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Nestmate discrimination and cuticular profiles of a temporary parasitic ant *Lasius* sp. and its host *L. fuliginosus* (Hymenoptera, Formicidae)

Received: December 13, 1999 / Accepted: April 25, 2000

Abstract Workers of the temporary parasitic ant *Lasius* sp. were perfectly compatible with their hosts *Lasius fuliginosus*. Aggression was never seen between allospecific nestmates in either field or laboratory. In the laboratory, trophallaxis and allogrooming between allospecific nestmates were statistically more frequent than that between conspecifics. These ants were highly aggressive toward individuals of either species from another mixed colony located 1 km away, indicating that they discriminated nestmates and nonnestmates regardless of the species to which they belonged. No aggressive actions, however, were observed between two neighboring mixed nests located 5 m apart. We have not considered, however, the relationship of the two neighboring nests: the two nests may have been two subunits of the same family or sister colonies, or may have been different colonies but discriminated from distant aliens. Gas chromatography analysis on the total cuticular extracts showed that *Lasius* sp. and *L. fuliginosus* possess distinct cuticular profiles, even though they were collected from the same mixed colony.

Key words *Lasius* spp. · Temporary parasitic ant · Naturally mixed colony · Nestmate recognition · Cuticular profiles

Introduction

Nestmate discrimination is almost legendary in ants (Fielde 1904). The adaptive function of nestmate discrimination

is the defense of a colony and its resources (Breed and Bennett 1987; Vander Meer and Morel 1998). As to the proximate mechanism, it is generally believed that ants recognize nestmates through chemical cues that are colony specific, and it has further been demonstrated that cuticular products, especially hydrocarbons, are probably the major chemicals involved (Bonavita-Cougourdan et al. 1987; Morel et al. 1988; Nowbahari et al. 1990; Dahbi and Lenoir 1998; Nielsen et al. 1999; Lahavi et al. 1999).

However, the occurrence of naturally mixed colonies composed of two or more species does not agree with the legend of nestmate discrimination in ants. Symbioses may occur among ant species or between ants and other arthropods. Although in general individuals of different origins apparently live a peaceful life in mixed colonies, the relationship between them is rarely mutualism, but rather commensalism or parasitism (Hölldobler and Wilson 1990). In ants, approximately 180 of 8800 species are social parasites that exploit temporarily or permanently the labor force of other ant species or other colonies of the same species (Hölldobler and Wilson 1990). Wilson (1971) categorized them into temporary parasitism, dulosis (slave-keeping), and inquilinism. Temporary parasitism has been found in genera of Formicinae and Myrmicinae (Hölldobler and Wilson 1990); it is a way in which a newly mated parasitic queen succeeds in entering into a host nest, and then kills and replaces its original queen. Unlike ants of dulosis and inquilinism that depend on their victims permanently for survival and reproduction, the queen and brood of temporary parasitism are reared by host workers only during the colony initiation. The mixed phase is soon over as the host workers die out from natural causes (Wilson 1971).

The cases of mixed colonies raise an interesting question about how parasites succeed in breaking through the defensive line of ant colonies and have attracted many field and laboratory studies (see review in Hölldobler and Wilson 1990; Breed and Bennett 1987; Vander Meer and Morel 1998). In this study, we carried out experiments to examine the relationship between workers of a temporarily parasitic ant, *Lasius* sp., and its domestic host, *L. fuliginosus*, their abilities to discriminate between nestmates and neighbor-

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strangers, and their cuticular profiles, which might explain how those two *Lasius* species can share the same nest.

Materials and methods

Materials

In July 1997, five mixed colonies of *Lasius* sp. (color, yellow; the species name of this ant is still uncertain because of the lack of queen specimens in the collection; Yamauchi, personal communication) and its host *L. fuliginosus* (black) were located in Mito, central Japan. All mixed colonies we found were in the early stage of parasitism, judging from that the host workers, *L. fuliginosus*, were still numerous. For example, in 39 visits to mixed colony A in summer 1997, workers of *Lasius* sp. were found outside of the nest only 6 times, and the average number of *Lasius* sp. workers accounted for merely 2.3% ($\pm 1.6\%$) of a total of foragers ($n = 3288$). These ants searched for food along foraging trails on both tree trunks and ground; they also attend aphids to collect honeydew. Three mixed colonies were chosen as experimental materials; colonies A and B were neighbors located only 5 m apart, whereas colony C was about 1 km away from the others. Colonies A and C nested at the base of pine trees and colony B at a maple tree.

Behavioral observations

To determine if any antagonistic or preference behaviors occur between allospecific workers sharing the same mixed colony, ten *Lasius* sp. and ten *L. fuliginosus* foragers were captured from colony A and kept in the laboratory in two petri dishes (diameter, 9 cm), each containing five *Lasius* sp. and five *L. fuliginosus* foragers. A piece of apple was provided daily as food. The interactions among nestmates in two petri dishes were recorded for a total of 6 h during 1 week (3 h per dish). The frequencies of interactions between conspecific and allospecific nestmates were analyzed with Pearson χ^2 test, by assuming that if there is no discrimination, theoretically the frequencies of the interactions between different pairs, i.e., *L. fuliginosus*–*L. fuliginosus*, *Lasius* sp.–*Lasius* sp., and *L. fuliginosus*–*Lasius* sp., should be equal.

Nestmate recognition and neighbor–stranger discrimination were assayed in the field by introducing foreign workers from colonies B and C near the entrance of colony A. In the field, quantification of behavior was difficult because often more than one resident worker encountered the introduced individual. In this case, the interactions between resident workers and “intruders” were simply categorized in a dichotomous manner as aggressive or nonaggressive. The behavior was recorded as aggressive if any of the following behaviors were observed in either a resident or an introduced ant: (1) threatening with mandibles opened, (2) biting and dragging the body and appendages of other ants, and (3) gaster flexion; although disregarded, antennation and trophallaxis were noted as nonaggressive behavior. In

total, 7 *Lasius* sp. and 8 *L. fuliginosus* workers from colony B, and 6 *Lasius* sp. and 11 *L. fuliginosus* workers from colony C, were released one by one near colony A.

Gas chromatographic analysis

Sixteen cuticular extracts, each containing the product of one worker from colonies A and C (four ants for each species and from each colony), were analyzed on a gas chromatograph (Shimadzu GC-14B) equipped with a flame ionization detector and 25 HiCap-CBP1 WCOT nonpolar capillary column (25 m long, 0.25 mm i. d.). To avoid the possible contamination of cuticular extracts by secretions from ant exocrine glands, ant samples were individually collected by carefully placing the opening of a glass vial around a target worker without directly touching it and waiting until the confined ant crawled into the vial. The samples were then brought alive to the laboratory and immediately stored in a freezer (-10°C). To prepare cuticular extracts, freeze-killed ants were individually soaked in 500 μl of hexane for 5 min. Before GC analysis, each extract was evaporated under a gentle flow of air to dry and then redissolved in 20 μl of hexane. For one GC analysis, 2 μl of redissolved sample, i.e., about 1/10 of the cuticular product from a worker, was analyzed. Helium was used as a carrier gas, and the oven temperature was programmed to increase at $10^\circ\text{C}/\text{min}$ from 100° to 300°C . Cuticular profiles were recorded on a Shimadzu Chromatopac C-R6A processor.

Results

Behavioral observation

During a 1-week observation (total, 6 h) in the laboratory, aggression was never seen either between conspecific or allospecific nestmates from mixed colony A, while trophallaxis and allogrooming were frequently observed between them. The Pearson χ^2 test showed that there were statistical differences in the frequencies of trophallaxis and allogrooming between conspecific and allospecific nestmates in both petri dishes, with this hierarchical order of interactions: *L. fuliginosus*–*Lasius* sp. > *L. fuliginosus*–*L. fuliginosus* > *Lasius* sp.–*Lasius* sp. (Table 1).

The mutual acceptance of allospecific nestmates was also confirmed in the field: aggression was never seen between allospecific workers belonging to the same mixed colony. All these observations indicated that the two *Lasius* species living in the same mixed colony were totally compatible. On the other hand, *Lasius* sp. and *L. fuliginosus* displayed a high level of aggression toward both conspecific and allospecific workers from another mixed colony located 1 km away, colony C. Introduction of workers from colony C of either species ($n = 17$) always induced violent attacks by several resident ants, sometimes involving both *Lasius* sp. and *L. fuliginosus* workers (3/17), and all “intruders” were killed. In contrast, *Lasius* workers of colony A disregarded completely their neighbors from colony B ($n = 15$).

Table 1. Pearson χ^2 test on the frequencies of allogrooming and trophallaxis between conspecific and allospecific nestmates of a temporary parasitic ant *Lasius* sp. and its domestic host, *Lasius fuliginosus*, raised in two petri dishes

Interaction pairs	Petri dishes	<i>Lasius</i> sp.– <i>Lasius</i> sp.	<i>L. fuliginosus</i> – <i>L. fuliginosus</i>	<i>Lasius</i> sp.– <i>L. fuliginosus</i>	Pearson χ^2 test
Allogrooming	1	15	29	36	8.58, <i>df</i> = 2*
	2	21	24	41	8.12, <i>df</i> = 2*
Trophallaxis	1	44	38	74	14.31, <i>df</i> = 2**
	2	22	51	54	14.67, <i>df</i> = 2**

Total ants observed: 20 workers, 10 from each species; total observation time, 6 h

* $P < 0.05$; ** $P < 0.01$

In the field, a hostile encounter was never recorded between colonies A and B near the entrance of colony A.

Gas chromatography

GC analysis showed that the cuticular profiles of both *Lasius* species were species specific: the compositions of the cuticular extracts were the identical for the same species, and cuticular profiles were similar regardless of their colony identity. However, workers of *Lasius* sp. had cuticular profiles totally different from that of *L. fuliginosus*, even though they were collected from the same mixed colony. In other words, if the peaks with the same retention time in the chromatogram represent the same chemical compound, no common compounds were shared between the two *Lasius* species. As the cuticular profiles of the two *Lasius* species were species specific without important variation between different mixed colonies, only the chromatogram of a single *Lasius* sp. and a *L. fuliginosus* worker from mixed colony A are presented (Fig. 1).

Discussion

Behavioral observations in both field and laboratory suggested that the temporarily parasitic ant *Lasius* sp. and its domestic host *L. fuliginosus* lived peacefully without any hostility; rather, they treated each other as true nestmates. If there any preference, it should be allospecific (see Table 1). In fact, the workers of the host ant *L. fuliginosus* seemed more active than their parasites *Lasius* sp. in the initiation of trophallaxis and allogrooming. The hierarchical order in the frequencies of interactions yielded the strong impression that workers of *L. fuliginosus* had been attending their parasites, *Lasius* sp. This result indicates that *Lasius* sp. has succeeded in cheating their victims recognition system. On the other hand, violent aggression toward both conspecific and allospecific workers from a mixed colony at a relatively distant place (1 km distant) showed that the workers of the two *Lasius* species were able to discriminate foreigners from nestmates, regardless to which species they belong. The mutual tolerance between neighboring colonies seems to be interesting because that it could be another example of the “dear enemies phenomenon” (Temeles 1994) that

had been reported in several ant species (e.g., *Acromyrmex octospinosus*, Jutsum 1979; *Leptothorax curvispinosus*, Stuart 1987; *Leptothorax nylanderi*, Heinze et al. 1996). Yet, before concluding that those ants display neighbor–stranger discrimination, we must note that colonies A and B were defined according to the foragers aboveground activities. We neither verified their genetic relatedness nor dug into the ground to see if there was any underground connection between the two nests. Thus, a possibility remains that colonies A and B were sister colonies or merely two subunits of the same colony.

Three hypotheses have been developed to explain by which means arthropod social parasites to cheat their victims nestmate discrimination system. The first hypothesis refers to chemical camouflage, i.e., the acquisition of host recognition cues via passive absorption or active contacts (Vander Meer and Wojcik 1982); the second one is the chemical mimicry of host recognition cues by biosynthesizing host recognition cues such as cuticular hydrocarbons (Howard et al. 1980); and the third possibility, known as the fellowship hypothesis developed by Jaisson (1988), is based on an observation that the nestmate recognition in most ant species is learned in the early development of an individual rather than genetically controlled. According to fellowship hypothesis, various parasites may well benefit from this behavioral plasticity to imprint their odor to young and ignorant victims.

Acquisition of host odor via contacts had been documented in a myrmecophilous beetle *Myrmecaphodius excavaticollis* (Vander Meer and Wojcik 1982); a myrmecophilous cricket, *Myrmecophilus* sp. (Akino et al. 1996); two slave-keeping ants, *Polyergus samurai* (Yamaoka 1990) and *Harpagoxenus sublaevis* (Kaib et al. 1993); the newly mated queens of *Polyergus rufescens* (Errard et al. 1998); two xenobiotic ants, *Formicoxenus provancheri* and *F. quebecensis* (Lenoir et al. 1997); and a workerless inquiline ant, *Leptothorax kutteri* (Franks et al. 1990). Chemical mimicry by biosynthesizing the requisite cuticular hydrocarbons was suspected in two termitophile beetles, *Trichopsenius frosti* and *Xenistusa hexagonalis* (Howard et al. 1980, 1982), and a fly, *Microdon albicomatus*, a predator of ants (Howard et al. 1990). Although all cases of natural mixed colonies suggest that nestmate recognition is based on a learning process and that the early experience may affect the mutual acceptance of a parasite ant (Errard et al. 1992), no direct evidence supports the idea that the accep-

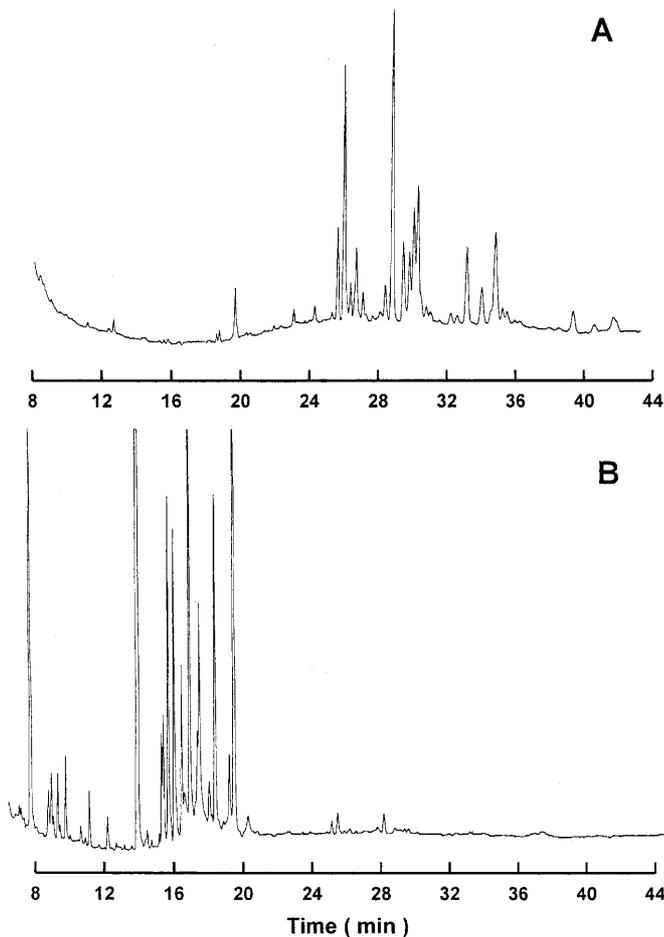


Fig. 1A,B. Examples of cuticular profiles of a single worker of the temporary parasitic ant *Lasius* sp. (A) and its host *Lasius fuliginosus* (B) collected from the same mixed colony (colony A)

tance of parasites is an imprinting-like learning which is characterized by an early sensitive period and long-lasting effect, as already been described in birds.

Assuming that cuticular products that appeared on the gas chromatogram (see Fig. 1) are also involved in nestmate recognition of the two *Lasius* species we examined, neither chemical mimicry nor chemical camouflage hypothesis could explain the data we obtained, because the cuticular profiles of *Lasius* sp. did not change toward their hosts. In other words, there is neither a new biosynthesis nor an odor transfer between allospecific nestmates, although they frequently engaged in physical contact and trophallaxis.

In fact, the homogeneity in cuticular profiles is not always true in naturally mixed colonies. In some cases, aggressive interactions were observed between individuals bearing different cuticular profiles (Heinze et al. 1994); in other cases, however, the compatibility seemed perfect between individuals bearing different cuticular profiles (Habersetzer and Bonavita-Cougourdan 1993; Bonavita-Cougourdan et al. 1996; Orivel et al. 1997). As symbiote species in the latter cases share a certain number of cuticu-

lar products, it can still be assumed that some, not all, cuticular hydrocarbons are responsible for recognition in those ants. In contrast, the two *Lasius* ants we examined had completely different cuticular profiles without any common products. Although the chemical structures of the cuticular products in the two *Lasius* ants have not yet been identified, a possibility that those products or part of them are hydrocarbons remains high because the chemical procedure we used in GC was a conventional one for characterizing cuticular hydrocarbons. Bearing this in mind, we thought that signals other than cuticular hydrocarbons may serve as recognition signals in those ants. Naturally, we cannot rule out that those ants do use cuticular hydrocarbons but based on a decision rule other than “common odor acceptance.”

Unquestionably, the case of the two *Lasius* ants, as all cases of mixed colonies, suggests that nestmate recognition was based on learning. Regardless of what kind of cue is used, some problems have to be solved for the fellowship hypothesis. Under this hypothesis, a brood of parasitic ants needs to be raised uniquely by young and ignorant hosts. Given this is the case, a question still remains open on what happens when a parasite individual encounters old host workers whose recognition templates had already been formed before the emergence of the first batch of parasite workers. As such encounters seem quite possible, we think that the success of parasitism must also depends largely on the behavioral plasticity of adult hosts in recognition. Meanwhile, we have to bear in mind that learning ability in recognition in ants is not without limitation, but rather is a selective learning program restricted by the ant’s genetics. Only a few ant species are vulnerable to parasitism, and parasite–host relationships occur in fixed species pairs belonging to phylogenetic ingroups (Emery’s rule; cited in Hölldobler and Wilson 1990). This restriction is also true in ants of the genus *Lasius* (Hasegawa 1998).

In short, the data we obtained suggest that the temporary parasitic ant *Lasius* sp. probably succeeds in cheating their victim’s colonial defense line by exploiting the learning nature of *L. fuliginosus* in the recognition of nestmate. The success of *Lasius* sp. may also suggest that the recognition template of *L. fuliginosus* is not the classical imprinting type of a duckling to its mother, but rather is a more open system capable of updating its content over the whole life of an individual.

Further research on the nestmate discrimination of *Lasius* sp. and *L. fuliginosus* is needed in respect to (1) the chemical structure of cuticular lipids by GC-mass spectrometry; (2) the modulation of cuticular profiles of colony members along with the continuous increase of *Lasius* sp. workers and decrease of *L. fuliginosus* workers in a mixed colony; and (3) the difference in cuticular profile between mixed-colony members and free-living ants.

Acknowledgments We are grateful to K. Yamauchi and Sk. Yamane for the identification of the ants, and to J. Kojima, S. Tanaka, and J. Billen for their comments on the manuscript. Our thanks are also due to two anonymous reviewers for their valuable suggestions. This study was supported in part by grants-in-aid from the Japan Ministry of Education, Science and Culture (no. 96266) and the Japan Society for

the Promotion of Science (the JSPS postdoctoral fellowship program for foreign researcher).

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