complete rejection, have been found in ants (e.g. Carlin and Hölldobler, 1986; Obin and Vander Meer, 1988; Crosland, 1990; Provost, 1991; Errard and Hefetz, 1997; Lenoir *et al.*, 2001a), wasps (e.g. Gamboa, 1988; Bura and Gamboa, 1994) and bees (e.g. Dani *et al.*, 2005; Harano and Sasaki, 2006). However, the relationship between dissimilarity and rejection response does not have to be a linear one. We propose that an S-curve will fit the experimental data best (Figure 11.4): a mild rejection response (prolonged investigation followed by extra grooming and trophallaxis) towards individuals with low dissimilarity (shaded area in Figure 11.4), followed by a steep increase in rejection (from mandible opening, to biting, to stinging or gaster flexing), after which the rejection response will remain approximately at the same level with increasing dissimilarity in hydrocarbon profiles. The slope and intercept of this S-shaped function may depend on the frequency of interaction between desirable and undesirable individuals and on the fitness consequences of rejecting these classes of individuals (Reeve, 1989). Shifting rejection responses have at least been found in *Polistes dominulus* (Starks *et al.*, 1998), *Apis mellifera* (Downs *et al.*, 2001), and *Formica rufibarbis* (d’Ettorre *et al.*, 2004).

Implicit to the U-present model is that a social insect is habituated (or desensitized) to its colony odor: to the odor of its nestmates. The observation that cross-fostered individuals are less aggressive to colonies related to their foster colony, as compared to control individuals, shows that social insects generally use nestmates as referents (e.g. Buckle and Greenberg, 1981; Errard and Hefetz, 1997; Downs and Ratnieks, 1999; Harano and Sasaki, 2006). Because labels change over time, the template must be continuously updated and thus continuously reinforced (Breed and Bennett, 1987; Vander Meer *et al.*, 1989; Lenoir *et al.*, 2001a). This seems to argue against a template stored in the long-term memory. Habituation in the antennal lobe is more flexible and can easily explain the pattern that social insects detect differences rather than similarities (Lahav *et al.*, 1999; Couvillon and Ratnieks, 2008). It can also explain how exposure to non-nestmate odor through non-associative learning can lead to a reduction in aggression towards these non-nestmates after >15h (Leonhardt *et al.*, 2007). Yet, it still leads to the essential discrimination between nestmates and non-nestmates. Furthermore, inside the colony, it seems maladaptive to continuously

have to compare nestmates with a template stored in the long-term memory, whereas filtering this information early in the nervous system and only passing specific sensory stimuli would avoid this overload (Wehner, 1987). We propose habituation to nestmate odor and subsequent detection of undesirable hydrocarbon profiles as a parsimonious explanation for the pattern of recognition of non-nestmates observed among social insects. In our view, acceptance of nestmates would be a simple consequence of non-rejection.

Acknowledgments

We would like to thank the editors for inviting us to contribute to this book. Furthermore, we are grateful to J. J. Boomsma, N. Bos, K. R. Foster, L. Holman, and V. Nehring for comments and discussion and to all members of the Copenhagen *Centre for Social Evolution* for providing a stimulating working environment. The work was supported by the EU Marie Curie Excellence Grant CODICES-EXT-CT-2004-014202 assigned to PdE.

References

- Akino, T., Yamamura, K., Wakamura, S. and Yamaoka, R. (2004). Direct behavioral evidence for hydrocarbons as nestmate recognition cues in *Formica japonica* (Hymenoptera: Formicidae). *Appl. Entomol. Zool.*, **39**, 381–387.
- Arnold, G., Quenet, B., Cornuet, J.-M., Masson, C., De Schepper, B., Estoup, A. and Gasqui, P. (1996). Kin recognition in honeybees. *Nature*, **379**, pp. 498.
- Bagnères, A.-G., Lorenzi, M. C., Dusticier, G., Turillazzi, S. and Clément, J.-L. (1996). Chemical usurpation of a nest by paper wasp parasites. *Science*, **272**, 889–892.
- Bonavita-Cougourdan, A., Clément, J.-L. and Lange, C. (1987). Nestmate recognition: the role of cuticular hydrocarbons in the ant *Camponotus vagus* Scop. *J. Entomol. Sci.*, **22**, 1–10.
- Breed, M. D. (1983). Nestmate recognition in honey bees. *Anim. Behav.*, **31**, 86–91.
- Breed, M. D. (1998a). Recognition pheromones of the honey bee. *BioScience*, **48**, 463–470.
- Breed, M. D. (1998b). Chemical cues in kin recognition: criteria for identification, experimental approaches, and the honey bee as an example. In *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*, ed. R. K. Vander Meer, M. D. Breed, K. E. Espelie and M. L. Winston. Boulder, CO: Westview Press, pp. 57–78.
- Breed, M. D. and Bennett, B. (1987). Kin recognition in highly eusocial insects. In *Kin Recognition in Animals*, ed. D. J. C. Fletcher and C. D. Michener. Chichester: Wiley, pp. 243–285.
- Breed, M. D., Diaz, P. H. and Lucero, K. D. (2004). Olfactory information processing in honeybee, *Apis mellifera*, nestmate recognition. *Anim. Behav.*, **68**, 921–928.
- Breed, M. D. and Stiller, T. M. (1992). Honey bee, *Apis mellifera*, nestmate discrimination: hydrocarbon effects and the evolutionary implications of comb choice. *Anim. Behav.*, **43**, 875–883.
- Breed, M. D., Williams, K. R. and Fewell, J. H. (1988). Comb wax mediates the acquisition of nest-mate recognition cues in honey bees. *Proc. Natl. Acad. Sci. USA*, **85**, 8766–8769.

- Buchwald, R. and Breed, M.D. (2005). Nestmate recognition cues in a stingless bee, *Trigona fulviventris*. *Anim. Behav.*, **70**, 1331–1337.
- Buckle, G.R. and Greenberg, L. (1981). Nestmate recognition in sweat bees (*Lasioglossum zephyrum*): does an individual recognize its own odour or only odours of its nestmates? *Anim. Behav.*, **29**, 802–809.
- Bura, E.A. and Gamboa, G.J. (1994). Kin recognition by social wasps: asymmetric tolerance between aunts and nieces. *Anim. Behav.*, **47**, 977–979.
- Butts, D.P., Espelie, K.E. and Hermann, H.R. (1991). Cuticular hydrocarbons of four species of social wasps in the subfamily vespinae: *Vespa crabro* (L.), *Dolichovespula maculata* (L.), *Vespula squamosa* (Drury), and *Vespula maculifrons* (Buysson). *Comp. Biochem. Physiol. B*, **99**, 87–91.
- Carlin, N. and Hölldobler, B. (1988). Influence of virgin queens on kin recognition in the carpenter ant *Camponotus floridanus* (Hymenoptera: Formicidae). *Insectes Soc.*, **35**, 191–197.
- Carlin, N.F. and Hölldobler, B. (1986). The kin recognition system of carpenter ants (*Camponotus* spp.) I: hierarchical cues in small colonies. *Behav. Ecol. Sociobiol.*, **19**, 123–134.
- Châline, N., Sandoz, J.-C., Martin, S.J., Ratnieks, F.L.W. and Jones, G.R. (2005). Learning and discrimination of individual cuticular hydrocarbons by honeybees (*Apis mellifera*). *Chem. Senses*, **30**, 327–335.
- Christensen, J.H., Hansen, A.B., Karlson, U., Mortensen, J. and Andersen, O. (2005). Multivariate statistical methods for evaluating biodegradation of mineral oil. *J. Chromatogr. A*, **1090**, 133–145.
- Couvillon, M. and Ratnieks, F.L.W. (2008). Odour transfer in stingless bee marmelada (*Frieseomelitta varia*) demonstrates that entrance guards use an “undesirable–absent” recognition system. *Behav. Ecol. Sociobiol.*, **62**, 1099–1105.
- Couvillon, M.J., Caple, J.P., Endors, S.L., Kärcher, M., Russell, T.E., Storey, D.E. and Ratnieks, F.L.W. (2007). Nest-mate recognition template of guard honeybees (*Apis mellifera*) is modified by wax comb transfer. *Biol. Lett.*, **3**, 228–230.
- Crosland, M.W.J. (1990). Variation in ant aggression and kin discrimination ability within and between colonies. *J. Insect Behav.*, **3**, 359–379.
- Crozier, R.H. and Dix, M.W. (1979). Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. *Behav. Ecol. Sociobiol.*, **4**, 217–224.
- d’Ettorre, P., Brunner, E., Wenseleers, T. and Heinze, J. (2004). Knowing your enemies: seasonal dynamics of host–social parasite recognition. *Naturwissenschaften*, **91**, 594–597.
- d’Ettorre, P. and Heinze, J. (2005). Individual recognition in ant queens. *Curr. Biol.*, **15**, 2170–2174.
- d’Ettorre, P., Mondì, N., Lenoir, A. and Errard, C. (2002). Blending in with the crowd: social parasites integrate into their host colonies using a flexible chemical signature. *Proc. R. Soc. B*, **269**, 1911–1918.
- d’Ettorre, P., Wenseleers, T., Dawson, J., Hutchinson, S., Boswell, T. and Ratnieks, F.L.W. (2006). Wax combs mediate nestmate recognition by guard honeybees. *Anim. Behav.*, **71**, 773–779.
- Dalton, P. (2000). Psychophysical and behavioral characteristics of olfactory adaptation. *Chem. Senses*, **25**, 487–492.
- Dani, F.R., Jones, G.R., Corsi, S., Beard, R., Pradella, D. and Turillazzi, S. (2005). Nestmate recognition cues in the honey bee: differential importance of cuticular alkanes and alkenes. *Chem. Senses*, **30**, 477–489.

- Dani, F. R., Jones, G. R., Destri, S., Spencer, S. H. and Turillazzi, S. (2001). Deciphering the recognition signature within the cuticular chemical profile of paper wasps. *Anim. Behav.*, **62**, 165–171.
- Downs, S. G. and Ratnieks, F. L. W. (1999). Recognition of conspecifics by honeybee guards uses nonheritable cues acquired in the adult stage. *Anim. Behav.*, **58**, 643–648.
- Downs, S. G. and Ratnieks, F. L. W. (2000). Adaptive shifts in honey bee (*Apis mellifera* L.) guarding behavior support predictions of the acceptance threshold model. *Behav. Ecol.*, **11**, 326–333.
- Downs, S. G., Ratnieks, F. L. W., Badcock, N. S. and Mynott, A. (2001). Honeybee guards do not use food-derived odors to recognize non-nest mates: a test of the odor convergence hypothesis. *Behav. Ecol.*, **12**, 47–50.
- Dreier, S., van Zweden, J. S. and D’Ettorre, P. (2007). Long-term memory of individual identity. *Biol. Lett.*, **3**, 459–462.
- Dronnet, S., Lohou, C., Christides, J.-P. and Bagnères, A.-G. (2006). Cuticular hydrocarbon composition reflects genetic relationship among colonies of the introduced termite *Reticulitermes santonensis* Feytaud. *J. Chem. Ecol.*, **32**, 1027–1042.
- Errard, C. (1994a). Development of interspecific recognition behavior in the ants *Manica rubida* and *Formica selysi* (Hymenoptera: Formicidae) reared in mixed-species groups. *J. Insect Behav.*, **7**, 83–99.
- Errard, C. (1994b). Long-term memory involved in nestmate recognition in ants. *Anim. Behav.*, **48**, 263–271.
- Errard, C. and Hefetz, A. (1997). Label familiarity and discriminatory ability of ants reared in mixed groups. *Insectes Soc.*, **44**, 189–198.
- Espelie, K. E. and Hermann, H. R. (1990). Surface lipids of the social wasp *Polistes annularis* (L.) and its nest and nest pedicel. *J. Chem. Ecol.*, **16**, 1841–1852.
- Espelie, K. E., Wenzel, J. W. and Chang, G. (1990). Surface lipids of social wasp *Polistes metricus* Say and its nest and nest pedicel and their relation to nestmate recognition. *J. Chem. Ecol.*, **16**, 2229–2241.
- Gamboa, G. J. (1988). Sister, aunt-niece, and cousin recognition by social wasps. *Behav. Genet.*, **18**, 409–423.
- Gamboa, G. J., Grudzien, T. A., Espelie, K. E. and Bura, E. A. (1996). Kin recognition pheromones in social wasps: combining chemical and behavioural evidence. *Anim. Behav.*, **51**, 625–629.
- Gamboa, G. J., Reeve, H. K., Ferguson, I. D. and Wacker, T. L. (1986a). Nestmate recognition in social wasps: the origin and acquisition of recognition odours. *Anim. Behav.*, **34**, 685–695.
- Gamboa, G. J., Reeve, H. K. and Pfennig, D. W. (1986b). The evolution and ontogeny of nestmate recognition in social wasps. *Annu. Rev. Entomol.*, **31**, 431–454.
- Getz, W. M. (1982). An analysis of learned kin recognition in Hymenoptera. *J. Theor. Biol.*, **99**, 585–597.
- Greene, M. J. and Gordon, D. M. (2003). Cuticular hydrocarbons inform task decisions. *Nature*, **423**, pp. 32.
- Greene, M. J. and Gordon, D. M. (2007). Structural complexity of chemical recognition cues affects the perception of group membership in the ants *Linepithema humile* and *Aphaenogaster cockerelli*. *J. Exp. Biol.*, **210**, 897–905.
- Guerrieri, F. J., Nehring, V., Jørgensen, C. G., Nielsen, J., Galizia, C. G., and d’Ettorre, P. (2009). Ants recognize foes and not friends. *Proc. R. Soc. B*, **276**, 2461–2468.
- Hamilton, W. D. (1987). Discrimination nepotism: expectable, common, overlooked.

- In *Kin Recognition in Animals* ed. D.J.C. Fletcher and C.D. Michener. New York: Wiley, pp. 417–437.
- Hannonen, M. and Sundström, L. (2003). Sociobiology: Worker nepotism among polygynous ants. *Nature*, **421**, 910.
- Harano, K.-I. and Sasaki, M. (2006). Renewal process of nestmate recognition template in European honeybee *Apis mellifera* L. (Hymenoptera: Apidae). *Appl. Entomol. Zool.*, **41**, 325–330.
- Hefetz, A. (2007). The evolution of hydrocarbon pheromone parsimony in ants (Hymenoptera: Formicidae) – interplay of colony odor uniformity and odor idiosyncrasy: a review. *Myrmecol. News*, **10**, 59–68.
- Heinze, J. and d'Ettorre, P. (2009). Honest and dishonest communication in social Hymenoptera. *J. Exp. Biol.*, **212**, 1775–1779.
- Hölldobler, B. and Michener, C.D. (1980). Mechanisms of identification and discrimination in social Hymenoptera. In *Evolution of Social Behavior: Hypotheses and Empirical Tests*, ed. H. Markl. Weinheim: Chemie Verlag, pp. 35–58.
- Holzer, B., Kümmerli, R., Keller, L. and Chapuisat, M. (2006). Sham nepotism as a result of intrinsic differences in brood viability in ants. *Proc. R. Soc. B*, **273**, 2049–2052.
- Howard, R.W. and Blomquist, G.J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.*, **50**, 371–393.
- Howard, R.W., McDaniel, C.A. and Blomquist, G.J. (1980). Chemical mimicry as an integrating mechanism: cuticular hydrocarbons of a termitophile and its host. *Science*, **210**, 431–433.
- Howard, R.W., McDaniel, C.A. and Blomquist, G.J. (1982). Chemical mimicry as an integrating mechanism for three termitophiles associated with *Reticulitermes virginicus* (Banks). *Psyche*, **89**, 157–168.
- Jaffe, K. (1987). Evolution of territoriality and nestmate recognition in ants. *Experientia Supplementum*, **54**, 295–311.
- Jaisson, P. (1991). Kinship and fellowship in ants and social wasps. In *Kin Recognition*, ed. P.G. Hepper. Cambridge: Cambridge University Press, pp. 60–93.
- Kaib, M., Jmhasly, P., Wilfert, L., Durka, W., Franke, S., Francke, W., Leuthold, R.H. and Brandl, R. (2004). Cuticular hydrocarbons and aggression in the termite *Macrotermes subhyalinus*. *J. Chem. Ecol.*, **30**, 365–385.
- Katzav-Gozansky, T., Boulay, R., Vander Meer, R. and Hefetz, A. (2004). In-nest environment modulates nestmate recognition in the ant *Camponotus fellah*. *Naturwissenschaften*, **91**, 186–190.
- Lacy, R.C. and Sherman, P.W. (1983). Kin recognition by phenotype matching. *Am. Nat.*, **121**, 489–512.
- Lahav, S., Soroker, V., Hefetz, A. and Vander Meer, R.K. (1999). Direct behavioral evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften*, **86**, 246–249.
- Lenoir, A., Cuisset, D. and Hefetz, A. (2001a). Effects of social isolation on hydrocarbon pattern and nestmate recognition in the ant *Aphaenogaster senilis* (Hymenoptera, Formicidae). *Insectes Soc.*, **48**, 101–109.
- Lenoir, A., D'Ettorre, P., Errard, C. and Hefetz, A. (2001b). Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.*, **46**, 573–599.
- Lenoir, A., Fresneau, D., Errard, C. and Hefetz, A. (1999). Individuality and colonial identity in ants: the emergence of the social representation concept. In *Information Processing in Social Insects*, ed. C. Detrain, J.-L. Deneubourg and J.M. Pasteels. Basel: Birkhäuser, pp. 219–237.

- Leonhardt, S., Brandstaetter, A. and Kleineidam, C. (2007). Reformation process of the neuronal template for nestmate-recognition cues in the carpenter ant *Camponotus floridanus*. *J. Comp. Physiol. A*, **193**, 993–1000.
- Liang, D. and Silverman, J. (2000). “You are what you eat”: diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. *Naturwissenschaften*, **87**, 412–416.
- Liebert, A.E. and Starks, P.T. (2004). The action component of recognition systems: a focus on the response. *Ann. Zool. Fennici*, **41**, 747–764.
- Liebig, J., Eliyahu, D. and Brent, C. S. (2009). Cuticular hydrocarbon profiles indicates reproductive status in the termite *Zootermopsis nevadensis*. *Behav. Ecol. Sociobiol.*, in press.
- Lorenzi, M. C., Bagnères, A. G., Clément, J. L. and Turillazzi, S. (1997). *Polistes biglumis bimaculatus* epicuticular hydrocarbons and nestmate recognition (Hymenoptera, Vespidae). *Insectes Soc.*, **44**, 123–138.
- Martin, S., Helanterä, H. and Drijfhout, F. (2008a). Colony-specific hydrocarbons identify nest mates in two species of *Formica* ant. *J. Chem. Ecol.*, **34**, 1072–1080.
- Martin, S. J., Vitikainen, E., Helanterä, H. and Drijfhout, F. P. (2008b). Chemical basis of nest-mate discrimination in the ant *Formica exsecta*. *Proc. R. Soc. B*, **275**, 1271–1278.
- Meskali, M., Bonavita-Cougourdan, A., Provost, E., Bagnères, A.-G., Dusticier, G. and Clément, J.-L. (1995a). Mechanism underlying cuticular hydrocarbon homogeneity in the ant *Camponotus vagus* (SCOP.) (Hymenoptera: Formicidae): Role of postpharyngeal glands. *J. Chem. Ecol.*, **21**, 1127–1148.
- Meskali, M., Provost, E., Bonavita-Cougourdan, A. and Clément, J.-L. (1995b). Behavioural effects of an experimental change in the chemical signature of the ant *Camponotus vagus* (Scop.). *Insectes Soc.*, **42**, 347–358.
- Monnin, T. (2006). Chemical recognition of reproductive status in social insects. *Ann. Zool. Fennici*, **43**, 531–549.
- Müller, J. K., Eggert, A.-K. and Elsner, T. (2003). Nestmate recognition in burying beetles: the “breeder’s badge” as a cue used by females to distinguish their mates from male intruders. *Behav. Ecol.*, **14**, 212–220.
- Nash, D. R. and Boomsma, J. J. (2008). Communication between hosts and social parasites. In *Sociobiology of Communication: an interdisciplinary perspective*, ed. P. d’Ettorre and D. P. Hughes. Oxford: Oxford University Press, pp. 55–79.
- Nowbahari, E., Lenoir, A., Clément, J. L., Lange, C., Bagnères, A. G. and Joulie, C. (1990). Individual, geographical and experimental variation of cuticular hydrocarbons of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): Their use in nest and subspecies recognition. *Biochem. Syst. Ecol.*, **18**, 63–73.
- Nunes, T. M., Nascimento, F. S., Turatti, I. C., Lopes, N. P. and Zucchi, R. (2008). Nestmate recognition in a stingless bee: does the similarity of chemical cues determine guard acceptance? *Anim. Behav.*, **75**, 1165–1171.
- Obin, M. S. and Vander Meer, R. K. (1988). Sources of nestmate recognition cues in the imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae). *Anim. Behav.*, **36**, 1361–1370.
- Ozaki, M., Wada-Katsumata, A., Fujikawa, K., Iwasaki, M., Yokohari, F., Satoji, Y., Nisimura, T. and Yamaoka, R. (2005). Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science*, **309**, 311–314.

- Provost, E. (1991). Nonnestmate kin recognition in the ant *Leptothorax lichtensteini*: evidence that genetic factors regulate colony recognition. *Behav. Genet.*, **21**, 151–167.
- Provost, E., Rivière, G., Roux, M., Morgan, E. D. and Bagnères, A.-G. (1993). Change in the chemical signature of the ant *Leptothorax lichtensteini* Bondroit with time. *Insect Bioch. Mol. Biol.*, **23**, 945–957.
- Ratnieks, F. L. W., Foster, K. R. and Wenseleers, T. (2006). Conflict resolution in insect societies. *Annu. Rev. Entomol.*, **51**, 581–608.
- Reeve, H. K. (1989). The evolution of conspecific acceptance thresholds. *Am. Nat.*, **133**, 407–435.
- Ross, K. G., Meer, R. K. V., Fletcher, D. J. C. and Vargo, E. L. (1987). Biochemical phenotypic and genetic studies of two introduced fire ants and their hybrid (Hymenoptera: Formicidae). *Evolution*, **41**, 280–293.
- Ruther, J., Sieben, S. and Schricker, B. (2002). Nestmate recognition in social wasps: manipulation of hydrocarbon profiles induces aggression in the European hornet. *Naturwissenschaften*, **89**, 111–114.
- Scott, M. P., Madjid, K. and Orians, C. M. (2008). Breeding alters cuticular hydrocarbons and mediates partner recognition by burying beetles. *Anim. Behav.*, **76**, 507–513.
- Sherman, P. W., Reeve, H. K. and Pfennig, D. W. (1997). Recognition systems. In *Behavioural Ecology: An Evolutionary Approach*, ed. J. R. Krebs and N. B. Davies. Oxford: Blackwell, pp. 69–96.
- Singer, T. and Espelie, K. (1996). Nest surface hydrocarbons facilitate nestmate recognition for the social wasp, *Polistes metricus* Say (Hymenoptera: Vespidae). *J. Insect Behav.*, **9**, 857–870.
- Singer, T. L. (1998). Roles of hydrocarbons in the recognition systems of insects. *Am. Zool.*, **38**, 394–405.
- Sledge, M. F., Dani, F. R., Cervo, R., Dapporto, L. and Turillazzi, S. (2001). Recognition of social parasites as nest-mates: adoption of colony-specific host cuticular odours by the paper wasp parasite *Polistes sulcifer*. *Proc. R. Soc. B*, **268**, 2253–2260.
- Smith, B. H., Carlson, R. G. and Frazier, J. (1985). Identification and bioassay of macrocyclic lactone sex pheromone of the halictine bee *Lasioglossum zephyrum*. *J. Chem. Ecol.*, **11**, 1447–1456.
- Soroker, V., Vienne, C. and Hefetz, A. (1995). Hydrocarbon dynamics within and between nestmates in *Cataglyphis niger* (Hymenoptera: Formicidae). *J. Chem. Ecol.*, **21**, 365–378.
- Soroker, V., Vienne, C., Hefetz, A. and Nowbahari, E. (1994). The postpharyngeal gland as a “gestalt” organ for nestmate recognition in the ant *Cataglyphis niger*. *Naturwissenschaften*, **81**, 510–513.
- Starks, P. T., Fischer, D. J., Watson, R. E., Melikian, G. L. and Nath, S. D. (1998). Context-dependent nestmate discrimination in the paper wasp, *Polistes dominulus*: a critical test of the optimal acceptance threshold model. *Anim. Behav.*, **56**, 449–458.
- Steiger, S., Peschke, K., Francke, W. and Müller, J. K. (2007). The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. *Proc. R. Soc. B*, **274**, 2211–2220.
- Steiger, S., Peschke, K. and Müller, J. (2008). Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: the chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*? *Behav. Ecol. Sociobiol.*, **62**, 1053–1060.
- Stuart, R. J. (1988). Collective cues as a basis for nestmate recognition in polygynous leptothoracine ants. *Proc. Natl. Acad. Sci. USA*, **85**, 4572–4575.

- Takahashi, S. and Gassa, A. (1995). Roles of cuticular hydrocarbons in intra- and interspecific recognition behavior of two Rhinotermitidae species. *J. Chem. Ecol.*, **21**, 1837–1845.
- van Zwenden, J. S., Dreier, S. and d’Ettorre, P. (2009). Disentangling environmental and heritable nestmate recognition cues in a carpenter ant. *J. Insect Physiol.*, **55**, 158–163.
- Vander Meer, R. K. and Morel, L. (1998). Nestmate recognition in ants. In *Pheromone Communication in Social Insects*, ed. R. K. Vander Meer, M. D. Breed, M. L. Winston and K. E. Espelie. Boulder, CO: Westview Press, pp. 79–103.
- Vander Meer, R. K., Saliwanchik, D. and Lavine, B. (1989). Temporal changes in colony cuticular hydrocarbon patterns of *Solenopsis invicta*: implications for nestmate recognition. *J. Chem. Ecol.*, **15**, 2115–2125.
- Wagner, D., Tissot, M. and Gordon, D. (2001). Task-related environment alters the cuticular hydrocarbon composition of harvester ants. *J. Chem. Ecol.*, **27**, 1805–1819.
- Waldman, B. (1987). Mechanisms of kin recognition. *J. Theor. Biol.*, **128**, 159–185.
- Wehner, R. (1987). ‘Matched filters’ – neural models of the external world. *J. Comp. Physiol. A*, **161**, 511–531.
- Weil, T., Hoffmann, K., Kroiss, J., Strohm, E. and Korb, J. (2009). Scent of a queen – cuticular hydrocarbons specific for female reproductives in lower termites. *Naturwissenschaften*, **96**, 315–319.