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Development of Interspecific Recognition Behavior in the Ants *Manica rubida* and *Formica selysi* (Hymenoptera: Formicidae) Reared in Mixed-Species Groups

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The purpose of this study was to determine the role of early social experience on the ontogeny of kin and nestmate recognition in ants by means of both behavioral and chemical analysis. Workers of two ant species, Manica rubida *(Myrmicinae) and* Formica selysi *(Formicinae), were reared in homospecific groups (control) or in artificial heterospecific groups (mixed), created less than 5 h after their emergence. Recognition was evaluated between unfamiliar individuals of different species reared in control and mixed groups for periods of 5, 12, 24, 48, and 72 h and 8, 15, 30, 60, or 90 days after emergence. Heterospecifically reared individuals of both species gradually became tolerant of allospecific individuals from control groups. Moreover, homospecifically reared individuals did not aggress allospecific individuals reared in mixed groups. During the course of familiarization between the species, there were modifications of the chemical recognition signals. In mixed groups, hydrocarbon profiles of both species acquire gradually some of the components characteristic of their heterospecific nestmates. These experiments showed that allospecific recognition required the acquisition of a minimal quantity of allospecific cues. The phenomenon provided an another example of the relationship between tolerance and the chemical cues displayed by both species. The results suggested that the individual recognized the allospecific cues borne on each individual's body surface and/or that each individual learned and memorized allospecific cues during its early life. Therefore, each individual might develop a template encoding the allospecific and the conspecific cues to characterize nestmates.*

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INTRODUCTION

Social insects discriminate between members of their colony and strangers by chemical cues (or odors) located on the cuticle (for reviews, see Breed *et al.*, 1987; Gadagkar, 1985; Gamboa *et al.*, 1986; Carlin, 1989). In general, the colony members have a common odor ("colony odor") which is composed of heritable and environmental odors (Carlin, 1989). In fact, the colony odor encompasses all the odors associated with a colony, including endogenously and exogenously derived odors and the nestmate recognition cues are drawn from these odor sources. Genetically derived odors can come directly from the individual (endogenous) or from interactions with other individuals (exogenous) (Greenberg, 1979; Buckle and Greenberg, 1981; Mintzer *et al.*, 1985; Moritz *et al.*, 1990). In this case, individuals in the colony can acquire the components of the colony odor from their queen (Carlin *et al.*, 1987; Provost, 1987; Keller *et al.*, 1989) or by exchanges with other colony members during mutual grooming or trophallaxis (Crozier *et al.*, 1979; Hölldobler *et al.*, 1980; Stuart, 1992). Environmental sources are always from exogenous sources. Colony members acquire them from environmental sources outside the colony such as food or nesting materials (Kalmus *et al.*, 1952; Wallis, 1962; Jutsum *et al.*, 1979; Jaffe *et al.*, 1985; Gamboa *et al.*, 1986; Breed *et al.*, 1988; Stuart, 1988).

In many social insects, the first hours of adult life, called the "sensitive period," seem crucial for the integration and the socialization of individuals into their maternal colony. Early in adult life, each colony member must learn nestmate recognition cues which are encoded in a sensory template (memory). This template is used for comparison and the determination of the colonial membership of individuals that are encountered (Breed *et al.*, 1987; Carlin, 1989; Hölldobler *et al.*, 1990; Stuart, 1992). Early experience may thus induce a preference for a particular social environment and may explain the natural associations between different species of ants (reviewed by Jaisson, 1991). The existence of these imprinting-like phenomena allows newly emerged workers to become well integrated in an alien conspecific colony as well as in an allospecific one (Wilson, 1971; Hölldobler *et al.*, 1980; Carlin *et al.*, 1986; Morel *et al.*, 1988; Stuart, 1992). This hypothesis is supported by studies of slave-making ant species (Buschinger *et al.*, 1980; Le Moli *et al.*, 1985) and others involving allospecific colonies created by assembling very young individuals (Fielde, 1905; Jaisson, 1980; Carlin *et al.*, 1983; Errard, 1984, 1986; Errard *et al.*, 1984, Vienne, 1993).

Some authors have assumed that for individuals from different colonies not to attack each other, they must have similar odor labels (Bonavita-Cougourdan *et al.*, 1987; Hölldobler *et al.*, 1990; Nowbahari *et al.*, 1990). It has also been suggested that the exact mechanism by which the inquilines achieve a successful integration into the life of the host colony implicates primarily chemical stimuli and a chemical mimicry phenomenon (Howard *et al.*, 1980, 1982, 1990; Vander Meer *et al.*, 1982; Breed *et al.*, 1992). However, how chemical mimicry of the host cuticular hydrocarbons would evolve in integration of parasites or predators is less clear. So we used artificial mixed colonies as an experimental paradigm, to investigate the origin of the olfactory cues mediating kin and nestmate recognition, specifically, the relative contribution of gestalt sources (chemical transferred between individuals) and a species genetic source.

With this model, we attempt to answer the following questions:

(1) What is the role of cuticular hydrocarbons in interspecific recognition? If the chemical signature changes during the ontogeny, would nestmate recognition be better? and

(2) What is the mechanism involved in recognition cues during early adult life?

MATERIALS AND METHODS

Subjects and Housing Conditions

The species studied, *Manica rubida* (myrmicinae) and *Formica selysi* (Formicinae), were collected in the same biotope (French Alps; altitude, 800 m) from May to August 1989. The experiments were conducted in the laboratory under the following conditions: 20 ± 3°C and the natural photoperiod of Paris. Each experimental group was reared in a test tube (180 × 17 mm), fitted at one end with a water container. Food (honey/apple mixture and mealworms) was introduced by the other end, which was closed with a cotton plug.

As soon as they emerged, young workers were selected and removed from their mother colonies (only one colony per species), in order to create homospecific (control) or heterospecific (mixed) groups. A control group consisted of about 20 young workers of a single species (either *M. rubida* or *F. selysi*), while a mixed group included about 15 young workers of each species (Errard, 1986). No group contained a queen or brood of either species. A total of 30 control groups for each species and 30 mixed groups was created in this way. The developmental periods were 5, 12, 48, and 72 h and 8, 15, 30, 60, or 90 days after emergence. For each duration of rearing, two control groups of each species and two mixed groups were created.

Observations

Recognition between individuals was evaluated by confronting individuals from the different rearing methods, aged, respectively, 5 h (after emergence), 12, 24, 48, 72 h, 8 days, 15 days 30 days, 60 days or 90 days in heterospecific encounters.

Two types of encounters were observed (see Fig. 1).

(1) Encounters between individuals from control groups: Control *F. selysi* were confronted with unfamiliar *M. rubida* reared in a control group (10 groups of each species).

(2) Encounters between individuals from control groups (10 groups of each species) and mixed groups (20 groups): *F. selysi* or *M. rubida* individuals of control groups were confronted with unfamiliar allospecific individuals from mixed groups.

In each encounter, or test, two ants, one of each species, were placed together into a clean petri dish (4.8 cm in diameter), which constituted a neutral arena. The two insects were always of the same age. In all tests, the two ants were observed simultaneously for 5 min. The time preceding the first contact was recorded (latency) and all interactions between the two partners were recorded every 5 s.

Observed behavioral events were grouped into three categories:

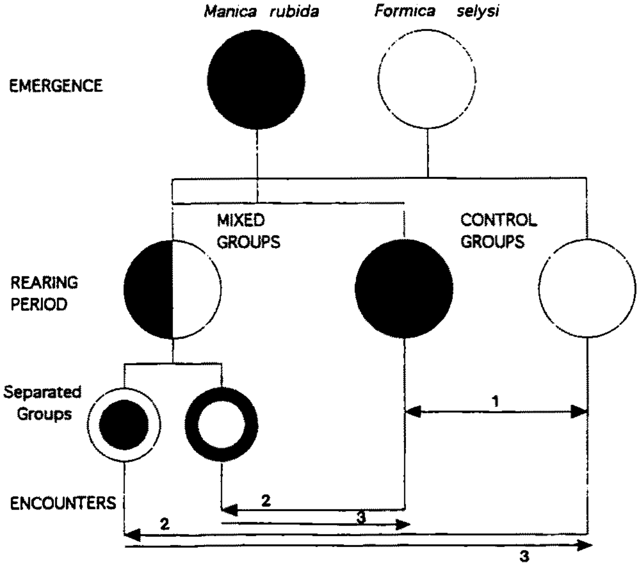

Fig. 1. Diagram of the different types of encounters: control–control (1) and control–mixed (2, control → mixed; 3, mixed → control).

(1) antennal contacts (exploration);
(2) agonistic—threats, gaster flexion, bites, stings; and
(3) retreats.

Fifteen tests were conducted for each age and for each type of encounter (15 pairs of individuals were tested in each condition: control/control and control/mixed). Individuals were tested only once, at a given age and in a given encounter, to avoid any possible effects of familiarization.

The results including all the data were analyzed using ANOVA (F). The three factors considered were the species (*M. rubida* and *F. selysi*), the types of encounters (control → control, control → mixed, and mixed → control), and the age (10 periods).

Chemical Measures

At each age, individual ants from each rearing group were killed by freezing, then weighed. Each ant was then immersed in pentane for extraction of the cuticular hydrocarbons (Bagnères *et al.*, 1991).

Extracts from 5 to 10 individuals of each species and each age were analyzed by gas chromatography (GC) using a Chrompak CPSIL 5 WCOT capillary column (25 m × 0.25 mm ID) that was temperature programmed from 100 to 300°C at 5°C/min. The quantities of each compound were assessed by peak integration. Nonacosane (nC19) was used as an internal standard. Hydrocarbons present in quantities greater than 0.2 ng/mg per insect were included in the analysis using Spearmann's correlation coefficient. Comparisons were performed with Mann–Whitney tests.

The identification of hydrocarbons has been reported previously (Bagnères *et al.*, 1991) and confirmed by Hefetz *et al.* (1992).

For each age, one mixed group and one control group of each species were preserved for these chemical studies (10 mixed groups and 10 control groups of each species).

RESULTS

Behavioral Results (Table I)

The latencies did not vary according to the type of encounters ($F = 0.56$, df = 2,84, NS) but decreased significantly with age ($F = 26.5$, df = 9,756, $P < 0.001$). This latency was longest 5 h after emergence (96 ± 7 s), decreased (57 ± 8 s) at 24 h, and fell to 7 ± 1 s at 48 h. These results reflect the immobility of young workers and a rapid increase in movement with age.

In all behavioral tests, the 72-h old workers did not differ significantly from the 48-h-old workers. Therefore, this age is not presented in the figures.

Table I. Results of ANOVAs Examining Frequencies of Behavioral (Antennal Contacts, Aggressive Acts, and Retreats)[a]

Effect	*df*		Antennal contacts		Aggressions		Retreats	
	Effect	Error	*F*	*P*	*F*	*P*	*F*	*P*
Encounter	2	84	35.14	0.001	5.84	0.004	119.22	0.001
Species	1	84	12.23	0.001	30.73	0.001	217.05	0.001
Age	9	756	30.17	0.001	7.47	0.001	21.22	0.001
Encounter × species	2	84	36.14	0.001	1.08	0.343	59.16	0.001
Encounter × age	18	756	3.10	0.001	1.04	0.408	5.10	0.001
Species × age	9	756	3.30	0.001	1.98	0.038	4.26	0.001
Encounter × species × age	18	756	4.61	0.001	0.85	0.629	3.73	0.001

[a] Means and standard errors are shown in Figs. 2, 3, and 4.

Antennal Contacts (Figs. 2a and b)

The mean number of antennal contacts between the two species increased with the age of the individuals ($F = 30.17$, df = 9,756, $P < 0.001$) and varied significantly with the type of encounter ($F = 35.14$, df = 2,84, $P < 0.001$) and with the species ($F = 12.23$, df = 1,84, $P < 0.001$). This behavior was always rare between controls and did not differ between the two control species ($F = 1.27$, df = 1,149, NS). Control *M. rubida* (especially older than 60 days) made more antennal contacts toward mixed *F. selysi* than toward control *F. selysi* ($F = 8.55$, df = 1,299, $P < 0.004$), and than control *F. selysi* toward mixed *M. rubida* ($F = 10.26$, df = 1,299, $P < 0.001$). This behavior in the latter encounters did not differ from that observed in the control encounters ($F = 0.01$, df = 1,299, NS).

The antennal contacts made by mixed *M. rubida* toward control *F. selysi* were lower than contacts made by mixed *F. selysi* toward control *M. rubida* ($F = 37.30$, df = 1,299, $P < 0.001$) and greater than in control *M. rubida* encountering control *F. selysi* ($F = 4.99$, df = 1,299, $P < 0.02$), especially when all were more than 8 days old. Mixed *F. selysi* made more antennal contacts toward control *M. rubida* than control *F. selysi* made toward control *M. rubida* ($F = 61.13$, df = 1,299, $P < 0.001$) and than control *F. selysi* made toward mixed *M. rubida* ($F = 62.73$, df = 1,299, $P < 0.001$).

F. selysi which had spent at least 15 days in mixed groups made numerous antennal contacts toward control *M. rubida*. A similar amount of interest or recognition appeared in control *M. rubida* toward mixed *F. selysi* that were 60 days old. However, mixed *M. rubida* did not seem to recognize *F. selysi* controls, regardless of their age.

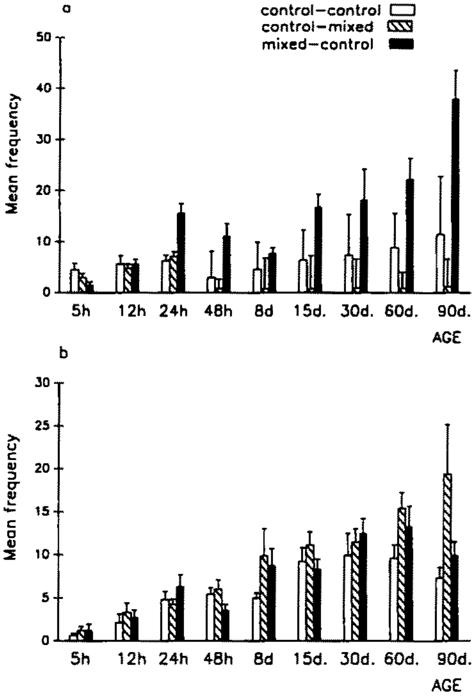

Fig. 2. Mean frequency of antennal contacts in the different types of encounters (control → control, control → mixed, mixed → control) as a function of age. (a) Acts by *F. selysi* toward *M. rubida*; (b) acts by *M. rubida* toward *F. selysi*.

Agonistic Behavior (Figs. 3a and b)

The mean number of agonistic interactions varied with the age of individuals, whatever the type of encounter ($F = 7.47$, df = 9,756, $P < 0.001$). Differences appeared between the different encounters ($F = 5.84$, df = 2,84, $P < 0.001$) and between the two species, with *M. rubida* always being more aggressive than *F. selysi* ($F = 30.73$, df = 1,84, $P < 0.001$). In control encounters, *M. rubida* began to show more aggressiveness from 48 h after emergence, and the frequency of this behavior increased until it reached a maximum at 8 days. *F. selysi*, however, became aggressive after reaching 8 days of age. When control *M. rubida* encountered control *F. selysi*, they inspected each other rapidly, then threatened, attacked, or fled. These attacks frequently led to injury or even death.

When control insects were confronted with individuals reared in mixed groups, the peak of aggression by control *M. rubida* toward mixed *F. selysi*

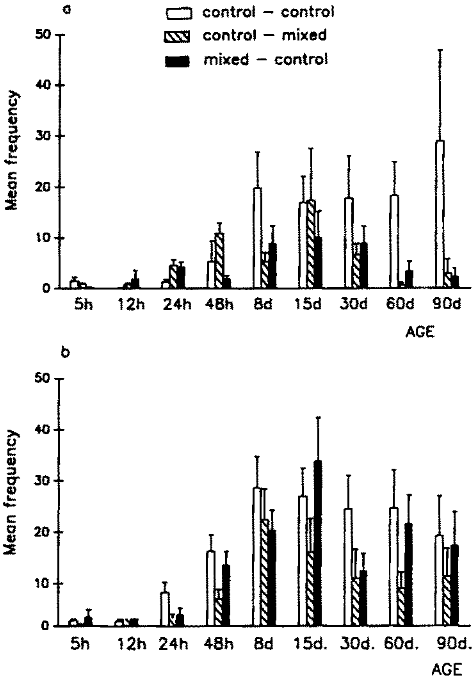

Fig. 3. Mean frequency of aggressive behavior in the different types of encounters (control → control, control → mixed, mixed → control) as a function of age. (a) Acts by *F. selysi* toward *M. rubida*; (b) acts by *M. rubida* toward *F. selysi*.

was at 8 days but decreased significantly by the time *F. selysi* had spent a month in a mixed group. This agonistic behavior was lower than in control encounters ($F = 11.12$, df = 1,299, $P < 0.001$). Similarly, in encounters between control *F. selysi* and mixed *M. rubida*, the agonistic behavior made by *F. selysi* increased until 15 days and then decreased when *M. rubida* had lived for a month in a mixed group. The agonistic behavior presented by *F. selysi* was also lower than in control encounters ($F = 24.06$, df = 1,299, $P < 0.001$).

Tested with control partners, individuals of both species reared in mixed groups showed agonistic behavior which increased in frequency up to the age of 15 days. This behavior decreased during the first month for *M. rubida* (no difference with control encounters: $F = 0.33$, df = 1,299, NS) and decreased only in the second month for *F. selysi* (lower than in control encounters: $F = 13.13$, df = 1,299, $P < 0.001$). In these encounters, mixed *F. selysi* was less aggressive than mixed *M. rubida* ($F = 34.85$, df = 1,299, $P < 0.001$). In the encounters between control and mixed groups, the aggressive actions were only threats, and never resulted in injury.

The ontogeny of agonistic behavior was more rapid in *M. rubida*. It was already well established at the age of 48 h, whereas its development required 8 days for *F. selysi*. This phenomenon may be explained by the fact that these species are from different genera. In addition, *M. rubida* attacked *F. selysi* more often than vice versa, showing that the defensive behavior of the two species differs.

Retreat Behaviors (Figs. 4a and b)

The mean number of retreats increased in both species with the age of individuals ($F = 21.22$, df = 9,756, $P < 0.001$) and varied significantly with the types of encounter ($F = 119.22$, df = 2,84, $P < 0.001$); individuals from mixed groups encountering controls retreated less. Differences appeared between

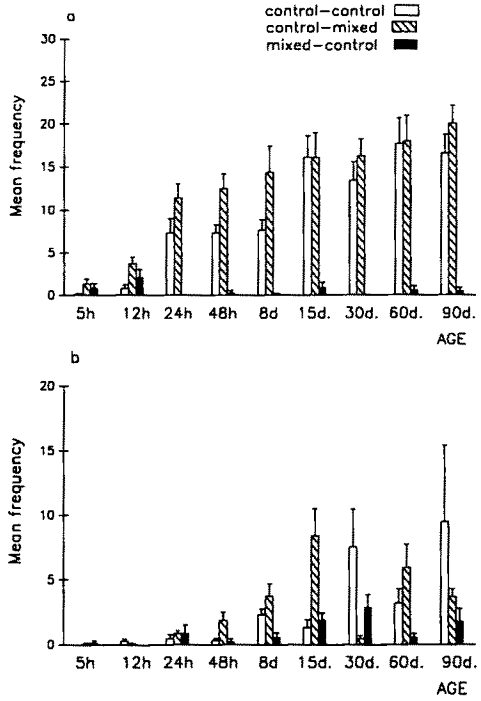

Fig. 4. Mean frequency of retreat behavior in the different types of encounter (control → control, control → mixed, mixed → control) as a function of age. (a) Acts by *F. selysi* toward *M. rubida*; (b) acts by *M. rubida* toward *F. selysi*.

the two species ($F = 217.05$, df = 1,84, $P < 0.001$); All *F. selysi* fled when attacked by *M. rubida*. These results confirmed that the defense strategy of *F. selysi* differed from that of *M. rubida*, which showed very few retreats.

Retreat behavior in encounters between controls increased from the age of 24 h for *F. selysi* species and from 1 month for *M. rubida* species. When controls encountered individuals from mixed groups, the incidence of retreat increased steadily up to the age of 3 months for control *F. selysi* (higher than in control encounters: $F = 3.94$, df = 1,299, $P < 0.04$) while in control *M. rubida* it increased up to the age of 15 days, then decreased (it did not differ from the control encounters: $F = 0.26$, df = 1,299, NS).

When ants from mixed groups were confronted with controls, there were no differences between the two species ($F = 2.20$, df = 1,299, NS). Individuals from mixed groups retreated less than controls (mixed *F. selysi* $F = 217.9$, df = 1,299, $P < 0.001$) (mixed *M. rubida* $F = 5.11$, df = 1,299, $P < 0.02$). Mixed *F. selysi* did not flee from control *M. rubida* after antennal contact when 24 h old. However, control *F. selysi* fled from antennal contact with mixed *M. rubida*.

Chemical Results

In homospecifically reared workers, analysis of chromatograms confirmed that both species already had their own specific cuticular profiles after the first 5 h (*F. selysi* species possessed large amounts of alkenes and alkadienes that were absent in the chromatograms of *M. rubida*) as found in previous studies (Bagnères *et al.*, 1991; Hefetz *et al.*, 1992). The analysis also confirmed that cuticular compounds increased quantitatively with the age of the workers (Errard *et al.*, 1991).

The two species possessed less cuticular hydrocarbons at 5 h (about 170 ng/mg per insect for *F. selysi* and 145 ng/mg per insect for *M. rubida*) than when 48 h old (300 and 400 ng/mg per insect, respectively. These quantities gradually increased till the age of 1 month, when they reached about 500 ng/mg per insect in *F. selysi* and 600 ng/mg per insect in *M. rubida*, and thereafter remained stable.

Heterospecifically reared workers possessed gradually greater quantities of cuticular hydrocarbons than controls (Mann–Whitney: *F. selysi* $U = 8$, df = 99, $P < 0.005$; *M. rubida* $U = 11$, df = 99, $P < 0.005$). By 8 days both species possessed about 600 and 800 ng/mg per insect when 1 month old and remained stable thereafter. Within a few hours, each species acquired some of the characteristic hydrocarbons belonging to the other. *F. selysi* from mixed groups gradually acquired ever-increasing amounts (until 8 days after emergence) of *M. rubida* specific hydrocarbons such as 5-methyl-C25 and 5,17-dimethyl-C27 (major specific cuticular hydrocarbons of *M. rubida*) (Fig. 5a)

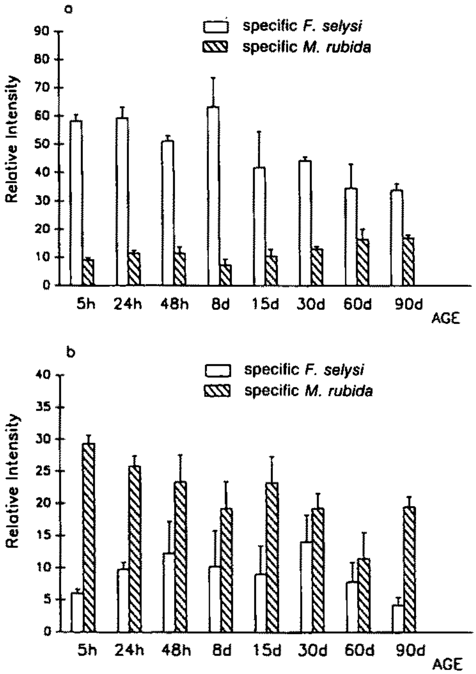

Fig. 5. Relative intensity (percentages) of the specific hydrocarbons of (a) *M. rubida* in *F. selysi* workers reared in mixed groups, (b) *F. selysi* in *M. rubida* workers reared in mixed groups, as a function of age.

Mixed *M. rubida* workers possessed increasing amounts of the major specific cuticular hydrocarbons of *F. selysi*, such as heptacosene (C27 : 1) nonacosadiene (C29 : 2), until 48 h after emergence (Fig. 5b).

To examine the patterns in species reared in mixed and control groups, the 10 most easily quantifiable hydrocarbons were selected. For each age, we compared the two species reared in mixed groups and, for each species, the mixed profiles with the control profiles (Spearmann correlation coefficient).

In control groups, the chemical results showed no correlation for all ages of *F. selysi* and *M. rubida* ($-0.59 < R < -0.32$, NS). In mixed groups, the chemical results of *M. rubida* were not correlated with those of the mixed *F. selysi* before the age of 15 days ($-0.61 < R$, NS). In contrast, when the species were reared in mixed groups during 15 days, the two mixed profiles were correlated ($R = 0.61$, $P < 0.05$) and the correlation was maintained during 2 months ($R = 0.75$, $P < 0.001$). After 2 months, the correlation between the two mixed profiles was positive ($R < 0.52$, NS) but not statistically significant.

The comparison between control and mixed groups revealed a difference between the species. The profile of mixed reared *M. rubida* was always correlated with the control *M. rubida* ($0.86 < R < 0.96$, $P < 0.01$) and never with the control *F. selysi* ($-0.70 < R < 0.05$, NS). The analysis also showed that the mixed *F. selysi* profile was correlated with the control *M. rubida* profile from the age of 8 to 90 days ($0.20 < R < 0.52$, NS) but never before 8 days, when it was similar to the profile of control *F. selysi* ($0.72 < R < 0.99$, $P < 0.005$).

DISCUSSION

While it is clear that workers possess their own genetically determined chemical profile, these results agree with several reports suggesting a strong exogenous influence on the chemical nature of these cues (reviewed by Carlin, 1989).

In control groups of both species, the odor is established within 48 h of emergence. The behavioral studies show that workers 48 h old (from conspecific groups) are highly aggressive in heterospecific encounters. Conversely, individuals reared in mixed groups do not display this aggressiveness. This result is corroborated by the chemical similarity of cuticular hydrocarbon profiles in the mixed groups, leading to a modification in the tolerance by unfamiliar allospecifics. In fact, workers reared in mixed groups partially take on the chemical cues of the other species (Errard *et al.*, 1987; Bagnères *et al.*, 1991; Hefetz *et al.*, 1992). These results, however, show that a complete chemical mimesis of cuticular hydrocarbon profiles is not necessary for acceptance, as Getz pointed out in 1982. There is, in fact, a progressive change in the chemical signature of each species, resulting in the creation of a ''collective odor'' or mixed odor composed of conspecific and allospecific chemical cues (Stuart, 1988, 1992). If we assume that the odor common to all members of a group results from continuous transfer of chemical cues (through social grooming or trophallaxis) between the group members [according to the ''gestalt model'' of Crozier and Dix (1979)], the amount of allospecific chemical cues carried by individuals will increase with the duration of association and probably gradually modify the recognition ability. We thus observe that the workers exposed to allospecifics during 1 month were more likely to accept an unfamiliar allospecific reared in a control group than those exposed for only 24 h. On the other hand, it appears that mixed *F. selysi* workers perform better than mixed *M. rubida* in recognizing control allospecifics and are more tolerated by the controls. This behavioral difference may be explained by the chemical results showing that the mixed odor does not develop in a comparable way in the two species. *F. selysi* workers reared in mixed groups acquire greater quantities of allospecific cuticular hydrocarbons than do *M. rubida*.

Three recognition mechanisms may be considered.

(1) Each individual produces specific labels and possesses a template encoding these labels. Therefore the template, innately specified, serves as a reference when encountering another individual. In our experiments, the template may be produced with out the involvement of any other individuals (self-based). Using the conspecific learned odors as a guide, workers accept or reject allospecific workers depending on the similarity of the allospecific workers' chemical cues to their own odor (Getz, 1982).

Since *F. selysi* reared in mixed groups acquire greater amounts of allospecific chemical compounds than *M. rubida*, they may be recognized by control *M. rubida* workers better than mixed *M. rubida* would be recognized by the control *F. selysi*. However, if this hypothesis explains the recognition of mixed individuals by controls, it does not explain the recognition phenomena of control ants by those raised in mixed colonies.

(2) The second hypothesis for a recognition system involves a template based on learning labels from individuals other than oneself, produced by smelling other individuals (conspecific and allospecific) belonging to the group (''non-self-based''). Mutual tolerance and recognition of the controls are thus possible because the ants develop their recognition model, through a phenomenon of learning, of the individuals that make up their social environment. In this case, the learning period may be independent of the quantity of allospecific chemical hydrocarbons borne on the cuticule. The recognition of allospecific workers depends only in the early learning. Each young member of the group learns the collective odor (recognitive cues) if exposed to it shortly after eclosion, through a process analogous to imprinting (Wilson, 1971; Jaisson, 1975, 1991). Therefore, the contribution of early mechanisms to the ontogeny of allospecific worker recognition appears to vary between the different ant species. This ability to recognize allospecific partners appears to be better in *F. selysi* than in *M. rubida* species.

(3) The third hypothesis proposes that workers reared in mixed groups may learn the odor of their own species (chemical labels produced by its own metabolic machinery) and the allospecific odors (or labels) they acquire on their cuticule during the association. After smelling themselves, they produce a template encoding conspecific and allospecific cues (''self-based'').

When a worker reared in a mixed group encounters an unfamiliar allospecific, it accepts the stranger if the amount of allospecific chemical compounds on its own cuticle is close to the stranger profile.

When mixed *F. selysi* encountered control *M. rubida*, the aggressive behavior decreased at 30 days, as the quantities of allospecific labels on its cuticle increased, whereas *M. rubida* reared in mixed groups never recognized *F. selysi* controls because the former had smaller amounts of alkenes on their cuticles. In *F. selysi*, when the individual possesses allospecific compounds on its cuticule, it can recognize allospecific partners even if these compounds are quantitavely low (Breed *et al.*, 1987).

These results demonstrate, therefore, a close relationship between tolerance and the chemical signature worn by both species, and they support the gestalt model (Crozier *et al.*, 1979), which assumes the "phenotype matching rule," that an individual recognizes the membership of an encountered individual based on whether it has one or a set of common cues (Carlin, 1989; Jaisson, 1991; Stuart, 1992).

In this study, we could not distinguish between the latter two hypotheses: A young worker created a mixed template directly from the allospecific (and conspecific) labels of the partners or from the allospecific (and conspecific) chemical cues acquired on its own cuticule. In both cases, the template may encode these labels early in adult life, during the first day after emerging, combining the effects of physiological maturation [young ants are not very mobile and contacts between group members are rare (Errard *et al.*, 1991)], and social experience (as observed also for intraspecific recognition by Morel *et al.*, 1988).

During the "sensitive period," there is rapid mutual familiarization of the two species reared together and the chemical profiles are flexible enough to be influenced by the perception of environmental odors perceived by the newly emerged individual (reviewed by Jaisson, 1991). This explains why the formation of the mixed groups must be attained within 48 h of emergence. We have confirmed that the chemical signature is already shaped within 48 h of emergence.

The very beginning of adult life is thus more important for the maintenance of the social group than for the establishment of the social group. Establishment of the social group occurs at the time of colony foundation when the first workers, the queen, and the surrounding environment collectively contribute to the new colony's unique colony odor, from which nestmate recognition cues are derived. After the colony foundation, the newly emerged callow worker enters an already established social group. This callow worker will contribute some small amount to the collective odor and will learn the already established and ever-changing colony odor of the moment.

Finally, these experiments provide another excellent exemple that "nestmate recognition" is chemically mediated through chemical compounds such as cuticular hydrocarbons which are required for discrimination (Bonavita-Cougourdan *et al.*, 1987; Morel *et al.*, 1988; Nowbahari *et al.*, 1990). Whether the elaboration of the template which encodes the labels, serving as a reference to characterize nestmates, is innate or has to be learned is still disputable. The template/cues system affects the two distinct mechanisms which appear in the results, recognizing and being recognized (Gadagkar, 1985). Studying the asymmetry of recognizing and being recognized may explain how templates form (on the criterion of acceptance and rejection) and how labels form in these two species. However, the chemical cues, in whole or in part, are probably the labels, but these data do not prove that they are.

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