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Olfactory recognition processes in the interactions between the slavemaking ant *Polyergus breviceps* and its *Formica* hosts

Zimmerli, Ellen Jeanne, Ph.D.

City University of New York, 1993

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OLFACTORY RECOGNITION PROCESSES IN THE INTERACTIONS BETWEEN THE SLAVEMAKING ANT *POLYERGUS BREVICEPS* AND ITS *FORMICA* HOSTS.

by

ELLEN J. ZIMMERLI

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1993

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree Doctor of Philosophy.

1/21/93 Howard Topoff
date Chairman of Examining Committee

1/26/93 Herbert D. Saltzstein
date Executive Officer

Dr. Howard Topoff
Dr. Peter Moller
Dr. Gerald Turkewitz
Dr. James Carpenter
Dr. Robin Stuart

Supervisory Committee

The City University of New York

ABSTRACT

OLFACTORY RECOGNITION PROCESSES IN THE INTERACTIONS BETWEEN THE SLAVEMAKING ANT POLYERGUS BREVICEPS AND ITS FORMICA HOSTS.

BY
ELLEN J. ZIMMERLI
ADVISOR: DR. HOWARD TOPOFF

In the complex world of ant societies, recognition processes allow the selective inclusion and exclusion of individuals from the colony. Recognition and discrimination are performed largely on the basis of olfactory characteristics, or chemical communication. These chemical recognition processes have played an important role in the evolution of social parasitism, such as in the slavemaking ant *Polyergus breviceps*. Slavemaking ants live in "mixed" nests with their "slave" hosts, who perform all colony functions. Slave and slavemaker recognize each other as nestmates due to olfactory imprinting in the nest, but recognition processes are also important in colony founding by slavemaking queens, the care of slavemaker brood by slave workers and in interactions between individuals from mixed nests. This dissertation reports the results of three investigations of olfactory recognition in the slavemaking ant *Polyergus breviceps*.

First, I studied slave odor as a component of colony odor and its effects on odor-mediated aggression in individuals from mixed nests. Individuals of slavemaking and slave species were placed in pairs in small (9 x 9 x 2 cm) arenas for 15 minutes and the following behaviors were measured: mutual investigation, latency to attack, accumulated attacking time, number of attacks, percentage of pairs fighting in each series, and percentage of pairs in each series that exhibited particular aggressive acts. Intraspecific aggression in the slavemaking ant *Polyergus* was significantly greater between individuals from colonies with different slave species of the genus *Formica*. Interspecific aggression between *Polyergus* and freeliving *F. occulta* individuals was low when the *Polyergus* had *F. occulta* hosts, and significantly greater when the *Polyergus* had *F. gnava* hosts. Intraspecific aggression among *F. wheeleri* slavemakers increased when we experimentally changed the slave species of one colony. Slave species odor did not affect aggression between individuals of different slavemaking species, however: aggression between *Polyergus* and *F. wheeleri* individuals was not reduced when both parasitized the same host species. This suggests that slave odors are important only in intraspecific interactions among slavemakers.

Second, through a pupa choice test conducted over 10 days, I studied the tendency to care for and nurse obligatory slavemaker (*Polyergus*) and facultative slavemaker (*Formica wheeleri*) pupae in freeliving and enslaved *Formica occulta* workers. Although nursing of slavemaker brood was facilitated in enslaved *Formica* workers, that have had prior exposure to the slavemaker brood, there was a natural

tendency for freeliving Formica workers to nurse Polyergus but not Formica wheeleri pupae. Freeliving workers of Formica occulta retrieve and nurse Polyergus pupae just as well as conspecific pupae. Formica neogagates, a sympatric species which is not parasitized by either slavemaker, preferentially retrieves and tends conspecific brood over that of Polyergus and F. wheeleri. It is proposed that brood of obligatory slavemaking species must possess an attractive pheromone for slavemaker colony foundation to be successful, since slavemaker brood must be nursed by adult slave workers with no prior exposure to slavemaker brood. An attractive pheromone is not necessary in the brood of facultative slavemakers, since this brood is cared for by newly-eclosed slave workers who imprint on the slavemaker brood.

Third, I studied the importance of host-queen killing and olfactory transfer during colony founding by Polyergus queens. Polyergus queens are incapable of rearing their own brood, and therefore require the assistance of host Formica workers soon after egg laying. Accordingly, a newly-mated Polyergus queen must penetrate a nest of Formica, kill the host queen, and become permanently accepted by the slave species' workers. Laboratory tests showed that a Polyergus queen will similarly attack and bite a dead (and therefore motionless) Formica queen. Immediately after attacking the dead host queen, the Polyergus queen will be accepted by

workers from any colony of *Formica* belonging to the same species of the dead queen (but will be attacked by workers from other *Formica* species). Preliminary results also indicated that any adoption-facilitating chemicals obtained by the *Polyergus* queen were still effective one week after killing the host *Formica* queen. When *Polyergus* queens raised in colonies containing *F. gnava* were introduced into nests of *F. occulta*, most showed little interest in attacking the resident *Formica* queen. In four of 10 tests, however, the *Polyergus* queen killed the foreign queen and was accepted by the *F. occulta* workers. *Polyergus* queens showed no interest in attacking newly mated *F. occulta* queens. However, *Polyergus* queens were successful in becoming adopted by several *Formica* colonies, killing the resident queen each time. Chemical transfer of foreign-queen odors may have played a key role in the evolution of social parasitism in *Polyergus*.

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GENERAL INTRODUCTION

Social Insects

Truly social (eusocial) insects are characterized by three traits: overlap of generations, cooperative rearing of young, and a reproductive caste which is served by sterile sister workers (Wilson, 1971). While various theories on the evolution of social behavior in insects differ in which of these steps evolved first (Wilson, 1971), the most fundamental step in the evolution of social behavior in insects is, by far, the recognition and tolerance of others of varying degrees of genetic relatedness: offspring, nestmates, conspecifics. Recognition allows some individuals to be included in the group and some individuals to be excluded or attacked. Recognition and discrimination in the insect world is performed largely on the basis of olfactory characteristics, or chemical communication. Pheromones, chemical signals which release specific behavioral responses in members of the same species, are used widely among ants (Hölldobler, 1984). Surface pheromones, hydrocarbons which adhere to the cuticle, are employed as signals among individuals, while groups of individuals collectively contribute to more salient colony odors (Wilson, 1971). Individuals that lack the colony odor, be they non-nestmate conspecific or isolated nestmate, are treated in a hostile manner; individuals who bear the colony odor, be they non-nestmate or heterospecific, are accepted. Colony odors stem from sources as varied as the queen (Carlin & Hölldobler, 1987), diet, nest material, or the workers themselves (Wilson, 1971; Hölldobler & Carlin, 1987; Provost, E., 1989).

Slavery in Ants

Chemical signals are also used widely in interactions between ant species (Hölldobler, 1983), but nowhere as interestingly as in the mixed nests of social parasites, where colony odor and nestmate recognition involve more than one species. Dulosis (or slavery) in ants is a form of social parasitism in which the dulotic (slavemaking, or parasitic) species conducts raids on freeliving colonies of a closely related (host, enslaved) species, driving the workers from the colony, and transporting the brood back to their home nest. Although a great deal of this stolen brood is consumed (Cool-Kwait & Topoff, 1984), some is raised to become slaves in the mixed nest. These slaves are accepted and behave as members of the slavemaker's nest, performing all of the usual worker duties. Slavemaking ants typically perform dramatic raids on freeliving colonies of the slave-species found in the slavemaker's nest. In general, these raids are not violent, because the workers of the target colony secrete an alarm pheromone, scatter, and thus do not put up much resistance to the raiders (Topoff et al., 1989). The slavemakers then proceed easily into the nest and remove whatever pupal brood they can carry. Slavemakers appear to follow the basic rule of parasitism: successful parasites do not kill their hosts. Their raids therefore, do not typically result in the death of the raided colony: the scattered workers (and queen) return to rebuild their colony and are often raided again at some later time (Goodloe et al., 1987).

Slavemaking occurs in two forms: facultative, and obligatory. The workers of facultative slavemaking species are not specialized, and maintain the ability to perform colony duties without the help of slaves. Workers of obligatory slavemaking species however, such as those of the amazon ant genus Polyergus, are specialized, and no longer perform any colony duties except for conducting slave raids. These slavemaking workers depend entirely on the slave workers for nest maintenance, brood tending, foraging, nest defense and even feeding.

The evolution of slavery may have followed any of several suggested paths. Emery's rule (attributed to Emery, 1909, in Wilson, 1971) states that social parasites are closely related to the species that serves them as hosts. In many social parasites this appears to be true, and it is not surprising, as two ant species can only coexist if they have similar biological needs and communication systems. Similar pheromones, and similar glands, are usually required for nestmate recognition, and for the efficient operation of a mixed nest (Buschinger, 1986).

Darwin (1859) suggested that slavery evolved out of brood predation on other species. In this scenario, the predatory colony would raid the colonies of other species, stealing the brood for food. Unconsumed pupae which hatch in the raiding colony, could contribute to the workforce in such a beneficial way that natural selection might benefit those colonies which raid and rear pupae of another species. Polyergus, an obligatory slavemaker, shows possible remnants of such an evolutionary path, consuming some of the stolen brood, and allowing the remainder to be reared (Cool-Kwait & Topoff, 1984; Topoff, 1985).

The territorial hypothesis (Wilson, 1975; Stuart & Alloway, 1982), in contrast, explains slave raiding behavior as evolving out of territorial interactions between neighboring colonies of the same species, with the dominant colony eradicating the other, stealing its brood. If the stolen brood is tolerated, which is likely among conspecifics, it will be raised in the victorious society. Once this behavior was established, inter-specific raids could have arisen from the extension of such raids to other species, or through sympatric speciation within the raiding group. Certain slavemaking Leptothorax species are often aggressive and territorial toward one another, demonstrating the link between intraspecific territoriality and interspecific raiding (Robin J. Stuart, personal communication).

Yet a third hypothesis claims that slavemaking behavior evolved from polygynous, polydomous ancestors, which allowed a constant exchange of workers and brood among nests (Buschinger, 1986). This hypothesis also assumes sympatric speciation, and seems unlikely since it requires a transformation of peaceful exchange of brood into very fierce slave-raiding behavior.

Olfactory Imprinting

The mixed nests of slavemaking ants have evolved because of the ability of some ant species to accept "others" into the colony. Forel (1874) and Fielde (1903, 1904) were the first to report success in forming artificially mixed colonies of ants, by mixing pupae and callow workers within 12 hours of eclosion. The phenomenon behind this success, which allows such colonies to exist in nature, has been interpreted by some as olfactory imprinting (Jaisson, 1975).

Imprinting is a process by which social preferences are narrowed, or social attachments are formed, as a result of experience with certain stimuli during a relatively early stage of development. Lorenz's original definition of "prägung" (imprinting) asserted that this form of learning is permanent and irreversible, and occurs during a narrowly defined "critical" period, as demonstrated by the lack of interest goslings showed their mother after they had imprinted on Lorenz (1937). Subsequent research has shown imprinting to be the result of diverse mechanisms which allow many forms of social attachment in many species to a variety of stimuli (Harlow & Zimmerman, 1959; Scott, 1958; Sluckin, 1968; Cairnes, 1966; Levy & Poindron, 1987; Kruijit, Ten Cate & Meeuwissen, 1983). In the diverse studies of imprinting, the "defining characteristics" of listed by Lorenz have not been found to be such: "classical" examples of imprinting have been shown to be reversible, and not confined to as narrow a time period as first thought (Sluckin, 1970). It is not surprising, therefore, that researchers in this area have felt uncomfortable with the term, instead using terms such as "imprinting-like learning" (Le Moli & Mori, 1987), or "olfactory learning" (Le Moli & Passetti, 1978; Isingrini, Lenoir & Jaisson, 1985; Carlin, Halpern, Hōlldobler & Schwartz, 1987). Bering in mind this inconsistency, I shall discuss the same phenomenon as these researchers, using the term imprinting only to imply a type of learning which occurs relatively early in development, in which social preferences are established based on exposure during a sensitive period.

Exposure to brood of another species was the method used by Forel (1874) in raising pupae of various Formica species, to enhance attraction and achieve mutual acceptance among callows on eclosion. Fielde (1903) recognized that callow ants accept whatever odors they first encounter, and accept these odors permanently. But although this olfactory learning is easiest to induce in a young callow, this process is not limited to this stage, and is not irreversible. Although exposing adults of different species to each other results in intense fighting, acceptance eventually occurs and mutual grooming and feeding is seen in the surviving workers (Wilson, 1971).

Ants recognize as nestmates those to which they are exposed as larvae and/or young callows (Jaisson, 1975; Jaisson & Fresneau, 1978; Carlin & Schwartz, 1989). In most natural situations, this recognition of nestmates is also of kin, since even polygynous colonies are made up of closely related queens. In a dulotic situation, however, both the slavemaking workers and the slaves eclose in a mixed nest, and are exposed to the odors of the other species before and after eclosion. Slaves are imprinted on the slavemakers, recognizing them as nestmates, and therefore perform for them all the behaviors common within a freeliving colony: brood tending, nest maintenance, foraging and even the feeding of other members of the colony (including, in this case, the slavemaking species).

An imprinting process similar to that of the slave species on the parasitic species can explain the preference of slavemaking ants for one slave species over the other. In the eastern United States the dulotic ant Polyergus lucidus can utilize any of three sympatric Formica species as slaves, but each Polyergus colony raids and keeps only one species of slave. Goodloe, Sanwald and Topoff (1987) suggested that this host specificity is caused by an imprinting of the *Polyergus* on the slaves present in the mixed nest, with raids simply conducted on other colonies with olfactory characteristics that match the home nest. A similar process of olfactory matching has been hypothesized in the colony founding behavior of *P. lucidus* queens, which successfully invade only colonies of the slave species found in their nest of origin, but it is unclear whether successful colony adoption was due to queen choice or slave worker selection (Goodloe & Sanwald, 1985).

Natural History of Polyergus breviceps

The western amazon ant, *Polyergus breviceps*, keeps different slaves in different habitats in the Chiricahua mountains of southeastern Arizona. In the oak-juniper woodland (altitude = 1700 m), *Polyergus* enslaves *F. gnava* hosts, and in the Ponderosa pine-Douglas fir habitat (altitude = 2650 m) the host is *Formica occulta*. A zone of overlap between the two slave species would be a natural situation to observe the host specificity mentioned above, but to date, no such zone has been found. Other *Formica* species which are not raided are found in both habitats, indicating the selectivity of raiding behavior.

The higher altitude site is also home to the facultative slavemaking species *Formica wheeleri*, which keeps *F. occulta* as slaves. *F. wheeleri* workers, which are not specialized as Polyergus workers are, have retained the ability to forage and maintain the nest, and therefore this species can potentially live independently of its hosts. No freeliving F. wheeleri colonies have been reported in this habitat, however, perhaps indicating an evolutionary step towards obligatory parasitism, or at least the abundance and ease of parasitizing F. occulta colonies.

Olfactory Recognition Processes

The evolution of slavery has depended on the manipulation of olfactory recognition processes in three different stages of the relationship between slavemaker and slave. First, the slavemaking queen cannot care for her own brood and must infiltrate and become accepted by a colony of the slave species. Second, the workers of the slave species must now care for the brood of this slavemaker queen. And third, the adult workers of slave and slavemaking species must live together peacefully.

Each of these three aspects of the slavemaker-slave relationship is important in the establishment of a mixed colony, and each involves olfactory recognition. This paper will describe three investigations of the odor mediated behavior in mixed nests. First, the nursing of slavemaker brood by slave-species workers was studied. Although it was expected that enslaved Formica workers would care for the brood of Polyergus slavemakers due to early exposure in the nest, it was discovered that no prior exposure was required.

Freeliving *Formica* workers displayed a natural tendency to care for *Polyergus* brood, but no brood nursing bias was found for the facultative slavemaker *Formica wheeleri*.

Second, the role of slave odor in aggression between individuals of slavemaking species and slave species was studied. Ants are generally aggressive toward individuals with different colony odors, and accept those with similar colony odors. Mixed colonies have "mixed" colony odors, and their inhabitants therefore exhibit unique patterns of aggression. Aggression among slaves and slavemakers (intraspecific and interspecific) was reduced between individuals exposed to the same slave species with the exception of interactions between individuals of different slavemaking species.

Third, the role of chemical transfer in colony takeover by *Polyergus* queens was investigated. Past research has indicated that during takeover of a *Formica* colony, a *Polyergus* queen fights with the resident *Formica* queen, possibly acquiring chemicals which facilitate her acceptance by the *Formica* workers. This study investigated the functional limits of the chemicals transferred between queens.

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PART I: THE EFFECT OF SLAVE ODOR ON AGGRESSION IN SLAVEMAKING ANTS.

ELLEN ZIMMERLI

Department of Psychology
Hunter College of CUNY
695 Park Avenue New York, NY 10021

Abstract

Ant colonies have characteristic colony odors, which allow ants to recognize nestmates. "Mixed" nests contain two or more ant species, a socially parasitic, slavemaking species, and one or more host species. The odor of slaves is an important component of colony odor in mixed species nests, and influences aggression, which is odor-mediated. This influence was explored in a series of experiments. Individuals of slavemaking and slave species were placed in pairs in small (9 x 9 x 2 cm) arenas for 15 minutes and the following behaviors were measured: mutual investigation, latency to attack, accumulated attacking time, number of attacks, percentage of pairs fighting in each series, and percentage of pairs in each series that exhibited particular aggressive acts. Intraspecific aggression in the slavemaking ant Polyergus was significantly greater between individuals from colonies with different slave species of the genus Formica. Interspecific aggression between Polyergus and freeliving F. occulta individuals was low when the Polyergus have F. occulta hosts, and significantly greater when the Polyergus have F. gnava hosts. Intraspecific aggression among F. wheeleri slavemakers increased when the slave species of one colony was experimentally changed. Slave species odor did not affect aggression between individuals of different slavemaking species, however: aggression between Polyergus and F. wheeleri individuals was not reduced when both parasitized

the same host species. This suggests that slave odors are important only in intraspecific interactions among slavemakers.

Introduction

Nestmate recognition in ants is accomplished through the detection of "colony odors" which elicit peaceful interactions between nestmates and aggressive interactions between non-nestmates. Colony odor is a unique composition of "surface pheromones", chemicals worn on the cuticle which are derived from the workers, the queen, and the environment (soil and diet) (Hölldobler & Wilson, 1990; Carlin & Hölldobler, 1986). In contrast to the ability to discriminate among kin with no prior exposure necessary, nestmate acceptance in ants is generally learned shortly after eclosion through an "imprinting-like process" on the colony odor (Jaisson, 1975; Carlin, Halpern, Hölldobler & Schwartz, 1987; Hölldobler & Wilson, 1990).

While the learning of nestmate odors allows for the acceptance of nestmates of varying degrees of relatedness, it also allows young ants to accept foreign individuals present during the "sensitive" post-eclosion period. This process has apparently played an important role in the evolution of social parasitism in ants. Through early exposure to odors of the "foreign" species, socially parasitic slavemaking ants and their slaves accept each other as nestmates and interact cooperatively (Le Moli, 1980; Le Moli & Mori, 1987). Slaves, comprising up to two-thirds of the colony population, contribute to the colony odor of the "mixed nest", providing a discernible cue which is used as a template when a slavemaking scout searches for a freeliving slave-species colony to raid and when a slavemaking queen searches for a colony to takeover (Goodloe, Sanwald & Topoff, 1987; Goodloe & Sanwald, 1985).

Mixed nests, therefore, provide a unique opportunity to study the relative effects of various components of nest odor on aggression. Ants usually behave aggressively towards other species, but in a mixed nest two species cohabit, most likely due to an expanded olfactory label for "nestmate". In the absence of territorial cues, aggression between different colonies of the same species can be relatively low, due to a species specific component to nest odor. But slavemaking colonies with different slave species will have significant differences in colony odor, perhaps resulting in greater inter-colonial aggression.

If mixed nests produce a "mixed" colony odor, this colony odor may also affect aggression between slavemakers and freeliving individuals of the slave species (Le Moli & Mori, 1984). Less aggression is expected between a slavemaker and a freeliving individual of the species it enslaves and resembles olfactorily, than between a slavemaker and an individual of a slave species not found in the slavemaker's nest. Different slavemaker species which enslave the same species would perhaps show reduced aggression based on the similar slave-odor component of colony odor.

The experiments described in this paper were designed to explore the relative influence of slave species and environmental odors on the aggressive behavior of slavemaking ants and their slaves.

Materials and Methods

The study was conducted 5 km west of Portal, Arizona. Colonies of the obligatory slavemaker Polyergus breviceps, with nest materials, were collected from two habitats: a Ponderosa pine-Douglas fir habitat (elevation 2510 m), where the slave species is Formica occulta, and an oak-juniper woodland habitat (elevation 1600 m), where the slave species is Formica gnava. Freeliving F. occulta colonies were collected from the high elevation site, as were colonies of the facultative slave maker Formica wheeleri (with F. occulta slaves). Ant colonies were maintained in the lab in boxes measuring 17 x 12 x 6 cm. Original nest soil, and 10 cm diameter inverted petri dish "nests" with dark removable cloth covers were provided. Ants were fed honey and insects (crickets).

Aggression Tests

Tests were performed in neutral arenas. The empty plastic boxes provided no territorial cues so that the responses of tested ants were derived only from cues coming from the other ant. To remove the possibility of social facilitation of aggression, for example, by the release of an alarm pheromone, aggression was measured between individuals and not in groups. This testing of individual pairs simulates the encounters between individuals common among foragers, nest defenders, and indeed, most ant workers. There is no reason to believe that aggression is altered by the artificial environment, since a series of experiments on aggression in European Formica species has shown that aggression in the laboratory is not significantly different from aggression in the field, albeit somewhat less pronounced (Le Moli & Parmigiani, 1981, 1982; Le Moli et al., 1982, 1983, 1984).

Individual ants were selected at random from laboratory colonies, and placed on opposite sides of diagonally divided square plastic boxes (9x9x2 cm). Individual ants were not used in more than one encounter. Cardboard separators were kept in place for 5 minutes. All aggression tests were performed under standard illumination, between 0800 and 1200 hrs (MST), at ambient temperature (22°C - 30°C). While the temperature at which the tests were performed varied, this variation was far less than that found in the field, and no inhibition or facilitation of aggression due to temperature was observed. The following behaviors were observed and measured using stopwatches for 15 minute periods:

a) Mutual Investigation (MI). Time (in seconds) spent in antennal inspection between the two individuals.

b) Latency to Attack (LA). Time (in seconds) from the first contact between the two ants until the onset of an attack (defined as the performance of at least one of the overt attack behaviors listed below: section f, v-viii). When no attack occurred, the LA value equaled the duration of the experiment (900 s).

c) Accumulated Attacking Time (AAT). Total time (in seconds) during which at least one ant was performing any of the overt attack behaviors listed below (section f, v-viii).

d) Number of Attacks (NA).

e) Proportion of fighting pairs across each series.

f) Proportion of fighting pairs across each series exhibiting the following ritualized (i-iv) and overt (v-viii) aggressive acts: i) startle, ii) threat (open mandibles), iii) upright posture (standing on hind legs), iv) gaster flexing (pointing of gaster towards opponent to spray formic acid or other glandular contents), v) seizing (biting), vi) dragging, vii) carrying, viii) rolling.

Each test series consisted of interactions between 12 dyads. Comparisons of aggression were made between test series. Because of the skewed nature of the data, non-parametric statistics were used. Measures of MI, LA, AAT and NA were compared with the Mann-Whitney "U" test. When pairwise comparisons were made between 3 or 4 test series (as in Tables 1 and 2) a Kruskall-Wallis Analysis of Variance was used first to determine that differences existed. Measures of the proportion of interactions involving each ritualized and overt aggressive act are shown merely to describe the aggression, and were not compared with statistics. Unless otherwise indicated, it was not possible to determine which individual in the dyad initiated the aggression.

Results

Hypothesis I. Intraspecific aggression in the slavemaking ant *Polyergus breviceps* will be greater between individuals from colonies with different host species.

The prediction was that slave odor plays a role in intraspecific aggression. This hypothesis was tested by comparing the aggressive encounters between *Polyergus* individuals from a) the same colony; b) different colonies with the same slave species; c) different colonies with different slave species (see Table 1).

Significant differences in aggression were found in heterocolonial (**different colony**) intraspecific *Polyergus* encounters between individuals from colonies with the same slave species and with different slave species (Table 1, comparison between b and c: $p<0.01$ for MI, AAT, LA, and NA). Heterocolonial interactions between dyads from colonies containing the same slave species (Table 1, b) were not aggressive. These encounters often involved a startle response (10/12 dyads), open-mandible "threat" (6/10 dyads), and upright posture (7/12 dyads), but no overt aggressive behaviors. Heterocolonial encounters between dyads from colonies with different slave species (Table 1, c) were very aggressive: immediate and prolonged aggression and no mutual investigation were observed between Polyergus individuals with different hosts. Out of 12 encounters, 11 involved fighting: moderate amounts of carrying (5/12 dyads), dragging (2/12 dyads) and threatening (4/12 dyads) were observed, as were great amounts of rolling (11/12 dyads), seizing (11/12 dyads) and gaster flexing (12/12 dyads). Five of the 12 dyads were still fighting one hour after the aggression test was conducted, and in two other dyads, one of the ants was dead after one hour.

Homocolonial interactions (between individuals from the **same colony**) were not aggressive, and no statistically significant differences in aggression were found between homocolonial encounters and heterocolonial encounters between individuals from colonies with the same slave species (Table 1, comparison of a and b). Homocolonial encounters (Table 1, a) were characterized by mutual investigation and a low rate of "startle" upon first contact (3/12 dyads), but no other ritualized or overt aggressive behaviors were observed.

Significant differences in aggression were found between homocolonial interactions and interactions between individuals from colonies with different-slave species (Table 1, comparison between a and c: $p<0.01$ for MI, LA, AAT and NA).

The hypothesis was therefore, supported: intraspecific aggression in the slavemaking ant Polyergus breviceps is greater between individuals from colonies with different host species.

Hypothesis II. Interspecific aggression between Polyergus and F. occulta nestmates will be low, while aggression in heterocolonial interactions between Polyergus and F. occulta will depend on the host species present in the slavemaker nest.

If aggression between nestmates is low due to exposure, and aggression between conspecific non-nestmates is of low intensity due to odor similarities, does olfactory familiarity reduce aggression between slavemakers and freeliving individuals of the host species? It was predicted that aggression between individual Polyergus and F. occulta workers would depend on the slave species in the Polyergus nest. This hypothesis was tested by comparing interactions between Polyergus and F. occulta nestmates, Polyergus (from colonies with F. occulta hosts) and freeliving F. occulta, Polyergus (from colonies with F. gnava hosts) and freeliving F. occulta, and, as a control, Polyergus nestmates (Table 2).

Significant differences in aggressive behavior were found in interactions between Polyergus and F. occulta nestmates as compared with heterocolonial Polyergus-F.

occulta (freeliving) pairings (Table 2, comparison between b and c: p<0.01 for AAT, LA and NA; no differences were found in MI). Eight of the 12 heterocolonial interactions (Table 2, c) involved fighting behaviors: startle (5/12 dyads), threat (6/12 dyads), gaster flexing (5/12 dyads), seizing (5/12 dyads) and dragging (4/12 dyads) were displayed. In four of these encounters the Formica worker was clearly the aggressor. One hour after the aggression test, one Formica worker was dead, and one dyad was still fighting.

Two very different patterns of aggression were seen in Polyergus-freeliving F. occulta interactions, with the Polyergus's host species the important factor. When Polyergus with F. occulta slaves interacted with freeliving F. occulta (Table 2, c), there was more MI (p<0.01) and the following low-level aggressive behaviors were observed: startle (5/12 dyads) and threat (6/12 dyads). When Polyergus with F. gnava slaves interacted with freeliving F. occulta (Table 2, d), there was greater AAT (p<0.05), and a greater NA (p<0.01,) and the following overt aggressive behaviors were observed: carrying (2/12 dyads), dragging (8/12 dyads), gaster flexing (11/12 dyads), rolling (11/12 dyads) and seizing (12/12 dyads). There was no difference in the LA. Within one hour of the aggression test, nine of these 12 encounters resulted in the death of one of the dyad: seven Formica and two Polyergus workers died.

The control comparison (Table 2, comparison between a and b) revealed no differences in aggressive behavior between homocolonial *Polyergus* interactions and homocolonial *Polyergus*-*F. occulta* interactions. No attacks were observed between nestmates and only 2 of 12 of heterospecific nestmate encounters involved a startle response. Heterospecific nestmates did, however, engage in considerably more mutual investigation than did *Polyergus* nestmates ($p<0.01$).

The pattern of aggression supported the hypothesis: interspecific aggression between *Polyergus* and *F. occulta* nestmates is low, while aggression in heterocolonial interactions between *Polyergus* and *F. occulta* depends on the host species present in the slavemaker nest.

Hypothesis III: Inter-slavemaker aggression between Polyergus *breviceps* and *Formica* *wheeleri* will be greater between individuals from colonies with different host species.

Since slave odor has been shown to affect aggression in both intraspecific and interspecific interactions, I next hypothesized that aggression between different slavemaker species (of different genera) would also be affected by their host species. To test this hypothesis I compared interactions between individuals from *F. wheeleri* and *Polyergus* colonies with *F. occulta* slaves and between *F. wheeleri* and *Polyergus* colonies with *F. occulta* and *F. gnava* slaves, respectively (Table 3).

No differences were found in measures of aggression in the inter-slavemaker interactions with the same or different slaves. All inter-slavemaker interactions consisted of immediate aggression and no mutual investigation. Nearly identical patterns of specific aggressive behaviors were displayed, involving little carrying (2/12 dyads), dragging (1/12 dyads), and startle (2/12 dyads), and much gaster flexing (8/12 dyads), rolling (9/12 dyads), seizing (9/12 dyads) and threat (9/12 dyads). Two of the encounters between slavemakers with the same slave species resulted in the death of the Formica wheeleri worker, in both cases within the first 7 minutes of the aggression test. Two of the encounters between slavemakers with different slave species displayed fighting one hour after the aggression test.

Thus, the hypothesis was **not** supported: Inter-slavemaker aggression between Polyergus breviceps and Formica wheeleri was found to be no greater between individuals from colonies with different host species than between individuals from colonies with the same slave species.

Hypothesis IV: A difference in host species will increase intraspecific aggression in Formica wheeleri.

As shown above, Polyergus from colonies with different slave species show more intense intraspecific aggression. Since intraspecific aggression is typically low, and slave odor contributes to the colony odor and therefore to aggression, I hypothesized that I could increase intraspecific aggression in *F. wheeleri* by artificially changing the slave species of a colony. In order to test this hypothesis, a *F. wheeleri* colony with *F. gnava* slaves was created. A year prior to the experiment, a newly mated *F. wheeleri* queen was given *F. gnava* brood and callow workers. The *F. wheeleri* queen produced *F. wheeleri* offspring which were tested the following year for aggression against conspecifics (see Table 4).

Differences in aggression were found between individuals from *F. wheeleri* colonies with the same slaves and between individuals from *F. wheeleri* colonies with different slaves. When *F. wheeleri* individuals from colonies with different slaves interacted, higher levels of aggression were found in less MI ($p<0.01$), shorter LA ($p=0.01$), greater NA ($p<0.05$) and greater AAT ($p<0.01$).

Intraspecific aggression between individuals from colonies with the same slaves showed these low-level aggressive behaviors: startle (6/12 dyads), threat (11/12 dyads), and upright posture (7/12 dyads). Encounters between individuals with different slaves showed the following overt aggressive behaviors: carrying (6/12 dyads), dragging (8/12 dyads), gaster flexing (12/12 dyads), and seizing (12/12 dyads). One hour after the aggression test, 2 dyads were still fighting, and one dyad contained a dead _Formica_.

The hypothesis was therefore supported: a difference in host species will increase intraspecific aggression in _Formica wheeleri_.

Discussion

The following is a brief review of our results:

Hypothesis I: supported. Intraspecific aggression in the slavemaking ant _Polyergus_ was significantly greater between individuals from colonies with different slave species of the genus _Formica_.

Hypothesis II: supported. Interspecific aggression between _Polyergus_ and freeliving _F. occulta_ individuals was low when the _Polyergus_ have _F. occulta_ hosts, and significantly greater when the _Polyergus_ have _F. gnava_ hosts.

Hypothesis IV: supported. Intraspecific aggression among _F. wheeleri_ slavemakers increased when the slave species of one colony was experimentally changed.

Hypothesis III: not supported. Slave species odor did not affect aggression between individuals of different slavemaking species: aggression between _Polyergus_ and _F. wheeleri_ individuals was not reduced when both parasitized the same host species. Although colony odor and nestmate recognition are frequently studied topics, this is the first investigation of the importance of slave odor as a component of nest odor in mixed nests. Our results show that slaves contribute significantly to the colony odor of Polyergus and Formica wheeleri slavemakers. The contribution of slaves to colony odor clearly affects the interactions between slavemakers and colonies of the slave species, and also, apparently, the interactions between conspecific slavemaker colonies. Slave odors do not, apparently, affect the interactions between Polyergus and Formica wheeleri slavemakers. Indeed, it is likely that the colony odors of these two species (genera) are sufficiently different to mask a common slave odor.

These experiments, conducted in a neutral arena, demonstrate the ants' responses in the absence of territorial and other environmental cues. The significant effects of slave odor on aggression in the laboratory suggests the possibility of even greater effects at work in the field, where a substantial body of literature has found aggression to be more pronounced (Le Moli & Parmigiani, 1981, 1982; Le Moli et al., 1982, 1983, 1984; Le Moli & Mori, 1986).

The odor of slaves in a mixed nest may function as a kind of template which Polyergus scouts use to recognize a target colony, and which Polyergus queens use to locate a potential host colony. Goodloe, Sanwald and Topoff (1987) have reported that _Polyergus lucidus_ workers will raid only the _Formica_ slave species found in the nest, despite the availability of other _Formica_ host species, due to the perceptions and actions of the scouts and queens. But slave derived components in the colony odor of slavemakers may also play an important role in modulating the aggression of slavemakers towards colonies of the slave-species during slave-raids. Slavemakers typically do not kill large numbers of host workers during raids (Topoff, Lamon, Goodloe & Goldstein, 1984). It is not clear whether slave-odors play a role in intra- and interspecific encounters between slavemakers. In these territorial battles aggression is extremely high and many ants are killed (Topoff et al., 1984).

It is also unclear what role slave derived odors play in the minority of slavemakers that often have two or more slave species present in the nest simultaneously (e.g., _Formica pergandei_, specimens in Museum of Comparative Zoology, Harvard University). A field study of sympatric slavemaking species with various hosts, should reveal a wealth of information on the complex relationship between "mixed" colony odor and aggression.

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Table 1. Medians and ranges for measures of intraspecific aggressive behavior between *Polyergus* individuals who were homocolonial, heterocolonial with same slave species, and heterocolonial with different slave species.

Trials	Proportion of fighting Pairs	MI (s)	LA (s)	AAT (s)	NA
a) Homocolonial					
Pb x Pb	0/12	35 (11-121)	900	0	0
b) Heterocolonial-same slave species					
Pb (Fo slaves) x Pb (Fo slaves)	0/12	74.5 (6-96)	900	0	0
c) Heterocolonial-different slave species					
Pb (Fo slaves) x Pb (Fg slaves)	11/12	0 (0-7)	1 (1-900)	140 (2-774)	9.5 (1-56)

MI= Mutual Investigation, LA= Latency to Attack, AAT= Accumulated Attacking Time, NA= Number of Attacks, Pb= *Polyergus breviceps*, Fo= *Formica occulta*, Fg= *Formica gnava*.

Kruskall-Wallis Analysis of Variance determined that the distributions of scores for the three groups were not identical ($p<0.01$ for MI, LA, AAT and NA). Mann-Whitney U tests were used to compare two groups at a time.

Comparisons made: a to b, b to c, a to c.

MI values: c differs from a and b, $p<0.01$ (Mann-Whitney 'U' Test).
a does not differ from b.

LA values: c differs from a and b, $p<0.01$ (Mann-Whitney 'U' Test).
a does not differ from b.

AAT values: c differs from a and b, $p<0.01$ (Mann-Whitney 'U' Test).
a does not differ from b.

NA values: c differs from a and b, $p<0.01$ (Mann-Whitney 'U' Test).
a does not differ from b.

Table 2. Medians and ranges for measures of aggressive behavior between homocolonial and heterocolonial *Polyergus* and *F. occulta*.

Trials	Proportion of fighting Pairs	MI (s)	LA (s)	AAT (s)	NA
a) Homocolonial Pb x Pb	0/12	35 (11-121)	900	0	0
b) Homocolonial Pb (Fo slaves) x Fo (slaves)	0/12	78.5 (50-119)	900	0	0
c) Heterocolonial Pb (Fo slaves) x Fo (freeliving)	8/12	65.5 (0-193)	1 (1-900)	6.5 (0-883)	1 (0-3)
d) Heterocolonial Pb (Fg slaves) x Fo (freeliving)	12/12	0 (0-16)	1 (1-65)	489.5 (11-839)	2.5 (1-42)

MI= Mutual Investigation, LA= Latency to Attack, AAT= Accumulated Attacking Time, NA= Number of Attacks, Pb= *Polyergus breviceps*, Fo= *Formica occulta*, Fg= *Formica gnava*.

Kruskall-Wallis Analysis of Variance determined that the distributions of scores for the four groups were not identical ($p<0.01$ for MI, LA, AAT and NA). Mann-Whitney U tests were used to compare two groups at a time.

Comparisons made: a to b, b to c, c to d.

MI values: a differs from b, $p<0.01$; c differs from d, $p<0.01$ (Mann-Whitney 'U' test).

LA values: b differs from c, $p<0.01$ (Mann-Whitney 'U' test).

AAT values: c differs from b, $p<0.01$, and d, $p<0.05$ (Mann-Whitney 'U' test).

NA values: c differs from b and d, $p<0.01$ (Mann-Whitney 'U' test).

Table 3. Medians and ranges for measures of aggressive behavior between Polyergus and F. wheeleri slavemakers, with same slaves and different slaves.

Trials	Proportion of fighting Pairs	MI (s)	LA (s)	AAT (s)	NA
a) Pb (Fo slaves) x Fw (Fo slaves)	12/12	0	1 (1-64)	55.5 (2-774)	9.5 (1-56)
b) Pb (Fg slaves) x Fw (Fo slaves)	12/12	0	1 (1-290)	20 (3-698)	6.5 (1-24)

MI= Mutual Investigation, LA= Latency to Attack, AAT= Accumulated Attacking Time, NA= Number of Attacks, Pb= Polyergus breviceps, Fw= Formica wheeleri, Fo= Formica occulta.

a and b are not statistically different in all measures.

Table 4. Medians and ranges for measures of aggressive behavior between *F. wheeleri* individuals from colonies with different slaves.

Trials	Proportion of fighting Pairs	MI (s)	LA (s)	AAT (s)	NA
a) Fw (Fo slaves) x Fw (Fo slaves)	7/12	69.5 (43-115)	118 (1-900)	1 (0-714)	1 (0-7)
b) Fw (Fg slaves*) x Fw (Fo slaves)	12/12	0 (0-128)	1 (1-292)	263 (7-896)	4 (1-22)

MI= Mutual Investigation, LA= Latency to Attack, AAT= Accumulated Attacking Time, NA= Number of Attacks, Fw= *Formica wheeleri*, Fo= *Formica occulta*, Fg= *Formica gnava*.

*= Artificially constructed colony - slave species switched in lab.

MI values: a and b differ, $p<0.01$ (Mann-Whitney 'U' test).

LA values: a and b differ, $p=0.01$ (Mann-Whitney 'U' test).

AAT values: a and b differ, $p<0.01$ (Mann-Whitney 'U' test).

NA values: a and b differ, $p<0.05$ (Mann-Whitney 'U' test).

PART II: THE ROLE OF AN ATTRACTIVE BROOD PHEROMONE IN THE OBLIGATORY SLAVEMAKING ANT, *POLYERGUS BREVICEPS*

Ellen J. Zimmerli

and

Alessandra Mori

Department of Psychology, Hunter College of CUNY
695 Park Avenue, New York, NY 10021

Instituto di Zoologia, Università di Parma
Campus di Via Langhirano, Viale delle Scienze, 43100 Parma,
Italy

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Abstract

Freeliving workers of *Formica occulta*, an ant species enslaved by the obligatory slavemaking ant *Polyergus breviceps*, retrieve and nurse *Polyergus* pupae just as well as conspecific pupae in a choice test. No such attraction was found toward pupae of the facultative slavemaker *Formica wheeleri*, which also enslaves *F. occulta*. *Formica neogagates*, a sympatric species which is not parasitized by either slavemaker, preferentially retrieves and tends conspecific brood over that of *Polyergus* and *F. wheeleri*. It is proposed that brood of obligatory slavemaking species must possess an attractive pheromone for slavemaker colony foundation to be successful, since slavemaker brood must be nursed by adult slave workers with no prior exposure to slavemaker brood. An attractive pheromone is not necessary in the brood of facultative slavemakers, since this brood is cared for by newly-eclosed slave workers who imprint on the slavemaker brood.

Introduction

Slavemaking ants conduct group raids on colonies of another species, driving away the adults and stealing the brood. The stolen booty is brought back to the slavemaker's nest and raised to perform the work of the colony (Wilson, 1975). Slavemaking species can be either facultative or obligatory. Obligatory slavemaking species are specialized and their worker castes have lost the ability to perform all colony functions beyond slave raiding. In contrast, workers of facultative slavemaking species have retained the ability to perform colony work and do so alongside their slaves (Topoff & Zimmerli, 1990). Colony founding also differs between the two types of slavemakers (Topoff, Weikert & Zimmerli, 1990; Topoff & Zimmerli, in press). A facultative slavemaking queen drives the resident queen and adult workers from a colony of the slave species and sits on the brood. The workers which eclose from the remaining slave species brood imprint on the slavemaking queen and care for her first offspring, which are then capable of assisting in the rearing of successive broods. Obligatory slavemaking queens however, do not drive away the adult workers of the slave species, but must obtain the care of these workers for herself, and for her brood. The first and subsequent slavemaker broods are tended by the queen's new slaves.

The role of early experience in pupa acceptance and nursing in various species of Formica is well documented.

Jaisson (1972, 1975) and Le Moli and Passetti (1977, 1978) elucidated a "sensitive period" in the early life of Formica workers during which discrimination in pupae nursing behavior is determined. These researchers found that exposure to pupae during the first week or two after eclosion determines pupae acceptance and nursing in F. polyctena and F. rufa. This "early learning" or olfactory imprinting hypothesis has been proposed as an explanation for the nursing of slavemaker brood by Formica slaves, who are exposed to the odors of their captors when they themselves eclose in the mixed nest (Jaisson, 1975, Le Moli and Passetti, 1978, Le Moli, 1980; Le Moli and Mori, 1987a, b). This hypothesis does not, however, explain how a colony of an obligatory slavemaking species can be founded, since nursing of the slavemaker's brood in this context must be by mature adult slave species workers with no prior experience with this brood.

The experiment reported in this paper tested the effect of being enslaved on brood retrieval and tending. Adult freeliving and enslaved Formica occulta workers were presented with pupae of their own species and pupae of the slavemakers Polyergus breviceps (obligatory) and Formica wheeleri (facultative). The brood-tending response of Formica neogagates, a species which is sympatric, but not parasitized, was also measured.

Materials and Methods

Seven experimental conditions were tested, with 6 replicates per condition, each replicate consisting of an artificial colony with approximately 35 adult workers and no brood. While this colony size is decidedly smaller than that of natural colonies, we found that the workers responded to the pupae placed in the arena shortly after their presentation. The colonies were kept in 18 x 12.5 x 6 cm plastic boxes with the rims lined with fluon to prevent the escape of the subjects. The bottoms of the boxes were covered with a thin layer of dirt, and inverted Petri dish "nests" (5 cm diameter) were provided, and covered with dark cloth which could be removed to observe nest activities. Colonies were fed honey water and insects, and the soil around the nests was moistened as necessary. Colonies were set up 12 hours prior to the experiment. Fresh pupae were removed from their colony of origin immediately before their use in the choice test, and placed in a evenly distributed manner on the soil in the arena. All conspecific pupae used were taken from the colony of origin of the workers.

Each of seven colony groups consisted of a different experimental condition (Table 1). Groups 1, 2, 3 and 4 were used to compare the reactions of freeliving and enslaved F. occulta workers to conspecific pupae and pupae of obligatory and facultative slavemaking species. In Group 1, six colonies of freeliving F. occulta workers were each presented with five conspecific pupae, and five Polyergus (obligatory slavemaker) pupae. In Group 2, six colonies of Polyergus-enslaved F. occulta were each presented with five Polyergus pupae and five F. occulta pupae from a freeliving colony. If prior exposure to brood (olfactory imprinting) is necessary for brood tending behavior, we would expect only the Polyergus-enslaved F. occulta workers to tend Polyergus brood, and the freeliving F. occulta workers would either ignore, consume, or destroy the Polyergus pupae.

In Group 3, six colonies of freeliving F. occulta were each presented with five F. wheeleri (facultative slavemaker) pupae and five F. occulta pupae from the worker's colony of origin. In Group 4, six colonies of F. wheeleri-enslaved F. occulta were each presented with five F. wheeleri pupae and five F. occulta pupae from a pure colony. Once again, if prior olfactory exposure affects brood tending behavior, we would expect F. wheeleri-enslaved F. occulta to tend F. wheeleri pupae, and freeliving F. occulta not to tend F. wheeleri pupae.

Group 5 was used to observe the reaction of freeliving F. occulta workers to the simultaneous presentation of pupae of both slavemaking species. In Group 5, six colonies of freeliving F. occulta were each presented with five Polyergus pupae and five F. wheeleri pupae. It was expected that neither type of slavemaker brood would be tended, since both would be foreign to the freeliving F. occulta.

Groups 6 and 7 tested the reactions of F. neogagates, a species which is not enslaved, towards conspecific and slavemaker pupae. In Group 6, six colonies of F. neogagates adults were each presented with five conspecific pupae and five Polyergus pupae. In Group 7, six colonies of F. neogagates workers were each presented with five conspecific pupae and five F. wheeleri pupae. Since F. neogagates is not a species known to be enslaved, it was expected that workers of this species would not tend slavemaker pupae.

On each day of the experiment, for each colony, we counted the number of pupae of each type which survived (were not destroyed) and the number of pupae which were retrieved (in the nest). To assess the worker's reaction to the pupae, the ratio of the survived pupae (or potentially retrievable pupae) which were retrieved into the nest was plotted over the 10 days (Fig. 1). This measure was chosen over survival or retrieval data alone, because survival data did not indicate whether the pupae were retrieved or left in the arena, and retrieval data did not indicate whether declines over time were due to removal of pupae from the nest or natural mortality of the pupae. A Wilcoxon signed-ranks test was used to compare mean retrieval ratios of the two types of pupae in each group.

Results

Retrieval ratio curves for the 7 groups are presented in Figs. 1 through 7.

In Group 1, freeliving F. occulta retrieved all surviving conspecific and Polyergus pupae (Fig. 1). There was no difference between the retrieval ratios of the two types of pupae (Wilcoxon test, N=0, T=0, NS).

The data were identical in Group 2: F. occulta workers which were enslaved by Polyergus retrieved all surviving Polyergus and conspecific pupae (Fig. 2). Once again, there was no difference in the retrieval ratios of conspecific and Polyergus pupae (Wilcoxon test, N=0, T=0, NS).

In Group 3, freeliving F. occulta workers preferentially retrieved conspecific pupae over F. wheeleri pupae (Fig. 3). The retrieval ratio curve shows complete retrieval of all surviving conspecific pupae, but incomplete retrieval of F. wheeleri pupae (Wilcoxon test, N=10, T=0, $p<0.005$).

In Group 4, F. occulta workers that were enslaved by F. wheeleri retrieved all surviving conspecific pupae and nearly all slavemaker pupae (Fig. 4). A Wilcoxon test on the retrieval ratios, however, found the very small but consistent differences to be statistically significant (N=8, T=0, $p<0.005$).

In Group 5, freeliving F. occulta presented with the brood of both slavemakers preferentially retrieved Polyergus pupae over F. wheeleri pupae (Wilcoxon test, N=7, T=0, p<0.01). The retrieval ratio curve (Fig. 5), however, shows an interesting temporal pattern: similar retrieval of both types of pupae on days 1 through 5, and a clear preference for Polyergus pupae from days 6 through 10. In spite of the appearance of the curve, a Wilcoxon comparison for data pooled over the first five days did find a significant difference in retrieval, with Polyergus preferred over F. wheeleri (T=0, p<0.025). The appearance of the retrieval ratio curve is difficult to interpret. This was the only group given a choice test between two unfamiliar pupae types, and it is possible that in the absence of conspecific pupae, retrieval is abnormal, with exposure necessary before retrieval. It is also possible that the pupae, which were maturing through the course of the experiment, developed or produced some discernable chemical cues after the fifth day. But despite the abnormal appearance of the retrieval ratio curve, Polyergus pupae were consistently preferred over F. wheeleri pupae.

In Group 6, the retrieval ratio curves show that F. neogagates workers retrieved conspecific pupae over Polyergus pupae (Fig. 6, Wilcoxon test, N=10, T=0, p<0.005).

In Group 7, workers of F. neogagates preferentially retrieved conspecific pupae over F. wheeleri pupae (Fig. 7, Wilcoxon test, N=10, T=0, p<0.005).

Discussion

Many ant species do not have innate recognition of their brood, but instead learn to care for the brood present in the nest upon eclosion. This early learning of brood labels has facilitated the evolution of slavery in ants (Hölldobler and Wilson, 1990). Le Moli (1980) has argued that only species in which brood recognition is based on learning (and not on innate recognition of conspecifics) are vulnerable to enslavement. Conversely, species with a bias in favor of conspecific brood, such as species of the formicine genus _Camponotus_, are generally not parasitized (Carlin _et_ _al_., 1987; Hölldobler and Wilson, 1990). The results of this experiment indicate that _F_. _neogagates_ has a bias in favor of caring for conspecifics, and is thus perhaps protected from parasitism.

Olfactory imprinting in a mixed nest appears to promote the nursing of _F_. _wheeleri_ pupae by _F_. _occulta_ slaves. Enslaved _Formica_ retrieve and nurse slavemaker brood. It is interesting that despite exposure to the facultative slavemaker, _F_. _occulta_ slaves retrieve conspecific pupae better than _F_. _wheeleri_ slavemaker pupae. Just as interesting is the fact that freeliving _F_. _occulta_ workers are attracted to and nurse _Polyergus_ brood, with no prior exposure. This argues that _Polyergus_ brood is naturally attractive to the slave species. It is unlikely that the _Polyergus_ pupae were retrieved through alimentary motivation (for the purposes of consumption) since very few Polyergus pupae were consumed, fewer in fact than the number of consumed F. occulta pupae. Rather, freeliving F. occulta nurse and retrieve Polyergus pupae as well as they do conspecific pupae. It is also not likely that Polyergus pupae were attractive simply because they are large; F. wheeleri pupae are larger than Polyergus pupae. While freeliving F. occulta did not destroy F. wheeleri pupae, these pupae were not retrieved, and thus not nursed. When presented with both types of slavemaker pupae, freeliving F. occulta retrieved and nursed Polyergus pupae, and consumed F. wheeleri pupae. Clearly Polyergus pupae are more attractive than F. wheeleri pupae.

Brood nursing behavior is elicited by chemical stimuli in F. rufa and F. cunicularia (Le Moli and Passetti, 1978; Mori and Le Moli, 1988). Workers of these species accepted and nursed foreign cocoons which were impregnated with pheromones derived from conspecific cocoons. Chemical analyses of gaster contents of the slavemakers F. sanguinea, P. rufescens, and their slaves F. fusca and F. rubifarbis have revealed odor similarities (Bergström and Löfqvist, 1968). It is therefore likely that the attraction to slavemaker brood is also based on its olfactory characteristics. The chemical attractant of the Polyergus brood may be attractive because it is **similar** to that of the F. occulta brood, or, it may be attractive in an entirely different way (which may be why Polyergus brood was not destroyed by F. neogagates). Chemical analyses and bioassays would be required to answer this question.

Chemical similarity has been shown to play a role in the first step of colony-founding by obligatory slavemaking queens: acceptance by host workers. Acceptance of Polyergus breviceps queens by host workers requires prolonged aggressive contact between the Polyergus queen and the resident Formica queen, most likely resulting in the transfer of pheromones between queens (Topoff et al., 1988). Although Dufour's gland secretions of Polyergus queens reduced aggression by Formica workers, successful colony takeover was significantly reduced in queenless colonies where a transfer of pheromones between queens was not possible.

Once the obligatory slavemaking queen has acquired the olfactory characteristics of the slave species' queen, and has been accepted by the adult slave workers, these workers must then care for her brood. There was likely strong selective pressure favoring pre-obligatory slavemakers with an attractive brood pheromone, for this would be the only way to ensure that the adult workers of the slave species would respond to the slavemaker brood, with no prior exposure required. In fact, this pheromonal brood attractant and the capacity to gain acceptance by adult slave workers may be the critical evolutionary steps separating obligatory from facultative slavemakers.

F. *wheeleri* queens do not require a pheromonal similarity to the slave species queen for successful colony founding. When introduced to laboratory host colonies, *F*. *wheeleri* queens sat on the host brood pile while host workers and resident queens abandoned the nests (Topoff *et al*., 1990). Likewise, the brood of the facultative slavemaker *F*. *wheeleri* does not exhibit a natural attractiveness (see fig. 1, Group 5). In similar research conducted on individuals from freeliving *F*. *cunicularia* colonies, eclosed in various social environments, *F*. *cunicularia* does not have a natural tendency to care for its frequent parasite, the facultative slavemaker *Formica sanguinea* (Le Moli and Mori, 1987). A brood attractant is not necessary for the success of a facultative slavemaking colony because the brood of the *F*. *wheeleri* queen is raised by newly eclosed slaves which will imprint on the slavemaker brood.

Acknowledgements

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Figure Legends

Fig. 1. Mean pupae retrieval ratios for group 1. Pb= P. breviceps, Fo= F. occulta. p. 58.

Fig. 2. Mean pupae retrieval ratios for group 2. Pb= P. breviceps, Fo= F. occulta. p. 59.

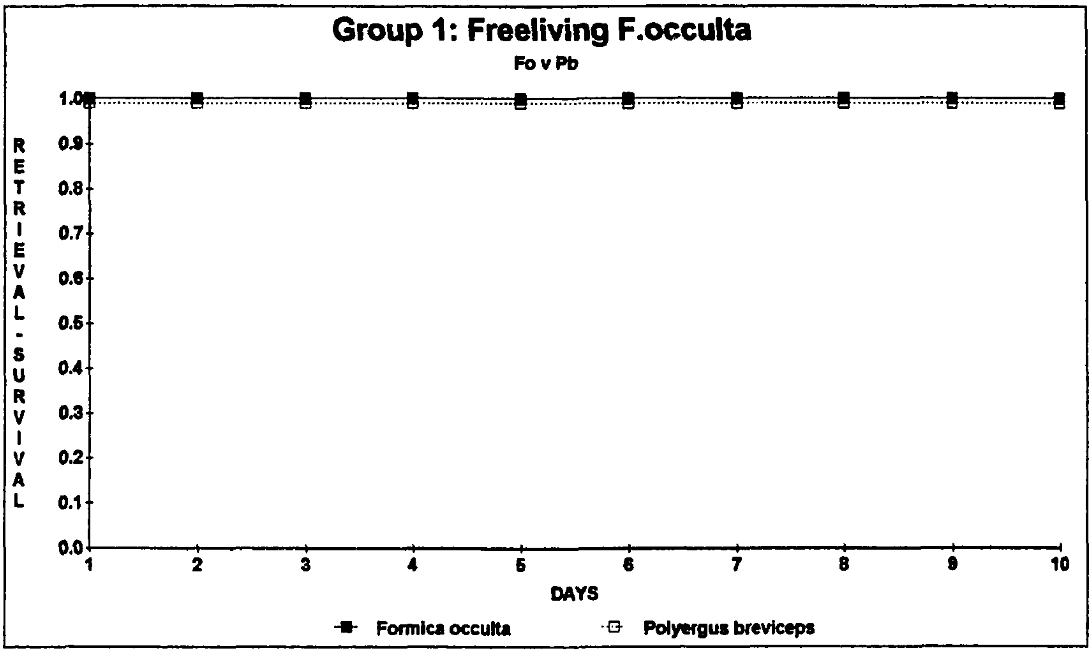
Fig. 3. Mean pupae retrieval ratios for group 3. Fo= F. occulta, Fw= F. wheeleri. p. 60

Fig. 4. Mean pupae retrieval ratios for group 4. Fo= F. occulta, Fw= F. wheeleri. p. 61

Fig. 5. Mean pupae retrieval ratios for group 5. Pb= P. breviceps, Fw= F. wheeleri. p. 62

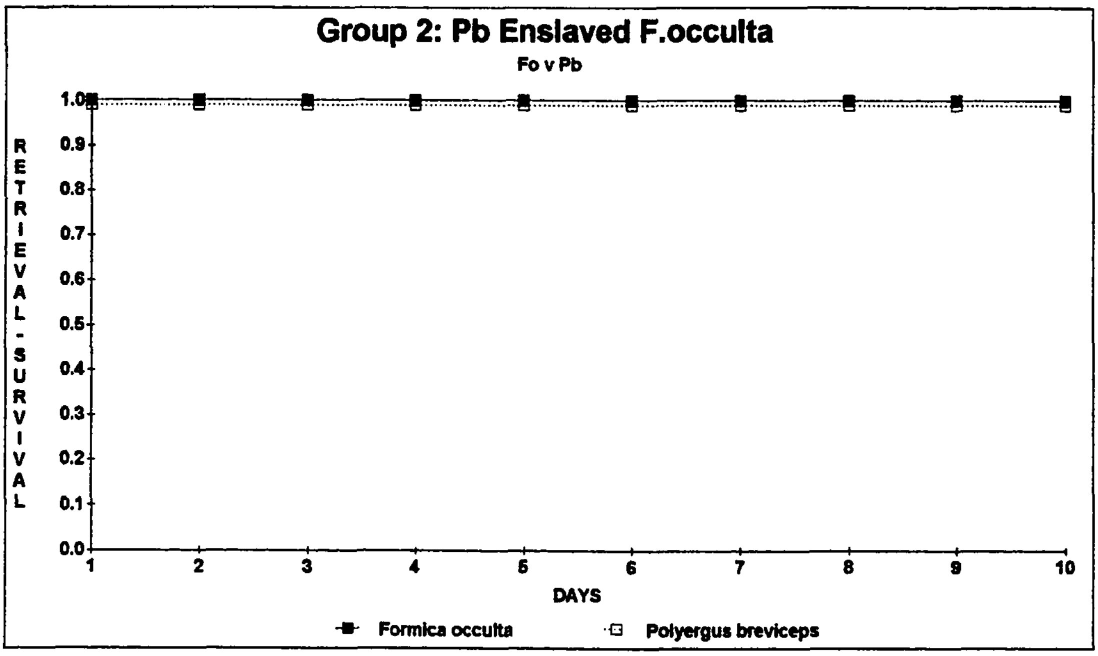
Fig. 6. Mean pupae retrieval ratios for group 6. Pb= P. breviceps, Fn= F. neogagates. p. 63

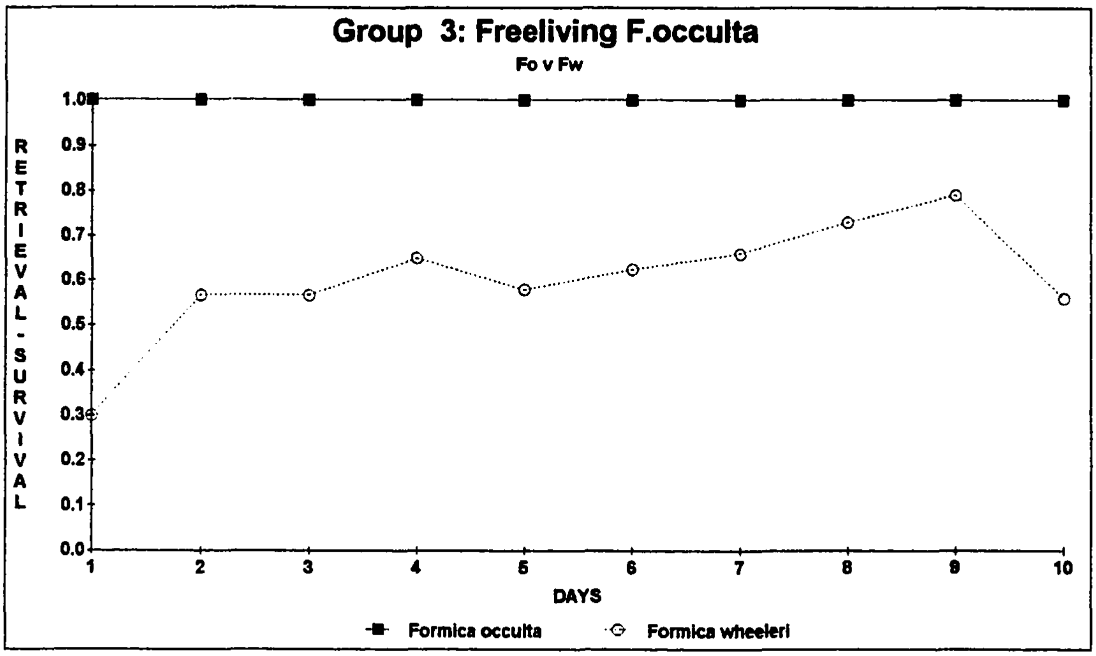
Fig. 7. Mean pupae retrieval ratios for group 7. Fw= F. wheeleri, Fn= F. neogagates. p. 64

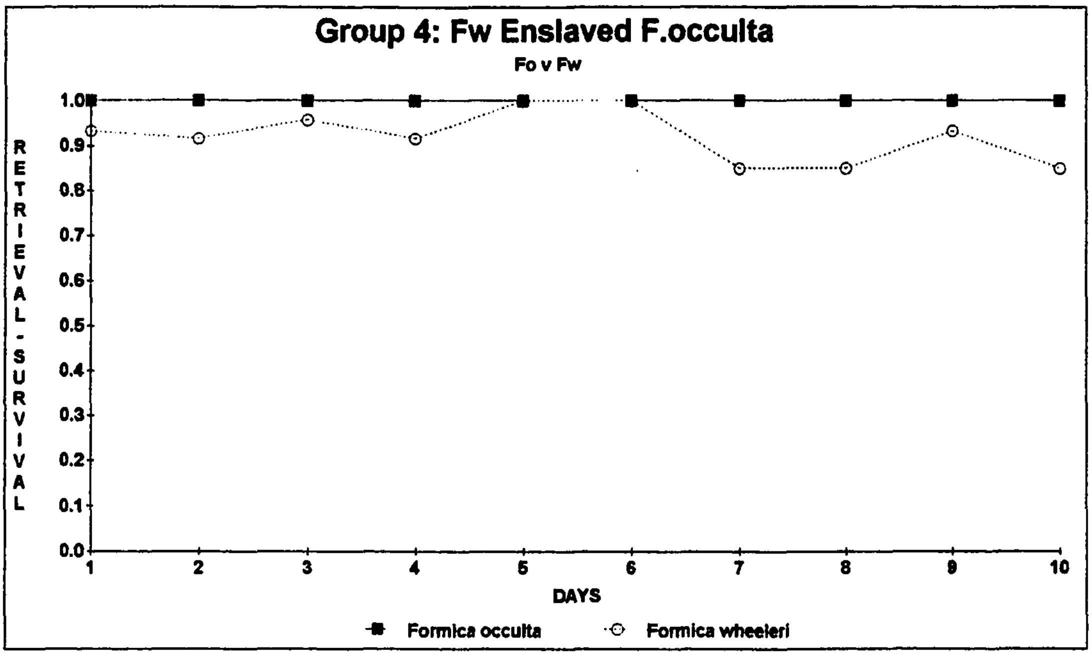


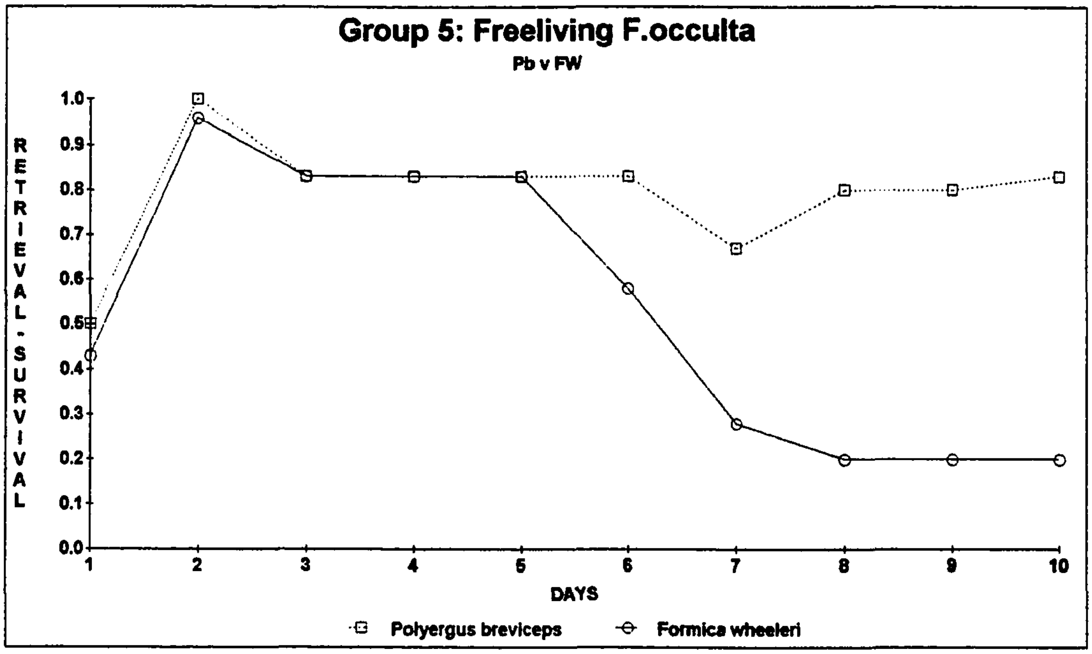
Group 1: Freeliving F.occulta

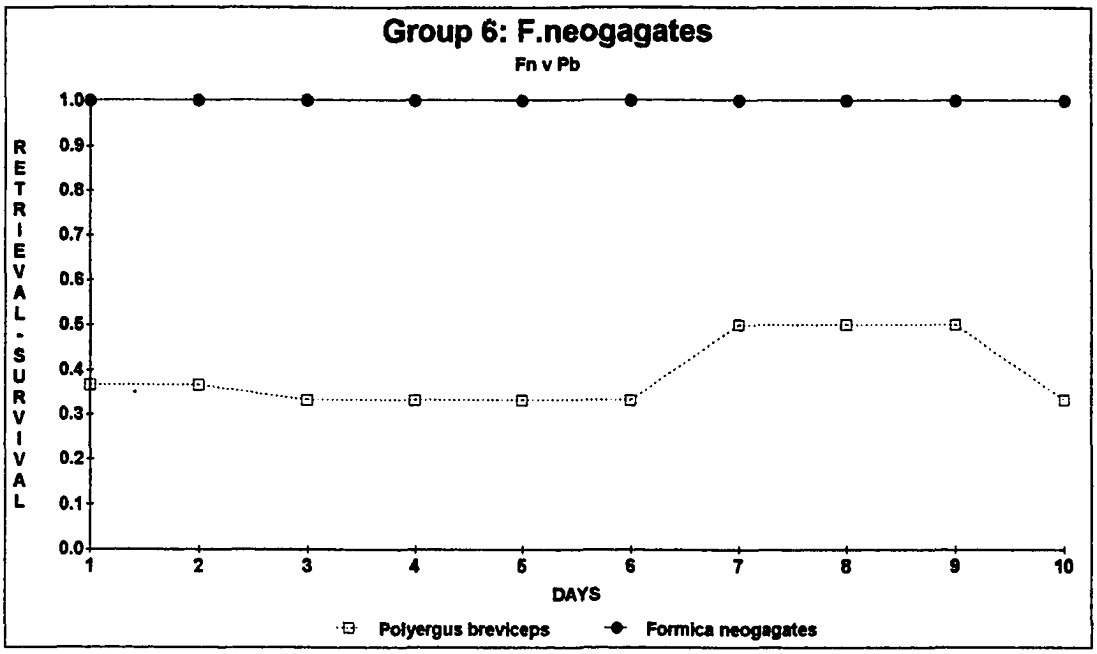
Fo v Pb


Group 2: Pb Enslaved F.occulta
Fo v Pb
RETRIEVAL-SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Formica occulta
Polyergus breviceps


Group 3: Freeliving F.occulta
Fo v Fw
RETRIEVAL - SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Formica occulta
Formica wheeleri


Group 4: Fw Enslaved F.occulta
Fo v Fw
RETRIEVAL - SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Formica occulta
Formica wheeleri


Group 5: Freeliving F.occulta
Pb v FW
RETRIEVAL, SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Polyergus breviceps
Formica wheeleri


Group 6: F.neogagates
Fn v Pb
RETRIEVAL - SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Polyergus breviceps
Formica neogagates

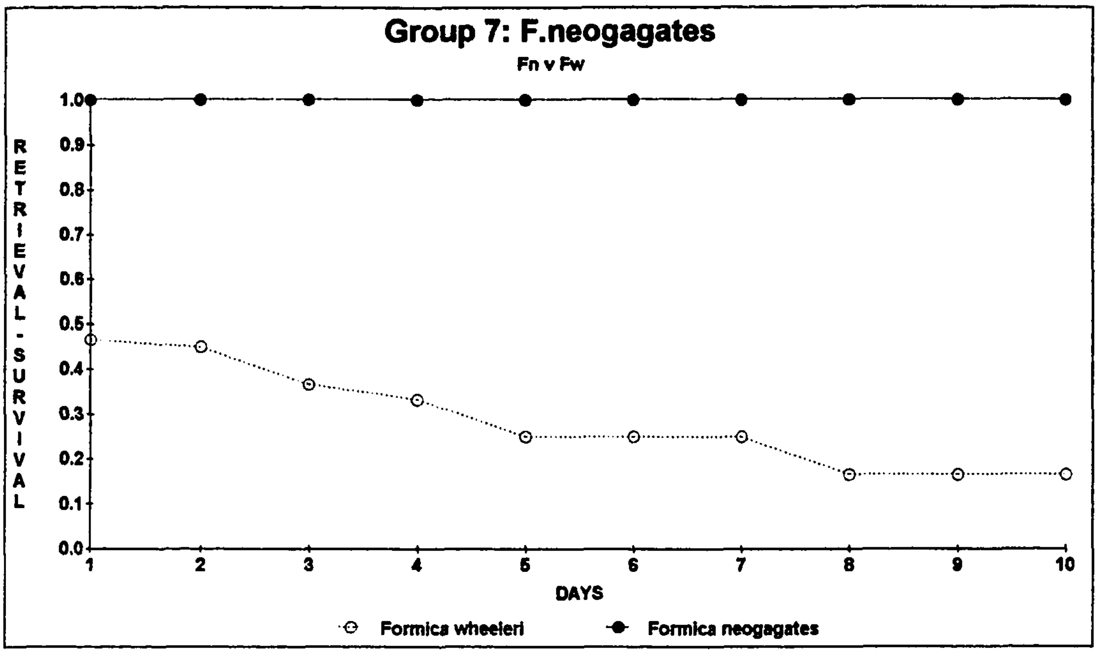

Group 7: F.neogagates
Fn v Fw
RETRIEVAL - SURVIVAL
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
1
2
3
4
5
6
7
8
9
10
DAYS
Formica wheeleri
Formica neogagates

Table I. Experimental groups used in the choice test.

GROUP	WORKERS	PUPAE
1	Freeliving *Formica occulta*	5 *Polyergus* pupae 5 *F. occulta* pupae
2	*Polyergus* enslaved *Formica occulta*	5 *Polyergus* pupae 5 *F. occulta* pupae
3	Freeliving *Formica occulta*	5 *F. wheeleri* pupae 5 *F. occulta* pupae
4	*F. wheeleri* enslaved *Formica occulta*	5 *F. wheeleri* pupae 5 *F. occulta* pupae
5	Freeliving *Formica occulta*	5 *F. wheeleri* pupae 5 *Polyergus* pupae
6	*Formica neogagates*	5 *F. neogagates* pupae 5 *Polyergus* pupae
7	*Formica neogagates*	5 *F. neogagates* pupae 5 *F. wheeleri* pupae

PART III: COLONY TAKEOVER BY A SOCIALLY PARASITIC ANT, POLYERGUS BREVICEPS: THE ROLE OF CHEMICALS OBTAINED DURING HOST QUEEN KILLING.

ELLEN ZIMMERLI

Department of Psychology
Hunter College of CUNY
New York, N.Y. 10021, U.S.A.

* A version of this manuscript, by Topoff and Zimmerli, has been accepted for publication by *Animal Behaviour*. Added here are experiments 6 and 7.

Abstract

Queens of the socially-parasitic ant Polyergus breviceps are incapable of rearing their own brood, and therefore require the assistance of host Formica workers soon after egg laying. Accordingly, a newly-mated Polyergus queen must penetrate a nest of Formica, kill the host queen, and become permanently accepted by the slave species' workers. Laboratory tests show that a Polyergus queen will similarly attack and bite a dead (and therefore motionless) Formica queen. Immediately after attacking the dead host queen, the Polyergus queen will be accepted by workers from any colony of Formica belonging to the same species of the dead queen (but will be attacked by workers from other Formica species). Preliminary results also indicated that any adoption-facilitating chemicals obtained by the Polyergus queen are still effective one week after killing the host Formica queen. When Polyergus queens raised in colonies containing F. gnava were introduced into nests of F. occulta, most showed little interest in attacking the resident Formica queen. In four of 10 tests, however, the Polyergus queen killed the foreign queen and was accepted by the F. occulta workers. Polyergus queens showed no interest in attacking newly mated F. occulta queens. However, Polyergus queens were successful in becoming adopted by several Formica colonies, killing the resident queen each time. Chemical transfer of foreign-queen odours may have

played a key role in the evolution of social parasitism in *Polyergus*.

Introduction

Social parasitism, in which the young of one species are raised by another, has evolved independently within the insect order Hymenoptera, and in several families of fishes (McKaye 1981; Baba et al. 1990) and birds (Friedmann 1960; Payne 1977). Despite obvious differences in morphology and the complexity of social organization, brood parasites have to solve at least two common problems: (1) the parasitic female has to infiltrate the host's nest and lay eggs; and (2) the parasitic young must establish interspecific social bonds with the hosts, and thus be reared by host individuals. Among ants, the genus Polyergus is an obligatory parasite, requiring workers of the related genus Formica for obtaining food, brood rearing, and colony maintenance. This dependence also holds for Polyergus queens, who are not capable of starting colonies on their own after mating. Accordingly, a newly-mated Polyergus queen attempts to penetrate a colony of Formica, kill the host queen, and become accepted by the slave species' workers. If successful, these resident workers rear the offspring of the parasitic queen until the Polyergus worker population is sufficiently large to supplement the slave force. This is accomplished by slave raids, in which Polyergus workers penetrate Formica nests, disperse the queen and workers, and capture the resident's pupal brood (Talbot 1967; Topoff et al. 1989). Although some raided brood is consumed, a part of it is reared through eclosion, and the emerging Formica workers thereafter assume total maintenance of the mixed-species nest.

In a recent study on the parasitic myrmicine ant Leptothorax kutteri, Allies et al. (1986) discovered a 'propaganda' pheromone used during colony usurpation of its host, L. acervorum. Originating from the queen's Dufour's gland, this exocrine secretion masked nestmate recognition and caused host workers to attack each other. Our laboratory study of P. breviceps (Topoff et al. 1988) showed that Polyergus queens also use a pheromone from their enlarged Dufour's glands to reduce aggression by workers prior to attacking the Formica queen. Immediately after the resident queen's death, however, Formica workers began constant grooming of the Polyergus queen. During host-queen killing, Polyergus queens spent a median of 26 min (range = 11-34 min, N = 13) biting the resident queen. Observations with a dissecting microscope disclosed that between bouts of biting, the attacking queen's mandibles were widely separated, as she continually touched her extruded hypopharynx to the dead queen. Perhaps the most significant result of our colony-adoption tests was that the success rate for colony takeover by P. breviceps queens dropped from 79% to 12% when no Formica queen was present. In the queenless condition, aggression by Formica workers towards the Polyergus queen typically resulted in her death. This suggests that during the act of biting the *Formica* queen, the parasitic *Polyergus* queen acquires one or more *Formica*-queen chemicals. In this manuscript, we report the results of laboratory tests that strengthen this hypothesis. First, we show that a *Polyergus* queen will just as vigorously attack and bite a dead (and therefore motionless) *Formica* queen. Second, we report that immediately after such an attack, the *Polyergus* queen will be accepted by workers from any conspecific colony of *Formica*. Thirdly, we demonstrate that a *Polyergus* queen will be immediately accepted by *Formica*, even after one week of isolation following the killing of the *Formica* queen. Fourth, we show that *Polyergus* queens do not attack and kill newly mated *Formica* queens. And finally, we report that not only is a *Polyergus* queen capable of multiple takeover success (in up to four different *Formica* colonies), but one *Polyergus* queen sequentially killed nine of ten *Formica* queens in a polygynous colony, over a period of weeks, while living peacefully among the living queens.

General Methods

We conducted this study during June and July, 1991, at the Southwestern Research station of the American Museum of Natural History, located 5 km west of Portal, Arizona. We collected colonies of *F. gnava* (with queens) from the Arizona oak-alligator juniper woodland surrounding the field station (elevation = 1646 m.), and colonies of F. occulta from a Ponderosa pine-Douglas fir habitat (elevation = 2510 m). Although these Formica species are allopatric, both are raided by P. breviceps, whose range extends across both habitats. We maintained colonies in the laboratory in plastic boxes (17 x 12 x 6 cm) filled with original nest soil. Newly-mated P. breviceps queens were collected from both habitats and kept until testing in individual plastic vials with moist cotton batting. The mated status of queens was affirmed at the end of the tests, by examining spermathecal contents in six randomly selected queens.

One day prior to each test, we placed groups of 15 Formica workers and 15 pupae in plastic petri dish nests (10-cm diam) containing two entrances 180° apart. The nests were placed in larger, plastic-box arenas (45 x 30 x 15 cm), with Fluon coated walls to prevent escape. At the start of each test, all Formica workers were motionless inside the nest, surrounding the pupal brood. We then introduced a Polyergus queen into the outside arena. Our criterion for successful takeover (when appropriate) was met when: (1) the queen was being groomed by colony workers; and (2) the Polyergus queen was sitting on or near the brood pile.

Test Series 1: Behaviour of *Polyergus* Queens Toward Dead *Formica* Queens, And Success Of Subsequent Takeovers

If *Polyergus* queens acquire adoption-facilitating chemicals during host-queen killing, we hypothesize that *Polyergus* queens will attack and bite *Formica* queens, even if they are already dead and motionless.

Methods

Immediately prior to testing, we froze a *F. gnava* queen for five min, thawed her for an additional five min, and placed her in a nest containing 15 workers and 15 pupae. A single *Polyergus* queen was then introduced into the arena surrounding the nest chamber. For each of five replications, we recorded the number of attacks (discrete bites) the *Polyergus* queen made on the *F. gnava* queen, the accumulated attacking time, and time until the takeover criterion was met. After each takeover trial, we removed and isolated the *Polyergus* queen.

After 24 h of isolation, we introduced each of the *Polyergus* queens to another queenless nest of 15 workers and 15 pupae from the same colony. Our objective here was to demonstrate that acceptance of a *Polyergus* queen by *Formica* is not limited to workers present when their queen was killed. We recorded the number of attacks by *Formica* workers on the *Polyergus* queen, and the time to successful takeover.

Results

Each of the five *Polyergus* queens successfully took over *F. gnava* colonies containing dead queens, with a median time to takeover of 140 min (Table I). The median accumulated attack time by the *Polyergus* queen was 23 min, which was not significantly different from the attack time (median = 26 min) recorded for our previous study using living queens of *Formica*. In each instance the *Polyergus* queen attacked the dead *F. gnava* queen repeatedly, directing bites over the *Formica* queen's head, thorax, legs, petiole and gaster. After biting and piercing the dead queen, each invading *Polyergus* queen used her hypopharynx to lick all bitten surfaces, followed by long bouts of grooming. The entrance of the *P. breviceps* queen caused some of the *F. gnava* workers to leave the nest and scatter in the adjoining arena. Those that stayed in the nest aggressively attacked the intruding *Polyergus* queen, often with prolonged bites to her limbs. After the *Polyergus* queen pierced the *Formica* queen, worker behaviour changed abruptly from attack to antennation and grooming.

Each of the five *Polyergus* queens was promptly accepted by the new population of workers from the colony taken over by the *Polyergus* queens; they immediately inspected and groomed the queens. Although the introduced *Polyergus* queens were always accepted by Formica workers, the large range in time to takeover (2 - 75 min) was due primarily to the variation in the time for the queen to locate and straddle the pupal brood.

Test Series 2: Acceptance Of Formica Queens By Foreign, Conspecific Workers

Many ant species are monogynous, and have kin recognition systems that are possibly developmentally unmodifiable, two characteristics which make them resistant to being parasitised (Hölldobler & Michener 1980; Buschinger 1986; Pollock & Rissing 1989). The kin recognition system of many Formica species, by contrast, is based on an olfactory imprinting process (Le Moli 1980), and mature colonies of F. gnava may contain more than 30 queens. If polygyny can be enhanced by established colonies accepting additional newly-mated queens from foreign nests, such unrestricted acceptance could easily extend to closely related, parasitic queens bearing an olfactory similarity. This study assayed the acceptance by workers of F. gnava and F. occulta of foreign, conspecific queens.

Methods

In each test, we introduced a single F. gnava or F. occulta queen to 15 adult workers from a foreign, conspecific colony. We recorded the first 10 interactions between the queen and the workers as aggressive (a bite, with or without gaster flexing) or non-aggressive (antennation, grooming). We conducted five replications for each of the following three conditions (1) F. gnava queens from established nests introduced to heterocolonial (from another colony) F. gnava workers; (2) F. occulta queens from established nests introduced to heterocolonial F. occulta workers; (3) newly-mated F. occulta queens introduced to F. occulta workers from an established colony.

Results

All F. gnava and F. occulta queens taken from established, polygynous nests (conditions 1 & 2) were readily accepted by conspecific colonies. In every instance, 10/10 interactions with workers were distinctly non-aggressive, involving only antennation, and grooming. In contrast, newly-mated F. occulta queens (condition 3) were not readily accepted by conspecific workers. In four of the five replications, 10 of 10 queen-worker interactions were aggressive, and in the fifth replication six of 10 interactions were aggressive.

Test Series 3: Adoption Of Polyergus Queens By Workers From Foreign Colonies

Test series 1 verified that killing a Formica queen enables a Polyergus queen to be immediately adopted by host workers. Test series 2 demonstrated that workers of F. gnava will readily accept mated queens from foreign colonies belonging to the same species. These results lead to the prediction, tested in this series, that a Polyergus queen who attacks and pierces even a dead Formica queen would also be accepted into any colony of Formica, as long as it is conspecific with the dead queen. A second hypothesis tested here was that the acceptance of Polyergus queens would not generalize to other species of Formica.

Methods

As in the previous test series, we allowed each of five Polyergus queens to attack and pierce a queen of F. gnava from an established nest. The Polyergus queens were then introduced to heterocolonial F. gnava nests (i.e. nests with ants from colonies other than the home of the dead queen), containing 15 adult workers and 15 pupae.

For the last manipulation in this series, we introduced each of five Polyergus queens who had attacked and mangled a dead F. gnava queen into a nest containing 15 workers and 15 pupae of the allopatric species *F. occulta*. We observed the behaviour of the *Polyergus* queens and the *F. occulta* workers continuously for 1 h, and then approximately every 6 h until the *Polyergus* queen was either dead or accepted by the workers.

Results

Polyergus queens introduced to colonies of *F. gnava* unrelated to the colony containing the dead queen were uniformly accepted by the adult workers. Median time to takeover for five replicates was 20 min (Table II). The *Formica* workers immediately inspected and groomed the *Polyergus* queens, and in two cases dragged the queens into the nest and onto the brood pile.

In contrast, each of the five *P. breviceps* queens were immediately attacked when introduced to *F. occulta* colonies (Table II). Although two *Polyergus* queens were eventually accepted by the *F. occulta* workers, this only occurred after many attacks by workers, with the *Polyergus* queens remaining non-aggressive.

Test Series 4: Does Formica-Queen Killing Provide Polyergus Queens With A Transient Or Long-lasting Ability To Usurp Host Colonies?

The behaviour of Formica workers towards Polyergus queens shifts abruptly from fighting to grooming after the Formica queens' death. If the Polyergus queen obtains chemicals for adoption directly from the Formica queen, we hypothesize that the behavioural effect would be transient, lasting just until the workers started grooming the queen. Our reasoning was that grooming by workers alone would provide chemical cues necessary to maintain worker-queen social bonds.

Methods

We placed five Polyergus queens in individual chambers, each with a freshly frozen and thawed F. gnava queen and 15 pupae. We recorded the duration of attacks between queens. When the attacks were completed and the Polyergus queens commenced autogrooming, we removed the queens from the chambers and isolated them for 24 h. We introduced each test queen to a nest containing 15 F. gnava adult workers and 15 pupae from the colony of the F. gnava queen, and recorded the first 10 contacts between the Polyergus queen and workers as aggressive (i.e. involving biting) or non-aggressive (antennating). We removed the Polyergus queen from the worker nest immediately after the first 10 encounters, and isolated her for 24 h in a plastic vial with moist cotton batting. We then introduced each queen to nests with a different group of 15 adult workers and pupae from the same colony, and again observed interactions until the first 10 contacts with workers. This procedure was repeated for seven days.

Using the same queens on each of seven days and terminating each trial after the first 10 queen-worker contacts was dictated by the limited number of *Polyergus* queens available. This procedure was clearly a compromise, because we wanted to assess acceptance by workers while minimizing the workers' opportunity to transfer their own chemicals to the queen. Individual encounters were therefore not permitted to last longer than 5 s, after which a blunt probe was used to separate the worker from the queen.

Results

All five *Polyergus* queens were consistently accepted by the *F. gnava* workers on each of the seven days. Resident *Formica* workers approached the queens slowly, and antennated them. Although the duration for the first 10 queen-worker interactions was short (median = 3 min, range = 1-9 min), we never observed a single aggressive encounter during the 35 trials.

Test Series 5: Takeover Of Formica Colonies By Polyergus Queens From Colonies With Different Slave Species

Test series 3 demonstrated that Polyergus queens who have taken over F. gnava colonies are not accepted by F. occulta workers, indicating that the chemical mechanisms of acceptance do not transfer across species. The question remains, however, whether a Polyergus queen could successfully takeover a colony of a different Formica species if given the opportunity to acquire the odour of the heterospecific queen?

Methods

We placed each of seven newly-mated Polyergus queens from colonies containing F. gnava slaves in a nest of F. occulta containing 15 adult workers, 15 pupae and one living queen. Conversely, we placed seven Polyergus queens from colonies containing F. occulta slaves in F. gnava nests containing 15 adult workers, 15 pupae and one queen. For each trial, we recorded the number of attacks by the Polyergus queen on the resident Formica queen, the accumulated attacking time, and time until the takeover criterion was met. We observed behavioural interactions between the Polyergus queen and Formica workers continuously for 2 h, then approximately every hour until the takeover criterion was met or the Polyergus queens died.

Results

Attempts by Polyergus queens to take over allopatric colonies were only partially successful (Table III). Four of the Polyergus queens showed no interest in attacking the Formica queens: they encountered the Formica queens, antennated them and then either ignored or avoided them. Three of these non-aggressive queens were killed by attacking Formica workers, and the fourth avoided attack by leaving the nest.

Of 10 Polyergus queens who attacked the Formica queens only four were successful in meeting the takeover criterion (range = 50 min - 3 days). The four successful takeovers were marked by little to no aggression after the Polyergus queen killed the Formica queen. In one case, the Polyergus queen died several days after takeover, most likely from injuries sustained during pre-takeover aggression. The six unsuccessful takeovers were marked by noticeably more acute aggression than seen in sympatric takeovers, involving repeated biting, rolling and gaster flexing. The deaths of the six unsuccessful and one successful Polyergus queens were largely the result of constant attacks by the Formica workers prior to the killing of the Formica queen.

Test Series 6: Will A Polyergus Queen Attack And Kill A Newly Mated Formica Queen?

The results of test series 2 indicated that a Polyergus queen with the odor of a Formica queen might be accepted by any colony of the same Formica species, and test series 4 showed that a Polyergus queen would have at least seven days to locate an appropriate colony of Formica. We hypothesized, therefore, that it may be possible for a Polyergus queen to acquire the Formica queen odor without invading a colony, by simply killing a newly-mated, egg-laying Formica queen. The mating flights of Formica occur slightly earlier and then simultaneously with those of Polyergus. Newly-mated Formica dealates are very abundant at this time. If a Polyergus queen would attack and kill such a Formica dealate, she may be immediately accepted by the workers of a Formica colony. The goal of this test was to determine whether a Polyergus queen would attack and kill a newly-dealate Formica queen.

Methods

Ten Polyergus queens were placed in individual chambers, each with a newly dealate F. gnava queen. The behavior of the queens was observed continuously for 2 h, and the accumulated time spent attacking or antennating was measured. If no aggression or attacking occurred, the

Polyergus queen was then removed from the chamber and placed in a small F. gnava colony containing a queen, 15 workers, and 15 pupae. In each case we recorded the time until takeover criterion were met. If these Polyergus queens would then attack established F. gnava queens, this would establish that the Polyergus queens were indeed capable and interested in attacking and taking over a colony.

Results

All interactions between Polyergus queens and newly dealate F. gnava queens were non-aggressive. Both queens inspected one another (median = 7 min, range = 14 s - 35 min) with no instance of aggression observed in two h of observation. Therefore, each Polyergus queen was placed in a small F. gnava colony. Nine of the ten Polyergus queens successfully took over the F. gnava nests, with a median time to takeover criterion of 80 min (range = 20 min - 19 h).

Test Series 7: Can A Polyergus Queen Take Over A Polygynous Formica Colony?

While we have had considerable success simulating colony takeover in the laboratory, using small Formica colonies with one queen only, the question remains whether a Polyergus queen could successfully takeover a polygynous colony. A large part of this question is whether a _Polyergus_ queen is capable of killing more than once, and if so, what is the limit? We therefore set out to answer this question in two ways: by introducing _Polyergus_ queens to several colonies for sequential takeover, and by introducing a _Polyergus_ queen to a polygynous _Formica_ colony.

Methods

Ten _Polyergus_ queens were placed in small, queenright colonies of _F_. _gnava_ and time to takeover criterion was measured. _Polyergus_ queens who performed successful takeovers were continually placed in other small colonies. Because of the limited availability of _F_. _gnava_ colonies and _Polyergus_ queens, it was not possible to perform ten full replications: ten _Polyergus_ queens were introduced to a first colony, four of these ten queens were introduced to a second colony, and two of these four queens were introduced to a third and a fourth colony.

One _Polyergus_ queen was introduced to a polygynous colony containing 10 _F_. _gnava_ queens and 100 workers. This colony was observed daily until either the _Polyergus_ queen died or all the _F_. _gnava_ queens were dead.

Results

Nine of ten _Polyergus_ queens were successful in their first takeover attempts; three of four queens were successful in their second takeover attempt; two of two queens were successful in their third attempts; and one of two were successful in their fourth attempts.

The Polyergus queen, placed in the polygynous F. gnava colony on July 20, 1992, attacked one Formica queen after 135 minutes. After 34 min. accumulated attacking time, the F. gnava queen was dead, and the Polyergus queen was no longer attacked by F. gnava workers. The Polyergus queen was then pulled by F. gnava workers towards the other nine F. gnava queens. The Polyergus queen then, over a period of weeks, killed all but one of the remaining F. gnava queens, with whom she had stayed in close, peaceful contact. The last remaining Formica queen was killed on October 4, 1992. The Polyergus queen continues to live with the Formica workers, but has not yet produced any eggs.

Discussion

Chemical communication is an important process regulating behavioural interactions between ant species (Hölldobler 1983). Parasitic worker ants use chemicals to disorganize and scatter resident host workers during group slave raids (Regnier & Wilson 1971; Topoff et al. 1989), and parasitic queens of Leptothorax and Polyergus secrete a pheromone to diminish aggression by resident workers during attempted colony takeover (Allies et al. 1986; Topoff et al. 1988). Studies of colony takeover by parasitic ant queens have revealed diverse queen-killing behaviours, including decapitation, antennal amputation, throttling, and stinging (Wilson 1971, 1975; Buschinger 1986; Buschinger & Klump 1988). It has been suggested that some species of parasitic queens (e. g. *Bothriomyrmex*) are pre-adapted for colony adoption by possessing chemical similarity to the host queen (Lloyd et al. 1986), and recent biochemical studies have indeed revealed similar enzyme patterns between *Harpagoxenus* and its *Leptothorax* hosts (Heinze 1991). An alternative hypothesis is that attacking queens obtain appropriate chemical stimuli directly from host ants. Invading queens of *Lasius umbratus*, for example, grasp and chew workers of their host *L. niger* (Crawley 1909), and a queen of *Epimyrma stumperi* captures a *Leptothorax* host worker, rubs it with her forelegs, and grooms herself with those forelegs (Kutter 1969). Our initial study of colony takeover with *P. breviceps* verified that a newly-mated queen kills a resident *Formica* queen by repeated biting of the head, thorax and gaster (Topoff et al. 1988), and touches her hypopharynx to all pierced surfaces of the dead queen. The hypothesis of chemical transfer arose from our surprising finding that successful adoption dropped significantly when the host *Formica* colony contained no queen, and from the occasional observation that the *Polyergus* queen actually placed the dead *Formica* queen on top of her. The results of the present study demonstrate that a newly-mated *Polyergus* queen will similarly attack, pierce and lick even a dead Formica queen, and will then be immediately accepted by workers from any colony of Formica belonging to the same species as the dead queen. Taken together, these results provide the strongest evidence to date in support of the chemical-transfer hypothesis, with the host queen being the source of the relevant chemicals.

Prior to our laboratory tests, we had thought that the death of the Formica queen (as indicated by the cessation of activity) might be the cue for Polyergus queens to terminate biting, and move from the dead queen to the brood. Although the notion of chemical transfer predicted some contact between queens, we were surprised that both the intensity and duration of the biting and licking were no different in the present study, when the host Formica queen was already dead (and therefore motionless). Perhaps Polyergus queens terminate contact with killed queens only after ingesting some minimal quantity of queen substances. We had also hypothesized that any chemicals obtained by Polyergus during host-queen killing would be quite volatile, allowing the parasitic queen to be accepted just long enough to activate social bonding via worker grooming. Although our seven-day long tests were each restricted to the first 10 queen-worker encounters (and should therefore be considered preliminary), we emphasize again that we never observed a single aggressive encounter. The results suggest that any chemicals transferred are still effective up to one week after the Polyergus queen kills the Formica queen. Although substances ingested by the Polyergus queen may be involved in the chemical synthesis of an attractive pheromone, the rapidity of the shift from biting to grooming by Formica workers suggests a more direct transfer of a crucial surface pheromone. The fact that Polyergus queens were not interested in attacking newly mated Formica queens may indicate that new dealates, without workers, do not yet have the crucial surface pheromone. This suggests that the source of the crucial surface pheromone may be grooming by adult workers.

Although acquiring foreign-queen chemicals broadens the Polyergus queens' ability for colony adoption (Test Series 3) this is not an open-ended process. Previous studies on P. lucidus in New York demonstrated that the slave species found in a queen's nest of origin may determine the host species she will subsequently invade (Goodloe & Sanwald 1985). This may explain the results of Test series 5, in which Polyergus queens reared in F. gnava colonies showed less interest in attacking queens of F. occulta, and vice versa. Nevertheless, even under these circumstances, four Polyergus queens did achieve adoption by killing foreign Formica queens.

While chemical transfer during host-queen killing is important in facilitating the acceptance of the Polyergus queen, the drive to kill *Formica* queens remains even after acceptance and grooming by workers. It is likely that this acceptance allows the *Polyergus* queen to live "peacefully" among workers and queens during the serial killing of *Formica* queens in a polygynous nest. The sequential killing observed in this study indicates that the *Polyergus* queen's drive to kill may not be limited to a narrowly defined time period immediately following mating.

The use of chemical transfer by *Polyergus* queens to achieve adoption in foreign colonies has important implications for theories of the evolution of social parasitism, which until recently have failed to account for colony founding behavior of slavemaking queens (Topoff, 1990).

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Table I. Aggression of *Polyergus* queens towards dead *Formica* queens, and success of colony takeovers.

	Formica workers present when queen killed			*Formica* workers not present when queen killed	
Colony	Successful takeover?	AAT (min)	Takeover time (min)	Successful takeover?	Takeover time (min)
1	+	30	180	+	60
2	+	26	140	+	60
3	+	7	165	+	75
4	+	19	59	+	7
5	+	23	45	+	2

AAT: accumulated attacking time by *Polyergus* queens.
+: successful takeover.

Table II. *Polyergus* queen takeover of unrelated *Formica gnava* colonies and of *F. occulta* colonies.

	Foreign *F. gnava* colony		*F. occulta* colony	
Colony	Successful takeover?	Takeover time (min)	Successful takeover?	Takeover time
1	+	15	-	NA
2	+	70	-	NA
3	+	1	+	2 days
4	+	60	-	NA
5	+	20	+	3 days

NA: not applicable.
+: successful takeover.
-: unsuccessful takeover.

Table III. Takeover of a *Formica* species not found in the *Polyergus* queen's nest.

Colony	Formica species	*Polyergus* slave sp.	Successful takeover?	AAT (min)
1	Fo	Fg	-	9
2	Fo	Fg	-	4
3	Fo	Fg	-	0
4	Fo	Fg	+	12
5	Fo	Fg	-	17
6	Fo	Fg	-	28
7	Fo	Fg	+	17
8	Fg	Fo	-	19
9	Fg	Fo	+	20
10	Fg	Fo	-	0
11	Fg	Fo	-	0
12	Fg	Fo	+	30
13	Fg	Fo	-	2
14	Fg	Fo	-	0

AAT: accumulated attacking time by *Polyergus* queen; Fo: *F. occulta*; Fg: *F. gnava*.
+: successful takeover.
-: unsuccessful takeover

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