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Nestmate Recognition in Ants

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Kin/Nestmate Recognition

All ants are highly eusocial, which means 1) individuals care for the young, 2) there are castes (reproductive division of labor), and 3) there is an overlap of at least two generations in which workers (normally sterile) assist their mother in rearing sisters and brothers. This altruistic (or nepotistic) behavior is thought to characterize kin selection (Hamilton 1964), defined by Wilson (1987) as "Differential survival or reproductivity that changes the proportion of genes through time due to the circumstance that individuals favor or disfavor relatives other than direct offspring." To achieve kin selection there must be kin recognition. Herein lies much of the driving force for the large volume of research over the past decade concerned with kin and nestmate recognition (e.g. Fletcher and Michener 1987). There are more pragmatic reasons to understand nestmate recognition.

Ants have developed a formidable array of active and/or passive semiochemical and non-chemical defenses (Hermann and Blum 1981). The many active defensive behaviors are initiated when an intruder is recognized as non-nestmate by resident workers. Thus, nestmate recognition represents the first line of defense for a colony. The chemistry and associated behaviors of kin/nestmate recognition are intrinsically interesting. Beyond that, knowledge of nestmate recognition is essential for a comprehensive understanding of both ant defenses and the organisms (symphiles) that have broken the recognition code and are able to infiltrate ant colonies and exploit colony resources (Kistner 1979). These areas of research may provide the basis of innovative control strategies for pest ant species.

What Are the Possible Recognition Scenarios?

The simplest ant colony situation is one where there is a single queen (monogyne), inseminated by a single male (monoandrous), the colony resides in a single nest (monodomous), and workers from each colony defend a territory (intolerant of workers from adjacent colonies). This scenario is found in nature, but so are polyandry (insemination by more then one male), polygyny (more then one queen), and polydomy (more then one nest). The latter conditions are common and are often accompanied by a lack of or diminished territoriality. This has profound effects on recognition possibilities.

Individuals within a colony may recognize each of the other individuals in their colony (**individual recognition**; Figure 4.1, Situation #1). This appears to be unlikely except where a colony is composed of only a few individuals. In small and large colonies **kin**

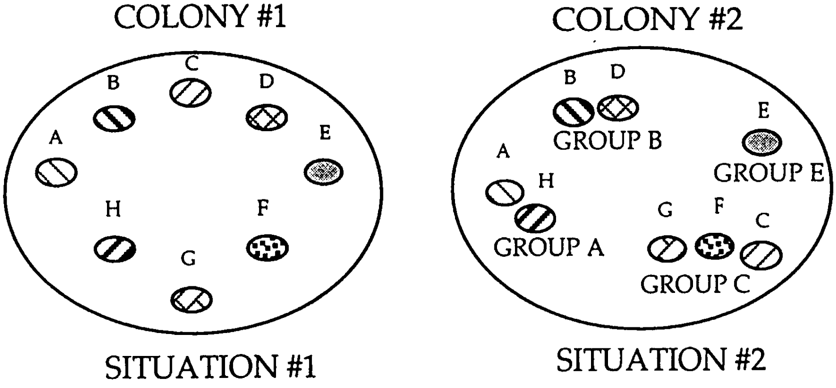

FIGURE 4.1. The diagrammatic representation of worker - worker recognition possibilities within a colony. Workers are represented by ovals filled with different patterns if recognized as different or the same pattern if not recognized as different. Situation #1: The colony is composed of eight workers, each of which recognizes all of the others as different, yet they also recognize them as nestmates. In large colonies this situation becomes difficult. Situation #2: Segregation of workers may occur based on worker recognition of a common mother or father in species that exhibit colony polygyny or polyandry. It is possible for members of each polygynous or polyandrous group to recognize each other as different, members of the other groups as more different, yet all members of the colony as nestmates. Since recognition is made up of chemical cues and neural templates, these diagrams can also be used to visualize the cue and template possibilities within colonies.

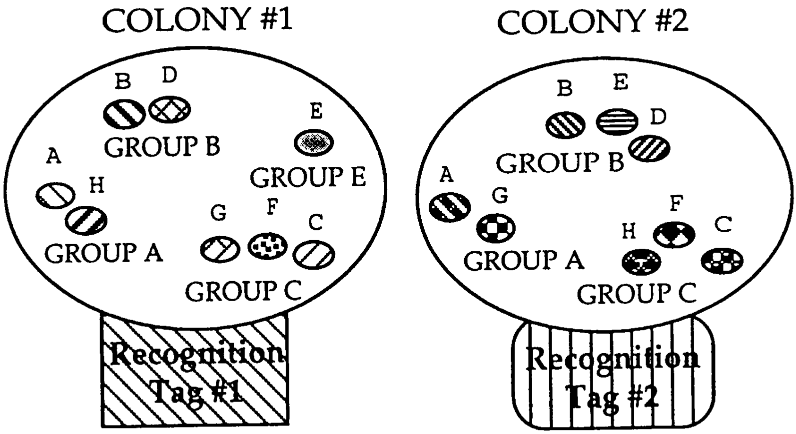

FIGURE 4.2. Diagrammatic representation of nestmate recognition for two polygynous or polyandrous colonies whose workers exhibit class recognition (Groups). The behaviors associated with class recognition are thought to be subtle in nature. Superimposed on class recognition is nestmate recognition - each colony member recognizes other colony members as nestmates. The commonality is represented by the colony TAG. Members of other colonies have different colony TAGs and are recognized as non-nestmate. Nestmate recognition is characterized by aggressive interactions.

recognition or **class (group) recognition** may occur. This category can be broken down into several subsections. Individual groups within a colony may be segregated to some degree based on recognition of a common maternal (**matriline recognition**) or a common paternal (**patriline recognition**) phenotype or both if polyandry and polygyny occur (Figure 4.1. Situation #2). At the next level is recognition of belonging to an individual nest (**nestmate recognition**; Figure 4.2). As indicated in Figure 4.2, it is possible to have intra-nest class recognition occur simultaneously with nestmate recognition. Class recognition may manifest itself in subtle interactions, such as preferential feeding of a worker's closest kin; whereas aggressive behaviors are elicited from resident workers when con-specific (in territorial species) or hetero-specific intruders are recognized. In theory, nestmate recognition also can involve subtle interactions, but these have not been observed. Nestmate recognition consolidates the colony against con- or hetero-specific ant competitors; whereas, kin recognition represents the optimization of gene flow of their nearest relative, the end result is presumably, kin selection.

Nestmate recognition is functionally equivalent to kin recognition in the special case where the species is monogynous and monoandrous, exemplified by the fire ant, *Solenopsis invicta*. Nestmate recognition may also promote fitness through kin selection if queens of a polygyne colony are more related to each other then are queens from other colonies. In all other situations of polygyny and/or polyandry, kin recognition may occur within the nest and inter-nest interactions are considered separately as nestmate recognition.

Most of the above discussion of kin recognition has been dogma for the last two decades with researchers working toward validation of the hypotheses. Recently, some interesting alternative views to the kin recognition story have surfaced in the literature. Carlin (1989), cautions about ascribing adaptive significance to results obtained under artificial laboratory conditions. He suggests that inter-colony discrimination may be based on any random odor idiosyncrasies and thus is not under selection. This is especially relevant for species demonstrated to use environmental cues in recognition. Also, within colony discrimination (kin recognition) may be a nonadaptive side-effect of clearly adaptive between colony discrimination (nestmate recognition). Grafen (1990) expands on this theme, stating that purported demonstrations of kin recognition are too weak to be effective and are again better explained simply as a side-effect of species recognition systems. We will proceed with these intriguing hypotheses in mind along with the realization that virtually all recognition studies on ants involve nestmate recognition rather than kin recognition.

The Recognition Process: Cues, Template, and Response

For recognition to occur each individual must have a label or set of cues (conceptually analogous to a bar code), a mechanism for detecting and interpreting the label of another individual (bar-code reader and central processing unit -- CPU), and a learned template of cues stored in memory (computer storage device that, as we shall see later, must have, read/write capabilities) with which to compare the incoming signal. The recognition process is completed with an appropriate behavioral response, which need not be overt.

The Cues: Where Do They Come From?

It is generally accepted that nestmate recognition cues are chemical in nature and are detected by a mere sweep of ant A's antennae across the cuticle of ant B (Wilson 1971). Recognition, especially inter-specific, may be elicited over a short distance (1-2cm) without antennal contact. As we shall see in detail later, nothing is known definitively about the chemical nature of nestmate recognition cues, in spite of the many publications implicating cuticular hydrocarbons. Thus, there are no chemical class / nestmate recognition associations to examine and the possibilities are open.

We define colony odor as all odors associated with a particular colony. Nestmate recognition cues are a subset of colony odor (Vander Meer 1988). The all-encompassing colony odor is composed of two main categories, odors derived from the environment and those from physiological sources. Environmental odors may be from the air, soil, food, or any other source not produced or modified by members of the colony. Physiological sources are derived from cuticular lipids, exocrine gland, excretory and regurgatory products, as well as other odors modified by the individual or group and released to the colony. Literature examples of nestmate recognition illustrate the importance of environmental cues, e.g. *Acromyrmex octospinosus* (Jutsum et al. 1979); physiological cues, e.g. *Psuedomyrmex feruginea* (Mintzer and Vinson 1985); and a combination of environmental and physiological cues, e.g. *Solenopsis invicta* (Obin and Vander Meer 1988). Thus, all combinations of the two broad categories are possible.

Cue Detection

The ant's antennae are analogous to our nose -- detecting volatile compounds in the air, but perhaps also capable of detecting "non-volatile" compounds by contact. The signal received is transduced and processed by the central nervous system, which then may trigger a behavioral response. The situation is similar to our personal experiences, where our nose detects many different odors but not all elicit a behavioral response. And similarly for insects, electroantennagram studies reveal that an insect's antennae may detect a compound, but observable behaviors may not be triggered. Antennectomized *Formica lugubris* workers were unable to recognize nestmates (LeMoli et al., 1983). After detection of an intruder's cue profile (a sub-set of the overall colony odor), the profile must be compared with the resident worker's neural **template** or memory pattern of its colony's recognition cues.

The Template

The hypothesis that the template associated with heritable cues is genetically determined and directly linked to the cues has not been supported experimentally. Thus, the following discussion only considers the development of a learned template. In all ant species nestmate changes in the cue profile. Therefore, a colony's nestmate recognition cue profile cannot be learned by newly eclosed workers (callows) as a fixed pattern but must be continuously updated through a process of iterative learning (Vander Meer 1988). Wallis (1963) proposed a similar scheme based on observations of *Formica* species. He suggested that each worker was probably "continually habituating to slight variations in the odor of its nestmates".

A generalized cue/template model is shown in top half of Figure 4.3 for territorial (monogyne) species -- they recognize con-specific workers from other colonies as different. Environmental cues are expected to vary quantitatively and qualitatively, whereas heritable cues will vary only in the relative intensity of the compounds involved (quantitative change only). Each colony in Figure 4.3 is designated by a letter. If colonies A to D were analyzed for recognition cues at some point in time they would each have a distinct profile derived from environmental and/or heritable sources, as shown in Figure 4.3. The template for each colony reflects the cues of that colony at that point in time. If these same colonies were sampled at another time, they would again have distinct cue profiles, but different from the previous sampling. For the system to work the template must reflect the cue changes. Hetero-colonial intruders have cues that do not match the template of the resident colony at any given time, resulting in aggressive behaviors.

Polygyne species often do not defend a territory and do not recognize members of other nests as different. There is free flow of environmentally derived odors from a larger area than expected for monogyne colonies and with free flow of workers from many matrilines and patrilines, workers experience a broad range of heritable cues. However, at any given point in time each polygyne worker has a distinct cue profile just as in the monogyne situation. The difference between monogyne and polygyne nestmate recognition lies in the template (Figure 4.3, bottom half), which is broader and less distinct than their monogyne counterpart. The fire ant, *S. invicta*, provides an excellent example of both extremes in a single species.

How do *S. invicta* polygyne and monogyne aggression bioassay data (Morel et al. 1990) conform to the model presented above? Polygyne residents do not recognize monogyne or polygyne intruders as different; however, monogyne residents are very aggressive toward both types of intruders (Figure 4.4). Thus, the cues of polygyne and monogyne intruders fit within the polygyne template. The response of polygyne or monogyne intruders introduced into monogyne colonies is small because the intruders are attacked so quickly they have no chance to respond recognition discriminators and the neural template are derived to some extent from all possible sources -- the environment, the individual, a class of worker, or queen from within the colony, or collectively from all individuals in a colony. The relative importance of each discriminator type may vary from species to species. Hölldobler and Carlin (1986) proposed just such a hierarchy for *Camponotus* species, involving queen, worker and environment derived discriminators.

Depending on the polyandrous and/or polygynous status of a colony, there may be many possible discriminator groups (also known as classes or referents; see Figure 4.1, Situation #2). It is easy to visualize a worker having to learn **multiple templates** (Bennett and Breed 1987), perhaps thousands, especially in polyandrous, and/or polygynous colonies, in order to assess the relatedness of nestmates. This presents an extremely complicated learning situation for individual workers. To simplify the task for both the ants and their human investigators the concept of **multiple mean templates** was proposed so that, for example, a worker from a polyandrous, polygynous colony could theoretically have mean templates for each queen and her offspring regardless of worker patrilineage. This is still a difficult experimental model and has been expanded further to a colony having a **single mean template.** The latter approach is experimentally indistinguishable from the "gestalt" model of Crozier and Dix (1979), which assumes that colony odors (nestmate recognition cues) are exchanged among colony workers through normal social interactions, such that all workers possess the same odor profile. Thus, genetically varied nestmates are anonymous to each other (Hölldobler and Carlin 1987) within the colony, but not between colonies. This relates also to the "fellowship" concept of Jaisson (1987). The "gestalt" model has been demonstrated in several ant species (*Pristomyrmex pungens*, Tsuji, 1990; *Camponotus* species, Carlin and Hölldobler, 1987). In reductionist terms, multiple templates, multiple mean templates and single mean template become an **individualistic model, multiple "gestalt" template model** and **colony "gestalt" model**, respectively. All of the above may occur; however, from an experimental point of view, devising an unambiguous experiment that measures subtle discrimination of non-kin via multiple templates or multiple mean templates has not yet been accomplished. So, we are left with experiments centered around the concept of a single mean template or colony "gestalt", which measures nestmate recognition rather than kin recognition, except the previously mentioned special case of monogynous, monoandrous species where kin and nestmate recognition are synonymous in that they both promote kin selection.

Are Cues and Templates Static or Dynamic?

We know that recognition cues can be derived from environmental and/or heritable sources. Environmental cues dynamically change over time, as food sources vary and the surrounding habitat changes with the seasons. Therefore, the part of the neural template based on environmental cues must also be dynamically changing. What about heritable recognition cues? One's first reaction is that heritable cues are genetically controlled, therefore, they should be static. It is well known that component ratios of lepidopteran sex pheromones are necessarily static, in order to maintain species separation. On the other hand, ant alarm pheromone components from *Crematogaster* species have been reported to vary considerably from individual to individual within the same nest (Brand, and Pretorius 1986), and head derived pheromones of *Tetramorium caespitum* vary with caste (Pasteels, et al. 1980), as do the mandibular gland products of *Atta sexdens rubropilosa* (Do Nascimentoi, et al. 1993). Pheromone variation within a colony led the former authors to suggest that alarm pheromones would not be useful nestmate recognition cues. What about the chemicals associated with an ant's cuticle? After all, recognition occurs with a simple sweep of the antennae across the cuticle of the intruder (Wilson 1971). Where quantitation has been reported, hydrocarbons are the major class of chemicals found on ant cuticle (Lok et al. 1975), but as should be expected, other lipid classes are also present. Cuticular hydrocarbons are under genetic control, as evidenced by high levels of concordance between the identification of *S. invicta* and *S. richteri* and hybrids based on hydrocarbon patterns and isozyme analyses (Vander Meer et al. 1985; Ross et al. 1987). Cuticular hydrocarbons are readily analyzed by gas chromatograph and have been used as a tool to study variability in the heritable component of colony odor. Vander Meer et al. (1989) used pattern recognition analyses to determine that at a given time, within colony cuticular hydrocarbon variation was less than colony to colony variation and nine *S. invicta* colonies sampled were readily distinguished. In addition, and perhaps most importantly, they found that colony hydrocarbon patterns varied over time. This phenomenon has been confirmed in *Leptothorax* species (Provost, et al. 1993). If the premise that cuticular hydrocarbons are representative of the heritable component of colony odor, and thus are heritable recognition cues, is correct, then heritable cues are not static but dynamically changing with time along with cues derived from the environment. This has profound effects on how we view the other half of the recognition process - the template. The neural imprint or template of colony recognition cues must also be dynamic rather than static and track

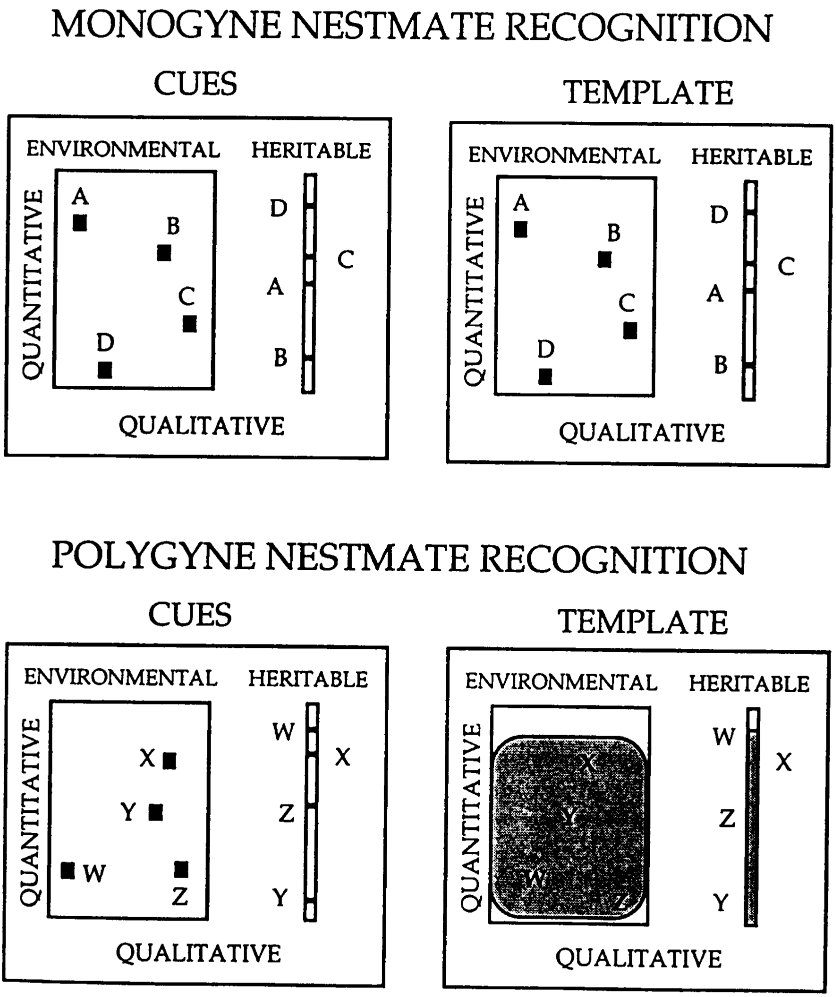

FIGURE 4.3. Diagrammatic comparison of the cue/template nestmate recognition system for monogyne and polygyne intraspecific populations. The upper cue/template pair are the same as seen in Figure 5. and represent the monogyne situation. The bottom cue/template pair represents the polygyne situation. For both types the cues part of the process is identical -- at any point in time each worker from each colony has a distinct cue profile. Differences in the two population types lie in the template. Multiple matrilines, patrilines, and lack of territoriality lead to a broader template that accepts intruders with a wider variety of cue profiles. Thus, polygyne and monogyne intruders are accepted into polygyne colonies, but polygyne intruders are not accepted into monogyne colonies.

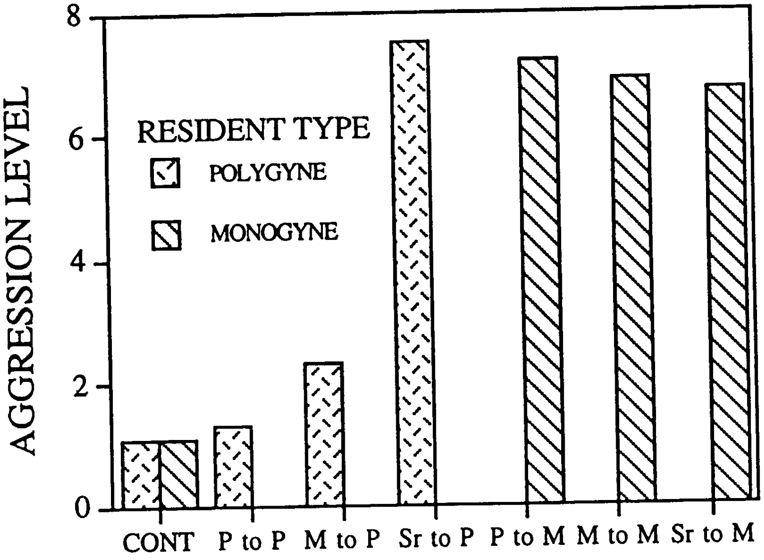

FIGURE 4.4. Aggressive responses of polygyne and monogyne *Solenopsis invicta* residents to intruders. M = monogyne; P = polygyne; Sr = *Solenopsis richteri*; cont = control; M>P = monogyne worker introduced into a polygyne colony. Level 1-3 = investigative; 4-6 = challenge; 7-9 = attack.

(Figure 4.5). For polygyne workers introduced into polygyne colonies there is acceptance in both directions and no aggression is elicited. However, monogyne intruders are not attacked by polygyne residents and have a chance to respond in encounters with residents whose cues do not match their templates. Heterospecific workers are readily recognized by both polygyne and monogyne *S. invicta* residents because the heritable component of their cues is qualitatively distinct. The model represented in Figure 4.3 accommodates the bioassay data. Further, queenright polygyne *S. Invicta* colonies brought into the laboratory for > three months, thus isolated from the polygyne population, developed acute intraspecific discrimination capabilities (Obin et al. 1993). Having isolated multiple queen colonies does not provide the necessary breadth of cue sources. Does this fit with the ontogeny of nestmate recognition?

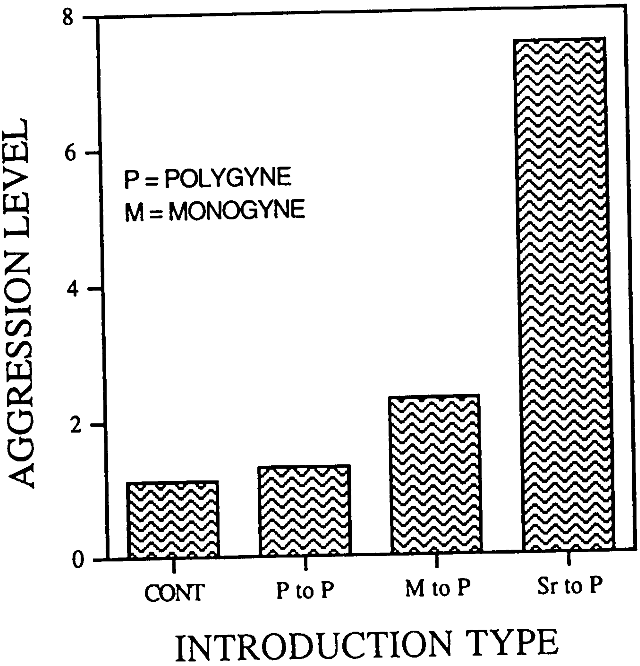

FIGURE 4.5 Aggressive responses of polygyne and monogyne *Solenopsis invicta* intruders to residents. M = monogyne; P = polygyne; cont = control; M to P = monogyne worker introduced into a polygyne colony; Sr = *S. Richteri*. Level 1-3 = investigative; 4-6 = challenge; 7-9 = attack.

The Ontogeny of Nestmate Recognition

Newly eclosed ant workers are called callows. Jaisson (1985) states the callow worker's situation succinctly: "...the newly emerged individual appears in an environment which is by definition hostile to anything alien, and must itself recognize its own colony as being an exception to this general hostility rule". Jaisson (1985) lists five callow worker attributes that help to explain the integration of young workers into the group: (1) The absence of aggressive behavior; (2) The presence of compounds that inhibit adult aggression; (3) Attractiveness for adults; (4) The potential for absorbing the colony odor; (5) Reduced mobility.

Colonies composed of two different species can be formed through the successful adoption of foreign brood or callow workers (Fielde 1903; Plateaux 1960 and Jaisson 1971). Thus, the callow worker characteristics listed above that account for colony integration, are non-specific, at least to a certain degree. The absence of aggressive behaviors and reduced mobility lend themselves to lessened aggression by mature resident workers, since it does take two to tangle. This allows the potential adoptee time in which to acquire the colony odor (nestmate recognition cues) of its new colony. Mechanisms that allow intruders (parasites, predators, etc.) to survive long enough to acquire colony odor will surface again in discussions of myrmecophiles and brood pheromones. Hölldobler and Michener (1980) suggest that successful callow worker adoptions can be explained by pupal pheromones contained in part in exuvial fluids. Jaisson (1985) more specifically believes the substances involved are only produced at the time of worker eclosion, because assistance from nurse workers is often necessary for successful eclosion. LaMon and Topoff (1985) give the following account of fire ant eclosion. At the initiation of eclosion, gross pupal movements occur, which loosen the pupal cuticle, often tearing at the joints. "At the same time the surface of the cuticle becomes covered with a sticky, viscous exudate. When adult workers are present they remove the eclosing pupae from the brood pile and avidly strip away the pupal skin..." Workers often consume the shreds of cuticle they tear off the eclosing pupae. This description of the social facilitation of eclosion in fire ants supports the contention of Jaisson (1985). Although potent phagostimulants appear to be present, it has not been demonstrated and it may not be necessary that attractants are involved. Vigorous movement in previously quiescent pupae may be adequate to get a brood tending worker's attention (LeMasne 1953). The intense interactions of adult workers with eclosing pupae and the resulting callow should accelerate the absorption of the colony's odor (Jaisson 1985). We think this along with reduced callow worker mobility and aggressive behaviors, but without invoking the presence of attractive compounds (pheromones), is enough to allow integration of the callow worker into its colony or adopted colony.

The net result of the above is that early social experience plays a role in colony integration of callow workers (Le Moli and Mori 1984, 1985; Morel and Blum 1988; Errard 1984, 1986) and in the acquisition of the colony label (Morel et al. 1988). In addition, a sensitive period can be defined, after which workers can no longer be successfully transferred to another colony. The mature worker's fully developed exocrine gland system and repertoire of aggressive behaviors precludes it from surviving long enough in another colony to acquire that colony's distinct odor. How does the above fit our scheme as presented in Figure 4.3? A newly eclosed callow worker has a distinct cue profile that differs from adult workers from the same colony, consequently, it should be attacked. Indeed, low levels of resident aggression toward callows are reported (Morel et al. 1988); however, through lack of reciprocal behaviors and immobility, callows gain time to acquire the constantly changing colony odor. Concomitant with the acquisition of colony odors, its repertoire of behaviors and mobility increase, such that successful adoption of this worker into another colony becomes increasingly difficult because it is losing the capacity to survive long enough to acquire a new colony's odor. Thus, the ontogeny of nestmate recognition fits very well into the general model shown in Figure 4.3, and further provides important insights into mechanisms of parasite and predator integration into ant colonies.

Cue -Template Matching Possibilities or How Are Recognition Decisions Made?

Cue similarity versus discrete odor matching models were investigated in *S. invicta* by Obin and Vander Meer (1989), using readily manipulated environmental cues (diet) to simulate the possible models described above. The data support a cue similarity model rather than models requiring discrete odor-matching (as in Getz 1982). Provost (1991) reported support for the graded response model, as non-related *Leptothorax lichtensteini* intruders induced a wide range of agonistic behaviors from resident workers. Provost (1991) also found 100% acceptance of related intruders and suggests this may be evidence in support of a threshold model. It seems to us that a threshold model must work in both directions -- acceptance and rejection. The Obin and Vander Meer (1989) paper did not address the question of graded response versus threshold response, however, there is ample evidence in the literature of ant nestmate recognition that graded responses occur in the real world (see the behavior section below).

Behavior Elicited

To study nestmate recognition and its associated chemistry an appropriate bioassay(s) must be developed. Assays have generally focused on the aggressive behaviors displayed by resident workers toward intruders that are recognized as non-nestmates. Interactions can be classified into two broad behavioral categories: non-aggressive (licking and trophallaxis) and aggressive (mandible opening, grasping, and flexing of the gaster with release of venom) (Morel, and Blum 1988, for *Camponotus floridanus*; Henderson, et al. 1990, for *Formica montana*). Aggressive behaviors in turn can be ranked in a hierarchical aggression scale (Table 2; Carlin, and Hölldobler 1986, for *Camponotus* spp.; Obin, and Vander Meer 1989, for *Solenopsis invicta*). The use of hierarchical behavioral units to score nestmate recognition is itself evidence against the threshold hypothesis for cue/template matching (Getz and Chapman, 1987) and evidence for a graded behavioral response depending on the degree of cue and template similarity. The caveat is that a worker introduced into a foreign con-specific colony may elicit a wide range of behavioral responses from encountered resident workers. Colony and individual variation in aggressive worker responses has been demonstrated for *Rhytidoponera confusa* (Crosland, 1990). Does this mean that each resident has a template different enough to promote the different behavioral responses to the intruder (support for the graded response) or are resident workers in different physiological modes that affect their behavior to the intruder (the effect is a graded response, but the cause is independent of the cue / template system)? To avoid this problem Obin and Vander Meer (1988) scored aggression assays using the most aggressive response shown out of 20 interactions. Bioassays have also been developed that utilize a surrogate worker, which makes it possible to address the chemistry of nestmate recognition.

Habituation Versus Active Recognition

It is possible that resident workers are habituated to their colony recognition cues and recognize non-nestmates rather than their nestmates, thus instead of nestmate recognition we would be dealing with **non-nestmate recognition**. Arguing against this proposition are examples where intra-specific, inter-nest recognition is dominated by heritable cue differences (Obin and Vander Meer 1988). In this situation only quantitative variation is expected for intra-specific heritable cues (as opposed to qualitative and quantitative variation for environmentally derived cues), thus in a habituated situation, small quantitative cue variation would not be expected to elicit a behavioral response. Therefore, recognition of nestmates will be assumed in this review.

The Chemical Cues

Some Problems with Nestmate Recognition Chemistry

Nestmate recognition in ants has been under intense investigation for well over a decade, yet, little is known about the chemical make-up of the recognition cues. There are many reasons for this. From our experience with the fire ant, *Solenopsis invicta*, it has been impossible to devise an appropriate nestmate recognition bioassay for this highly aggressive and sensitive species, although other species are more amenable (Bonavita-Cougourdan et al., 1987). Another problem, usually unrecognized, is that a solvent rinse of an ant's cuticle contains exocrine gland products, regurgatory and/or excretory products, as well as the expected cuticular lipids. The fire ant is a good example of this, because it produces easily analyzed alkaloids in its poison gland. Large amounts of these alkaloids are released when the ants are placed, dead or alive, in hexane. These compounds have many behavioral and physiological properties (Obin and Vander Meer 1985). In this case the surface lipids are grossly contaminated. For other studies we have made many unsuccessful attempts at obtaining alkaloid free fire ant body parts. The same problems probably apply to other less readily detectable behavior modifying exocrine gland products that can not be analyzed by gas chromatography.

What Is the Nature of Cue Chemistry?

Intra-Specific Examples. The surface lipids of most ants investigated thus far contain a large proportion of hydrocarbons -- straight chain, methyl, dimethyl, trimethyl branched and unsaturated. Early papers on the integration of myrmecophiles and termitophiles into their host colony showed a correlation between host hydrocarbon pattern and integration. These papers led to the "hydrocarbon bandwagon effect", which has delayed our coming to grips with the difficult nestmate recognition problem. Howard et al. (1980) demonstrated that the termitophile, *Trichopsenius frosti*, biosynthesized a cuticular hydrocarbon mixture qualitatively equal to that of its host, *Reticulitermes flavipes*. Without behavioral evidence the authors stated that cuticular hydrocarbons probably serve as the primary integration mechanism for this termitophile. Later Howard et al. (1982a) showed an asymmetric behavioral response from one of two sympatric termite species presented with critical-point dried *R. virginicus* worker surrogates treated with cuticular hydrocarbons from the two species. Other cuticular lipids were not tested to determined if a similar response would have been obtained. Howard et al. (1982b) reported three other termitophiles that appeared to use cuticular hydrocarbons as an integrating mechanism. There were no associated bioassays. Vander Meer and Wojcik (1982) got the wagon rolling further in their paper on the integration of a myrmecophile into fire ant colonies. Unfortunately, they wrote about hydrocarbon patterns and beetle integration in the same sentence and only at the end did they state that integration involves a passive defense that enables the beetle to survive long enough to acquire the species and environmental components of colony odor. As we will illustrate in the next paragraphs, a preoccupation with hydrocarbons as nestmate recognition cues has been a major handicap faced by researchers in this area.

Obin (1986) demonstrated that *Solenopsis invicta* nestmate recognition cues were both environmentally and heritably derived. Thus, aggression between colonies reared in the laboratory under identical conditions was reduced, compared to aggression between field colony workers. He also demonstrated differences between the patterns of the five major cuticular hydrocarbons found on workers from laboratory and field colonies; however, a correlation between similarity of hydrocarbon pattern and aggression was not obtained. Although this was an informative study regarding the source of nestmate recognition cues in *S. invicta*, no definitive conclusions could be reached regarding the role of cuticular hydrocarbons.

Bonavita-Cougourdan et al. (1987) studied the chemistry of nestmate recognition in *Camponotus vagus*. They washed worker heads and thoraces in pentane, analyzed the washes by GC, GC/MS and argentation TLC. As stated earlier, hydrocarbons dominate the chemical classes in cuticular washes, but they are not the only chemical class present. Bonavita-Cougourdon et al. (1987) found readily analyzed hydrocarbons in their GC and GC/MS analyses, and concluded that the total cuticular rinse contained only saturated hydrocarbons. Bioassays of the total rinse demonstrated differences in aggressive behavior between lures treated with resident worker versus alien worker rinses. They conclude that in *C. vagus*, nestmate recognition cues are composed of cuticular hydrocarbons. The lack of direct behavioral assays with the isolated hydrocarbons detracts from the authors' claims. However, they were able to devise a lure (surrogate worker) and bioassay that is amenable to testing the chemistry of nestmate recognition cues.

Bonavita-Cougourdan et al. (1989) showed that in choice experiments *C. vagus* workers preferentially retrieve larvae from their own colony rather than foreign conspecific larvae. They also determined that foreign larvae kept for 20 days with test colony sisters are no longer recognized as different and that the cuticular GC profile of the foreign larvae becomes more like that of test colony larvae. This is a good example of using hydrocarbons as a tool to study transfer and/or changes in the surface chemistry of individuals. However, the authors assume that the readily analyzed cuticular hydrocarbons are the responsible agents for worker recognition of larvae, as well as, worker-worker recognition and species recognition. Similarly, Bonavita-Cougourdan et al. (1990; 1993) found from principle component analysis of cuticular hydrocarbon profiles that dimethylalkanes are responsible for colonial chemical signature and the larvae, workers, sexual males or females and the queen possess characteristic chemical signatures composed from the n-alkanes and monomethylalkanes. They hypothesize that cuticular hydrocarbons are the cue that allows foragers to differentiate brood-tender and forager sub-castes. It is interesting that statisitical methodologies are capable of making these distinctions; however, until the behavioral bioassays are executed to demonstate a behavioral correlation, the "chemical signatures" are only useful to the human observers.

Henderson et al. (1990) studied internest aggression and nestmate recognition in the polygynous ant, *Formica montana*. They used pentane, hexane, ether, isopropanol, and water as cuticular rinses. Aggression bioassay results for pentane, hexane, and ether washes were not different for either nestmate or non-nestmate interactions, but were different from water and isopropanol washes. These authors assumed that pentane, hexane, and ether only remove hydrocarbons from the cuticular surface, and they hypothesize that hydrocarbons are used by this species in nestmate recognition. Pentane, hexane, and ether will dissolve all classes of non-polar lipids, thus no conclusions can be made about their hypothesis.

Nowbahari et al. (1990), investigated the individual, geographical and experimental variation of cuticular hydrocarbons in the ant, *Cataglyphis cursor*, and discussed their use in nestmate recognition. They cite the previous work, sometimes inappropriately, e.g. to support their contention that hydrocarbons are involved in nestmate recognition. Then they repeated the now classic mistake of assuming that if GC and GC/MS analysis of a worker rinse only detects what can be interpreted as hydrocarbons, then this rinse must contain only hydrocarbons. They conclude that intercolony aggressiveness in *C. cursor* is regulated, at least in part, by cuticular hydrocarbons. "In part", because application of the total rinse did not induce full levels of aggression.

Morel et al. (1988) made hexane soaks of *Camponotus floridanus*, and carried out chemical and pattern recognition analyses on the hydrocarbons that dominated the GC profile. However, they recognized that the ant rinse contains more than hydrocarbons. Thus they state "...although hydrocarbons are the major class of GC detectable compounds in *C. floridanus* soaks, we cannot say that they are responsible for nestmate recognition." Here the hydrocarbons and their GC profiles were used as a model for heritable cues known to dominate nestmate recognition in this species (Carlin and Hölldobler, 1986).

Vander Meer et al. (1989) studied the temporal changes in colony cuticular hydrocarbons of *S. invicta*. These authors did not state that cuticular hydrocarbons were involved in nestmate recognition but used them as a general model for heritable cues. Multivariate analyses were used to determine that at any point in time individual colonies are distinguishable through their hydrocarbon pattern; however, these patterns change over time for a particular colony (see the template section for details).

Interspecific Mixed Colonies. GC analyzed solvent rinses of workers from mixed colonies of *Formica selysi* and *Manica rubida* showed that hydrocarbon profiles had characteristics of both species (Errard and Jaisson 1991; Bagneres, et al. 1991). They assumed that these hydrocarbon changes permitted the two species to inhabit the same nest. Most interesting is the suggestion that heterospecific adoptees may be capable of switching epicuticular hydrocarbon biosynthesis to match that of the host colony. This certainly is an intriguing hypothesis that requires further investigation.

Habersetzer and Bonavita-Courgourdan (1993) investigated the cuticular components from mixed colonies of the slave-making ant, *Polyergus rufescens* and the slave species, *Formica rufibarbis*. In contrast to the above, the GC profiles of enslaved *F. rufibarbis* workers did not take on the characteristics of the *Polyergus* slave-maker. However, enslaved *F. Rufibarbis* workers lost their colony characteristic components. These authors assumed that the observed GC components they were hydrocarbons, based on retention time range, use of a non-polar solvent and a non-polar GC column. Unfortunately, these criteria are inadequate to support the hydrocarbon assumption. They conclude that one should bear in mind that chemicals other than the cuticular hydrocarbons may be involved in the recognition process.

All of the studies that have included chemical analyses have focused on hydrocarbons. Cuticular hydrocarbons no doubt function to prevent insect dessication, and they can have other functions, e.g. sex, alarm, and thermoregulation pheromones; defense; alarm; and as kairomones (see review Howard and Blomquist, 1982). More recently, Blum (1987) reviewed and speculated on the use of epicuticular hydrocarbons in social insects. He pointed out that epicuticular hydrocarbons exhibit enough variation between species and colonies to "qualify admirably" as species-specific signals and colonial signatures (of course other lipid classes would also qualify). These same epicuticular hydrocarbons (plus other lipids) should readily absorb exocrine gland products (and environmental odors), acting as a slow release matrix and adding information to the already chemically diverse epicuticular surface. Indeed, cuticular hydrocarbons may be involved in nestmate recognition, but the critical bioassays directly linking the chemistry and behavior have yet to be done.

Alarm Pheromones as Recognition Cues?

Jaffe and Marcuse (1983) found that *Odontomachus bauri* workers use volatile compounds as nestmate recognition cues. An aggression bioassay gave positive results for all body parts. Volatiles were detected by gas chromatography from the gaster and head but not the thorax. The proportions of volatile compounds differed from colony to colony, but no behavioral assays were done to test whether or not these compounds were a part of the recognition cue system. Jaffe and Sanchez (1984) investigated nestmate recognition in the ant *Camponotus rufipes*. They determined that introduction of worker heads but not thoraces or gasters were bitten more often by non-nestmates than nestmates. Freeze-dried workers were not effective in the bioassay, thus the authors conclude that cephalic odors, most likely alarm pheromones, are responsible for nestmate recognition. Similarly, alarm pheromones were suggested to be nestmate recognition cues for *Solenopsis geminata* (Jaffe 1986).

Jaffe (1987) brought together his ideas about nestmate recognition and alarm pheromones. He classifies nestmate recognition as advanced, if a specific chemical signal is used, e.g. alarm pheromone, and less advanced if non-specific signals are used, e.g. environmental odors, cuticular hydrocarbons, and/or more than one exocrine gland blend. According to Jaffe (1987) the gradation from non-advanced to advanced tracks the evolutionary advancement of the species considered. Thus, primitive Ponerinae, such as *Odontomachus bauri*, use non-specific signals, whereas the more complex *Ectatomma ruidum* specifically uses its alarm pheromone for nestmate recognition. In view of what we know now, there are problems with this scheme. "Advanced" species do utilize what Jaffe would call non-specific nestmate recognition signals, most notably *S. invicta*. We have already cited references to the intracolonial variation of alarm pheromones (Pasteels et al. 1980; Brand and Pretorius 1986; Do Nascimentoi, et al. 1993), which detracts from their use as recognition cues. It is difficult to understand the utility of alarm pheromones as nestmate recognition cues. A resident worker may release alarm pheromone in response to an intruder, but release must come after the recognition process occurs. Since recognition assays measure aggression, it is easy to visualize how presentation of an alarm pheromone could result in heightened activity and responsiveness to subsequently encountered cues, such as recognition cues.

For alarm pheromones to be operable as nestmate recognition cues they would have to be present on the surface of worker ants at levels below the alarm response threshold, otherwise alarm behavior would occur all the time. Jaffe (1987) astutely brings territoriality into the nestmate recognition picture, by postulating that an intruder experiencing a foreign territory will release alarm pheromone, thus initiating the recognition process. If this is true, recognition of being in the wrong place must still occur prior to release of the pheromone. Alarm pheromones are intimately linked with nestmate recognition, but in nature their release most probably is a result of recognition rather than the cause of recognition.

Interspecific adopted workers present an intriguing problem if alarm pheromones are involved in nestmate recognition. There is no question that the interspecific adoptee has an alarm pheromone profile that is qualitatively distinct from members of the host colony. How can it survive and what happens when the adoptee is in a situation that induces release of its alarm pheromone? It should be perceived as different by the normal workers in the colony and one would expect aggression to ensue.

The Bottom Line

Much has been theorized and learned about nestmate recognition in the past two decades. It is an essential ingredient in the recipe for eusociality and as such will continue to be studied extensively in the decades to come. An area of considerable interest to us is the unambiguous elucidation of the chemistry of nestmate recognition. Usually chemical techniques are far ahead of the development of suitable bioassays, but not in this case. The time is right for us to develop a clearer understanding of the nature of nestmate recognition cues, which will lead to a better understanding of the entire process. It is evident that more and more social insect researchers are developing expertise in molecular techniques, which can be brought to bear on many social insect questions, among them -- the issues centered around the elusive kin recognition problem. Ants are as diverse a group of insects as any, providing exceptions to every rule, thus generalizations can be dangerous. There are many examples of workers isolated during eclosion being attacked when returned to their mother colony (they did not acquire the colony-specific odor). However, Stuart (1987) reports that for *Leptothorax curvispinosus*, isolated workers produce persistent, colony-specific recognition cues after eclosion and are accepted when returned to their mother colony. Another divergence example, queen discriminators play a dominant role in nestmate recognition for

Camponotus spp. (Carlin, and Hölldobler, 1983, 1986, 1987), whereas they do not in *S. invicta* (Obin, and Vander Meer 1989) or *Rhytidoponera confusca* (Crosland 1990). These results are not contradictory (Carlin and Hölldobler 1991), but highlight the diversity of mechanisms and dangers of generalizations. Ant nestmate recognition is complex enough to keep the reductionist happy and to remind us of the sign in RVM's office, "Heisenberg may have slept here". As we more on to the next level of nestmate recognition understanding, it is clear that the future of this area of research has to be as exciting as the past has been.

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