



Individuality and colonial identity in ants: the emergence of the social representation concept

*Alain Lenoir, Dominique Fresneau, Christine Errard
and Abraham Hefetz³*

Summary

Colonial identity in social insects is based on nestmate recognition which is mediated through cuticular substances. Although this is considered to be distinct from kin recognition, it is possible that through evolution the signal mediating kinship was replaced by the signal mediating “nestmateship”. Cuticular hydrocarbons in *Cataglyphis niger* are responsible for modifying the ant’s aggressive behavior and are considered to have a similar function in other ants species. In ants, the postpharyngeal gland (PPG) serves as a storage organ for these cues and functions as a “gestalt” organ, with the gestalt being permanently updated. Its content is constantly being exchanged with nestmates through trophallaxis and allogrooming. We hypothesize that already in the primitive ponerine ants the PPG evolved as a gestalt organ even without trophallaxis. We discuss two alternative primary selective pressures for the evolution of trophallaxis: facilitating food exchange versus exchanging recognition cues. Callow workers seem to be characterized by a “cuticular chemical insignificance” followed by a “chemical integration” period when they acquire the gestalt of the colony and learn the associated template. We hypothesize that the template has evolved from a simple personal chemical reference in primitive species with small colonies to an internal representation of the colonial identity in larger colonies.

Introduction

In this paper we focus on nestmate recognition in ants and address questions pertaining to the nature of the signals (called labels or cues) at the basis of recognition, and their production and dissemination in adult ants and throughout

ontogenesis. We discuss the mechanisms (decision rules) underlying the behavior of an individual facing another individual: whether amicable or aggressive behaviors following comparison of the perceived signal with its own reference (called a template). We further discuss how this template is constructed. Finally we introduce the notion of social representation: does the ant have an integrative representation of its social environment or does it react according to simple rules?

Nestmate versus kin recognition

Nestmate recognition is not to be confused with kin recognition. These two phenomenon are indeed different. Nestmate recognition is typically manifested by rejecting alien intruders or preferentially transporting nestmate brood. Kin discrimination, on the other hand, is defined as the differential treatment of relatives according their level of relatedness so as to increase their fitness (nepotism) [1-3]. Since in most ant colonies relatedness is greater than zero, nestmate recognition can constitute a form of kin discrimination. However the evidence that the template is acquired by exposure and learning at the callow stage, suggests that these two phenomena are not necessarily linked. Indeed there is only limited evidence of nepotism, the expressed behavior of kin discrimination in social insects. Demonstrating kin recognition is still hampered by many methodological difficulties, and the obtained results have been criticized [4]. In honeybees there is evidence for kin recognition [5,1], but the degree of preference shown by workers for close kin is small and whether it has an impact on the fitness of the larvae is still uncertain. In ants and wasps the few studies conducted could not find evidence for within-colony discrimination [6-9]. Recently DeHeer and Ross [10] failed to demonstrate nepotism in multiple-queen colonies of the fire ant *Solenopsis invicta*. In agreement with Vander Meer and Morel [11] we consider that “virtually all recognition studies on ants involve nestmate recognition rather than kin recognition”.

Although this dearth of evidence is due to the lack of necessary tools for measuring kin discrimination, we would nonetheless like to present an alternative explanation. The primary selective pressure for developing a recognition system may indeed have been the process of kin selection. However, when more complex societies evolved, i.e., multiple mating by the queen and polygyny, selection pressures led to developing a nestmate recognition system that was not

necessarily linked to kin. For example, Keller [3] hypothesized that polyandry which increases intracolony variability could explain the low level of nepotism in bees. It was parsimonious to adapt the system that had already developed for kin recognition for nestmate recognition, and therefore kinship became replaced by *nestmateship* (called also *fellowship* [12]). If this is indeed true, it means that these societies have lost the ability to recognize kin, which would explain many of the observed phenomena. For example, kin recognition is demonstrable in the primitively social bee *Lasioglossum zephyrum* [13] (and possibly honey bee), whereas in the ants all efforts to demonstrate this phenomenon have failed.

Signals of nestmate recognition: the role of cuticular hydrocarbons

It is a basic observation that when two ants encounter they may already recognize each other from a very short distance (1-2 cm), but generally physical contact is needed. The contact can be made anywhere on the partner's body, indicating that the signal is widely spread on the cuticle. Cuticular lipids, including hydrocarbons, have a primordial role in protecting the insects against desiccation and invasion of micro-organisms or toxins. In social insects their hydrocarbon constituents may also have a determinant role in nestmate recognition [14,15]. This point is largely controversial as indicated in recent review papers[4,11]. Two approaches have been developed to test this role of hydrocarbons: correlation and experimental studies.

Correlation studies

Cuticular hydrocarbon composition is highly diverse and modern techniques permit the identification of more and more substances. For example, early analysis of cuticular lipids of *Myrmica incompleta* revealed 19 hydrocarbons [16] whereas further analyses resulted in the identification of 111 substances [17]. Recent analyses of the postpharyngeal gland secretion, representative of the composition of cuticular hydrocarbons (see below), in seven species of *Cataglyphis* resulted in the identification of a total of 242 different hydrocarbons [18]. Generally 30 to 60 substances are found in a given species. Similar to those found in other insects, they are species specific exhibiting qualitative variations,

and also present intraspecific quantitative variations. They were accordingly used for chemosystematic studies [14,18].

The hydrocarbon profile can also be characteristic of the population, rendering it a good index of speciation as was studied, for example, in the genus *Cataglyphis* [18,19]. More important is the fact that they are also colony, caste, and subcaste specific [14,20]. In all these studies the authors assessed the differences in hydrocarbon composition using a multivariate analysis, demonstrating that the colonies were well discriminated. A correlation was also found between the hydrocarbon pattern similarity and the closed nature of the colonies in *Cataglyphis cursor* [19]. All these findings do not provide unequivocal proof of the role of hydrocarbons, but they are indicative, taking into account the limitations of correlation studies.

Experimental studies.

The removal and replacement of cuticular compounds have provided indications for their role in nestmate recognition. Hydrocarbons are efficiently collected by rinsing the ant with apolar solvents. Corpses of workers treated thus were consequently not recognized as nestmates but considered as neutral. Upon application of the extract to surrogates, these became considered as alien workers and were consequently aggressed [see 11]. These results are difficult to interpret because a total body extraction contains additional exocrine products, as was observed in *Solenopsis invicta* which contained large amounts of alkaloids. In this latter ant it was impossible to devise an appropriate bioassay for nestmate recognition, probably due to this high contamination [11]. A solvent rinse also includes lipids other than the hydrocarbons that are found on the cuticle. Therefore this kind of experiment can at most lead to the conclusion that cuticular lipids are involved in recognition, but not necessarily hydrocarbons (fatty acids seem to be involved in the cuckoo ants [21]). Nevertheless it does confirm that the substances present on the cuticle are involved in recognition, and hydrocarbons by virtue of their dominant presence are likely candidates for constituting the signal. Recognition pheromones are spread throughout the body surface and, even if they constitute a monomolecular layer on the epicuticle there is a need for large quantities of material.

Chemical supplementation experiments comprise another approach and have been conducted in bees [see 4] in which nestmate recognition was modified by applications of a C₃₂ hydrocarbon. In the ant *Camponotus vagus*, applications of (Z)-9-tricosene induced some aggressive behaviors and intense antennations from the nontreated nestmates, indicating that the recognition cues had been modified [22]. These experiments are not conclusive since many compounds that are applied exogenously may elicit aggression toward the ants.

The PPG

Recent studies on this gland (idiosyncratic to the Formicidae) have enabled considerable progress in our understanding of the elaboration of colonial odor in ants. The PPG contains hydrocarbons that are congruent with the cuticular ones [23-25]. This suggests a link between these two body parts, and that the PPG contains the recognition cues. Several studies using the PPG content (and thereby avoiding contamination of secretions from other sources) have confirmed its role as a modifier of aggressive behavior. This was shown in *Cataglyphis niger* (Formicinae) and *Manica rubida* (Myrmicinae) where application of a nestmate's PPG on an alien ant reduced the aggression generally exhibited towards the latter, whereas application of an alien ant PPG secretion on a nestmate resulted in augmented aggression by her nestmates [26,27]. The complementary results obtained with ants from two different subfamilies suggest that the role of the gland as a modifier of aggressive behavior may be a general phenomenon in ants.

Causative experiments confirming the role of hydrocarbons in nestmate recognition

To test the specific role of hydrocarbons in nestmate recognition, PPG secretion of *Cataglyphis niger* was fractionated by column chromatography into hydrocarbons (hexane elution) and more polar lipids (chloroform: methanol elution) (Lahav et al, in prep.). Of these two fractions, only the hydrocarbons modified the ant's aggressive behavior. Interestingly, augmented aggression towards a nestmate applied with an alien ant hydrocarbon fraction was much more pronounced than decreased aggression towards an alien ant applied with a

nestmate's hydrocarbon complex. This may suggest that the mechanism of recognition relies on detecting differences rather than sameness.

A model of the production and dynamics of colonial odor: the gestalt model. A revisit of the role of trophallaxis

The use of the PPG as a source for nestmate recognition cues is very adaptive. Since it opens to the mouth cavity the secretion can be readily applied onto the body surface by self-grooming. The position of the gland also facilitates exchange of substances between members of the nest, promoting the rapid distribution of the scent within the colony. Moreover, any new substance that is introduced into the colony can be quickly incorporated and distributed among the nest members and thus become a part of the recognition system. Thus the PPG can be regarded as a perfect gestalt organ.

The gestalt model was first proposed by Crozier and Dix from theoretical considerations [28], and later behaviorally demonstrated in various *Leptothorax* species [29,30]. Recent studies using radioactive tracers have confirmed this role of the PPG. In *Cataglyphis niger* the gland would appear to be only a place for storage, rather than synthesis, of hydrocarbons [25]. According to these authors

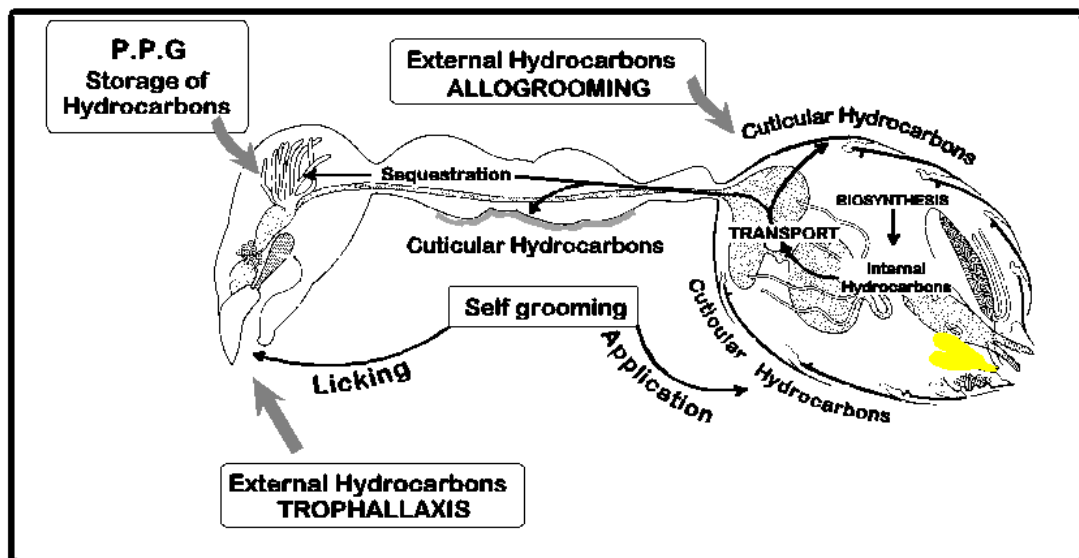


Figure 1 Intra- and interindividual hydrocarbon flow in the ants

the hydrocarbons are synthesized elsewhere in the body, perhaps in the oenocytes like in other insects, and are either secreted directly to the epicuticle, or transported through the haemolymph to the PPG. Encounters between a pre-labeled ant and an individual, or group of non-labeled nestmates, further confirmed that hydrocarbons can be transferred from an individual during trophallaxis, directly to the PPG of the partner(s) and then reapplied on its cuticle through autogrooming [25,27]. By constructing encounters in which one or both of the ants had blocked mouthparts, these authors further showed that allogrooming also plays a role, albeit secondary, in the transfer of chemicals. Similar conclusions were reached by deposition of (Z)-9-tricosene on the cuticle of a *Camponotus vagus* worker and finding this substance on the cuticle and in the PPG of the non treated nestmates [31].

As mentioned earlier, the use of the PPG as a gestalt organ facilitates the incorporation of new or external odors into the colony. It explains nicely how odors from environmental sources are used in nestmate recognition as found in fire ants [32], or how queen derived odors can circulate within the colony (see below). Glandular exchanges also seem to be the key to the ability of artificially mixed species to cohabit [33,34], and may reflect the situation in naturally mixed species colonies (e.g. parasite, slave maker; see later for additional discussion).

The finding that trophallaxis has a cardinal role in the creation of a homogenous colony odor, sheds new light on the function of this behavior. Despite the early hypothesis of Wheeler [35] that social life evolved in insects through trophallaxis between adults and their progeny, there is little documentation on the possible role of this behavior in social evolution. It is not cited at all in the two recent books on social evolution in ants by Bourke and Franks, and Crozier and Pamilo [1,36] and was not used as a factor in the evolutionary processes of ants discussed by Baroni Urbani [37]. It was recently suggested that food offered as part of an appeasement behavior may have selected for more elaborate trophallaxis in social Hymenoptera [38]. Having learned the role of trophallaxis in the exchange of semiochemicals, it was interesting to study how this process occurs in species that do not trophallax. Such a study was recently conducted with the ponerine ant *Pachycondyla apicalis* which has a normal size PPG [39]. Using radioactive tracers and dyadic encounters it was confirmed that the ants exchange hydrocarbons between PPG and cuticular surfaces. In contrast to *Cataglyphis niger*, the amount of transfer to the PPG was comparable irrespective of whether the donor had freed or blocked mouthparts. This indicates that all the transferred hydrocarbons to the donor were the result of

allogrooming. Behavioral observations confirmed the absence of trophallaxis in this species. Cue exchange by allogrooming seems to be less effective than trophallaxis, as revealed by the finding that the magnitude of transfer in the 24 hr dyadic encounters of species that employ trophallaxis such as *Manica rubida* or *Cataglyphis niger* is more than three-fold greater than in *P. apicalis*. Nonetheless, transfer by allogrooming may be sufficient for effective cue distribution in the small colonies of *P. apicalis* (80 individuals) [39-40]. Allogrooming is also probably involved in the chemical mimicry of the guest ant *Formicoxenus* which licks its host *Myrmica* very frequently to obtain its cuticular hydrocarbons [41,17].

If the case of *P. apicalis* can be considered as typical to ponerine ants, it would seem that the role of the PPG as a gestalt organ evolved earlier than trophallaxis. It can be postulated that as ant colonies became larger recognition by individual odors became impossible, favoring the evolution of a colonial odor [28]. This in turn necessitated an effective means of odor sharing and the development of an exocrine gland in which the different odors could be admixed. The use of the PPG with its external opening to the mouth cavity was adaptive. Early signs of the role of trophallaxis in cue exchange are observed in *Ponera coarctata* [38] where droplets may comprise postpharyngeal gland secretion rather than food [39]. At a later stage, when colonies evolved to be even larger, transfer by allogrooming became inefficient and transfer by trophallaxis became the major pathway for odor distribution within the colony. Whether trophallaxis evolution resulted from selective pressures to facilitate cue transfer in the colony, or primarily as a means of food transfer that was secondarily adapted for cue transfer, remains an open question.

The role of the queen in the composition of recognition cues

As mentioned above, ants from different colonies bear idiosyncratic compositions of hydrocarbons, even in cases in which they are reared under equal conditions and diet. This suggests a genetic component influencing their composition (see below). The question thus arises as to whose genes affect the colony odor: those of the queen or of the workers? In the case of monogynous and monandrous species the genetic makeup of the colony is rather homogenous, and unless the recognition cues constitute queen specific products, both the

queen or the workers can equally contribute to colony odor. In polyandrous monogynous species the genetic makeup of the colony is more heterogeneous and the question regarding the impact of the queen becomes more acute. It is definitely relevant in polygynous species. The solution to this biological problem may not be uniform. The impact of the queen may differ in different species with different social structures.

Of the few studies that have addressed this question, two possible models emerge. The first, based on behavioral experiments in *Camponotus floridanus*, suggests that the queen is the origin of the gestalt [42]. The second, based on biochemical studies in *Cataglyphis niger*, suggests that the queen acquires the average colony odor and therefore positions herself in the center of the gestalt, rather than being its origin (43). These models may not be mutually exclusive, especially in view of the fact that *C. floridanus* is monogynous whereas *C. niger* is polygynous.

The basic hypothesis behind the experiments done with *Camponotus floridanus* was that in a mixed species group consisting of an unequal number of workers from each species and a queen of the minority species, if the workers are responsible for the colony odor then this should have the odor of the majority species. Through a series of elegant studies with such mixed colonies it was demonstrated that whatever the respective number of workers, the colony still bears the odor of the species to which the queen belongs.

The study with *Cataglyphis niger* utilized a more direct approach to address this question (43). Through a series of dyadic and group encounters it was shown that the queen receives more PPG secretion that she tends to give away. It was further shown, that when given the choice, workers preferred to transfer PPG material to the queen rather than to their nestmates. The end result is that the queen possesses three times as much secretion in her gland and, moreover, she always has an average colony odor. This central position of the queen is also suggested from the experiment in which polygynous colonies were split into several daughter monogynous colonies. After several months of separation the hydrocarbon profiles of the daughter colonies diverged (see below), but the queen was always at the average of each respective colony (although the workers in the colony were not necessarily her descendants; Lahav et al., unpublished data).

Genetic and environmental origins of the hydrocarbons

Many studies have shown that colonial recognition is at least partly determined by genetic factors [4,11]). There is also evidence that hydrocarbon composition is genetically determined. Intermediary cuticular profiles that have been found in areas cohabited by two *Solenopsis* species, are apparently the result of hybridization between these two species [44,45]. In honeybees different patrines have discriminable cuticular profiles, emphasizing again the genetic components delineating hydrocarbon composition [5].

An environmental origin of the colonial odor must also be considered because the lipid layer of cuticle is a veritable trap for all odors. Some myrmecophiles rub their body against the walls of the nest to acquire the odor of the host colony as camouflage. In bees and wasps nesting material was described to influence colonial recognition [46,47], while in attine ants the quality of food leaves influences the nest odor [48]. In *Leptothorax nylanderi*, which nests in sticks on the forest ground, nestmate recognition is largely influenced by the origin of the stick, being either pine or oak [49]. Ants living in pine wood probably incorporate and use the characteristic conifer odors trapped by their cuticle.

Ontogeny of the PPG secretion and changes of hydrocarbon profile with age

Cataglyphis niger callow ants have a visibly structured, but not yet fully developed PPG. There is a clear structural as well as chemical age-dependent development. The epithelial thickness increases with age, reaching its maximum within the first week after emergence, and also showing a progressive development of microvilli and occurrence of lamellar inclusions. Concomitantly there is an increase in the quantity of hydrocarbons present in the gland [50]. A similar chemical ontogeny is also described for *C. iberica* [51]. This glandular ontogeny corroborates nicely with the studies pertaining to the ontogeny of nestmate recognition. Since the early work of Fielde [52] who hypothesized the existence of a progressive odor change with age in ants, other authors have confirmed that there is an age dependent quantitative variation in the production and release of the cues implied in the formation of colonial odor [see 11, 51]. In workers of *Manica rubida* and *Formica selysi*, chemical analysis showed that both species already had their own specific cuticular profiles within 48 hours of emergence, and that their total amount increased with age until they stabilized at the age of one month [53,54]. This quantitative variation in the production of

chemical cues is also observed in *C. iberica* where worker chemical profiles are distinctive according to age group and gradually converge from a “callow profile” with specific hydrocarbons to a profile characteristic of mature workers [51].

Callow workers are more likely to be accepted into alien colonies than a one week old worker [29,55-58], which may be linked to the weak aggressive behavior, but also due to the weak signal they possess on their cuticle. The lack of external chemical characteristics, which we term “*cuticular chemical insignificance*”, also seems to be the basis of the successful formation of artificially mixed species groups of *Manica rubida* and *Formica selysi*. This is followed by a period of “*chemical integration*” that results in a pseudosocial colony, and is possible only with callow workers [53], thus supporting the observations that at this stage the PPG is rather undeveloped (Hefetz and Errard, unpublished data). It is interesting that in more phylogenetically remote species workers must be younger in order to achieve a successful mixed species group [59]. We can speculate that in these cases the qualitative differences between their respective hydrocarbons are high, and the small amount of hydrocarbons that the callow workers possess is enough to signify the differences between the individual species in question. On the other hand, when the species are more related phylogenetically, qualitative differences in the recognition cues are smaller and there is a need for higher quantities to detect the differences between the species. A “*cuticular chemical insignificance*” phenomenon may also be the basis of the successful usurpation by slave maker and parasitic ants. We would like to emphasize that the processes of “*cuticular chemical insignificance*” followed by “*chemical integration*” that we observe in artificially mixed colonies do not necessarily reflect the mechanisms employed by naturally mixed societies. In artificially mixed societies the ants employ, once mutual tolerance has been established (the *cuticular chemical insignificance* phase), their predisposed behavioral repertoire, i.e., cue exchange by trophallaxis and allogrooming, and achieve a uniform colonial odor. Slave makers and parasites on the other hand have seemingly coevolved with their host and any of various mechanisms, from chemical camouflage to the maintenance of independent chemical signatures, could have been selected for.

Template acquisition

The template is an “internal representation of the labels expected in kin within the mind of the discriminating individual” [36]. Many ethological observations have shown the existence of a learning period for template acquisition. In all the species studied so far, nestmate recognition cues appear to be learned shortly after eclosion into the adult stage even in species utilizing cues that are primarily genetically determined, as in sweat bees *Lasioglossum zephyrum* [13,2]. Workers reared in mixed groups learn and memorize during their early social experience the allospecific chemical cues, incorporating them into their template and keeping them for at least one year [53,60]. The supposition of an early learning period does not exclude the possibility of template updating (see below).

The decision rules

When encountering another ant, the individual has to compare his own template with the received cues and choose between amicable or aggressive reactions. The response can be evoked according to a threshold rule or can be graded (these are by no means mutually exclusive). In some species (e.g. *C. cursor*, *Myrmica rubra*) ants exhibit scaled aggression from complete rejection to acceptance [19,26,27,32,55]. On the other hand in *Cataglyphis iberica* allocolonial workers are always immediately rejected [61], suggesting that the ants react according to a threshold. A behavioral response according to threshold does not mean that discrimination does not follow a gradual scale. In *C. iberica* young workers at the onset of hibernation possess a PPG composition that diverges from the colonial odor [62]. This difference is apparently recognized by older workers since the young workers are preferentially transported to other satellite nests. Since the difference in glandular composition does not elicit aggression, we can assume that they are below the threshold. Thus in this ant we have at least three levels of discrimination with a parallel gradation of dissimilarity in cuticular hydrocarbons: alien (behavioral response expressed as aggression), young (adult transport) and old (various amicable behaviors such as trophallaxis, allogrooming etc.). Although the role of hydrocarbons in nestmate recognition was not demonstrated in this species we draw a parallelism from the experiments with *C. niger*.

Additional evidence for a graded model in discrimination is the longer antennation time observed when the signature differs slightly from the template. This was shown in various *Solenopsis* and *Leptothorax* [63,64] in which nestmates are

recognized immediately when encountered, but any slight difference induces longer inspections and recognition is more difficult. Longer antennation, before actual recognition, presumably the result of slightly diverging cuticular compositions, was also observed in adult *C. iberica* that were reunited after a period of separation [65]. In a colony composed of non related workers mutual antennation was longer than normal [66,67], which was not the case when the workers constituted different matrilineal lines [7].

The conclusion arising from all these studies is that the gestalt colonial odor has a dynamic component and is modified with time according to the composition of the group and/or food, as has been observed in other ants [63,64]. It is further emphasized that there is a graded response to odor/template matching up to a threshold which is variable according to the species.

A new insight into the graded response was achieved in studies that tested the reaction of ants from mixed species colonies that were treated with postpharyngeal secretions under various contexts. Accordingly, it was hypothesized that the deciphering of the signal in the recognition process may be hierarchical and the reaction inverse to the familiarity of the signal. It was further demonstrated that the template to which the signal is compared is acquired, and since the label is dynamic template plasticity must follow. Thus the template is not fixed after the sensitive period, but has to be reinforced via constant perception of nestmate odors [29,68]. In some highly polygynous and pandemic species ants generally accept introduced alien workers. It is possible that the workers are habituated to be confronted with various odors due to low relatedness (the lowest known case is $r=0.02$ in *Linepithema humile* [69]) and more variable genetic cues [70]. They learn a less restrictive template and consequently their threshold response is very weak, or even entirely absent in pandemic tramp species [71].

Discussion and perspectives

Nestmate recognition is undoubtedly one of the major selective forces in social evolution, and has provided a multidisciplinary subject for research. Although it was a natural theoretical outcome from kin selection considerations, when it came to elucidating the proximal mechanisms, it proved to be a complex question. In order to progress in our understanding of the system, we have on the one hand to differentiate between the label and the template, but at the same time we also have to appreciate that the mechanisms controlling them are

interlinked. Our understanding of the nature of the label and its origin has improved considerably in recent years, but we are far from understanding these features regarding the template.

Concerning the label, the theoretical suggestion that a gestalt odor must exist in complex social insects was experimentally substantiated in all the species studied so far. While the theory of the gestalt provided an excellent explanation for the establishment of a colonial odor, biochemical experiments have outlined the processes involved, including the involvement of hydrocarbons as a part of the recognition system, and mutual exchanges as a means for creating the gestalt.

A basic feature of the label is its dynamic character and the ability of the ants to update the cues through their mutual sharing with a subsequent refreshment on the cuticular surface.

There are still open questions regarding the evolution of the label, its glandular origin and its mode of dissipation within the colony, in particular in very populous colonies. Was there a functional evolution of the PPG and comparable glands? Is there a cuticular lipid storing gland in solitary species, or has it evolved specifically in ants? An indication for this kind of convergent evolution comes from a recent study on the biosynthesis of the hydrocarbon sex pheromone of the tiger moth. It appears that the pheromone is biosynthesized in association with the abdominal integument and carried by a specific lipophorin to the pheromone disseminating gland in the abdomen [72].

The chemical gestalt present in the ants can be paralleled to a network. The gestalt is based on a constant flow of “information” and is naturally influenced by the number of participants. While optimizing the system so as to create uniformity, it is obvious that as the system becomes more complex it is liable to more breakdowns. To what extent, then, can the gestalt odor function in large colonies? Does it break down or are there alternative methods for achieving a cohesive closed colony?

The idea of a template has been around for a long time (e.g. hypotheses pertaining to learning and sensitive period), but little experimental progress has been achieved. This is not surprisingly since the experimental paradigms for testing the nature of the template are extremely difficult and multidimensional. We still lack understanding of how nestmate recognition cues are perceived, and at what levels the information is processed. At the behavioral level we know that the manifestation of the tested chemical comprises a complex behavior that is largely dependent on the context of their perception. For example, in the ponerine ant *Pachycondyla apicalis*, the outcome of a dyadic encounter depends

on whether the ants are resident or intruders in accordance with the theory of games [40, Fresneau et al. in prep]).

Another largely unsolved problem regarding the template is the impact of memory on the integration of the perceived signals. Adoption experiments point to the existence of a learning sensitive period during which the sensory imaging of the colony is formed. But this memory evidently has to be plastic rather than fixed, since the experimental evidence demonstrates that the label is continuously updated. Therefore an efficient system has to possess a comparably flexible template system. Returning to the case exhibited by the ponerine ants, ants that are adopted into an alien colony, following experimental manipulation, participate after a while in the defense of their adoptive nest. This means that they have substituted their original template for a new one. There are two alternative hypotheses to explain this phenomenon: the template is memorized, but if it is not reinforced or the stimulus permanently changes, the old memory is erased by a new one; alternatively, the template is not neural but chemical. The ants constantly compare the odor on their body surface (or part of it, e.g. antennae) to the odor perceived at any given moment [73]. This latter system is simpler since the information is already filtered at the peripheral system without a need to refer to memory embedded within the brain. These systems are not mutually exclusive. We hypothesize that the simpler chemical reference could have been selected for in the primitive social species with small colonies. In larger colonies the complexity of the systems increases exponentially and may have also required an internal representation of the colonial identity. We hope that with the advances in insect neuroscience answers to such questions will be resolved.

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