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# HOST SPECIFICITY AND PROXIMATE MECHANISMS IN RAIDING BEHAVIOUR OF THE SLAVE-MAKING ANT POLYERGUS LUCIDUS

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# HOST SPECIFICITY AND PROXIMATE MECHANISMS IN RAIDING BEHAVIOUR OF THE SLAVE-MAKING ANT *POLYERGUS LUCIDUS*

BY

Linda Pike Goodloe

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Faculty in Psychology in partial
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# GENERAL INTRODUCTION

Interspecific slavery in ants (dulosis) has evolved independently at least three times in two subfamilies (Wilson, 1971): in the genera Formica, Polyergus, and Rossomyrmex of the subfamily Formicinae, and in the genera Harpagoxenus-Leptothorax, and Strongylognathus -Tetramorium of the subfamily Myrmicinae. Within these groups exists a broad spectrum of slave-keeping behaviors. Briefly described, one ant species invades the nest of another closely related species and captures developing brood which is transported to the raiders' nest. The captured workers eventually eclose and perform all tasks necessary for colony maintenance in the service of the slave-makers. Dulosis may be either facultative, where the raiders are capable of functioning without their slaves and are often free-living, or obligatory, in which case the behavior of the raiders has become highly specialized for raiding and degenerate in such areas as nest maintenance, brood care, and even feeding. Obligatory slave-makers are incapable of surviving without the slaves who perform these tasks for them. Facultative dulosis is considered the primitive condition and obligatory dulosis the evolutionarily derived state.

Several hypotheses have been advanced to explain the evolution of this parasitic relationship and each must account for the diverse behaviors observed in slave-making workers, colony founding queens, and slave species. Central to these hypotheses is the observation by Emery, subsequently labeled "Emery's rule": "The dulotic ants and the parasitic ants, both temporary and permanent, generally originate from the closely related forms that serve them as hosts" (Wilson, 1971, p.360). Emery's rule reflects the observation that parasitic species usually bear a close resemblance to their hosts, and initially suggests that parasitic species evolve from the forms which later serve them as hosts. This is considered improbable because it appears to invoke sympatric speciation. An evolutionary pathway that avoids this difficulty by postulating the host-parasite relationship develops between two derivative species of a non-parasitic species has been suggested by Wilson (1971).

The first hypothesis for the evolution of dulosis was set forth by Darwin (1859). Based on his observations of Formicine ants, he suggested that slavery initially developed as a by-product of brood predation among related species. Instead of being totally consumed, some of the captured brood managed to survive and eclose within the predators' nest. Wilson (1971) has noted that a close phylogenetic relationship would increase the probability that the behavioral repertoire of the captive group would be compatible with their captors, and if their labors in the nest led to increased reproductive success of their captors, then the factors which made this relationship possible would provide a selective advantage.

A second hypothesis focuses on territorial interactions, with opportunistic brood predation, as the main pathway to dulosis (Alloway, 1980; Hölldobler, 1976; Stuart & Alloway, 1982; Wilson, 1971). Experiments by Kutter (discussed in Wilson, 1971), in which *Formica naefi* colonies were placed near colonies of *F. fusca*, a common slave species, resulted in the *F. naefi* workers overrunning the *F. fusca* colony and capturing both brood and workers. Alloway (1980) performed convincing experiments in which he manipulated three species of *Leptothorax* commonly found as slave species. He found, from the territorial interactions that resulted from pairs of these species, all possible combinations of brood predation including evidence in two instances of intraspecific slavery. Survival of some of the raided brood was seen in all but one of the nine pairs used. Hölldobler (1976) has described the occurrence of intraspecific slavery in the context of territorial contests in the Formicine desert ant *Myrmecocystus mimicus*. Intraspecific aggression and ant brood capture between adjacent colonies has also been observed in the western slave-raiding species Polyergus breviceps (Topoff, LaMon, Goodloe, & Goldstein, 1984).

Within the above mentioned studies, the use of the term "territoriality" is never clearly defined. However, the context in which it is invoked indicates that its usage is intended to describe aggressive interactions among neighboring colonies of closely related species or conspecifics. Although brood predation is a central feature of the evolution of slavery in both hypotheses, by itself it fails to account for the directed nature of slave raiding. Raids occur only to colonies containing the appropriate host species. Territorial interactions presumably resulting from competition among closely related species demonstrate that some selective recognition mechanism already exists. The territorial hypothesis thus appears to account for more of the components in the sequence of behaviors that comprise a slave raid.

The third hypothesis concerning the evolutionary origins of dulosis centers on the colony founding behavior in slave-making ants. The queens of all known species of slave-makers are social parasites, and unlike the queens of host species, cannot initiate new colonies by themselves. Instead, a queen of slave-keeping ants will enter into a nest of the slave species. Once accepted by the workers, she kills the resident queen.

Some dulotic queens have been noted to form colonies by moving into a raided nest accompanied by a number of raiders (Marlin, 1968). In either case, the remaining workers and brood become slaves to the parasitic queen and her subsequent brood. In cases of facultative dulosis, the original workers and brood may eventually die out if not replenished by new slave raids and the parasitic species can become free-living.

Some theorists (Buschinger, discussed in Alloway, 1980; Creighton, 1950) have postulated that the raiding behavior exhibited by slave-making workers is derived from the aggressive colony founding behavior shown by dulotic queens. In this view, the inheritance by the workers of the predatory behavior and the host-specificity observed is accounted for by the dulotic queen's aggressiveness and choice of host species in which she is most likely to succeed in being accepted. This view has been expressed by Creighton (1950) in a discussion of the Formica sanguinea species group in which facultative dulosis is common. He states: "..all that is necessary is that the impulse to enter a foreign nest should manifest itself simultaneously in most of the workers of the raiding column." The slave raid itself is seen as "...an abortive attempt on the part of the workers to carry out the nest founding reactions of the female" (Creighton, 1950, p.461). To explain the recovery of brood and the return to the nest, he suggests that foraging impulses then predominate. This evolutionary explanation, in addressing the aggressive slave-raiding behaviors, is less parsimonious than previous conjectures, involving major contextual shifts and reorganization of the components of raiding behavior. However, the previous hypotheses fail to address the obvious relationship between colony founding and slave-raiding behavior.

Of the models proposed so far, not one satisfactorily accounts for the full range of behaviors in slave-keeping species. Alloway (1980) attempts to deal with this problem by proposing a tripartite theory in which three factors must be present simultaneously for dulosis to occur: (1) the presence of closely related species in which this intimate host-parasite relationship can evolve, (2) a preadaptation for raiding such as territoriality and/or brood predation, and (3) a preadaptation for non-independent colony founding. Concerning the latter requirement, Alloway suggests that the condition of facultative polydomy and polygyny would be conducive to temporary parasitism and its extension to dulosis. Where polydomy and polygyny exist, colonies often reproduce by budding, and newly mated queens are often adopted by existing colonies. It is these behaviors in particular that he considers relevant preadaptations.

Although this is the most inclusive theory so far, it still does not provide for a functional relationship between non-independent colony founding and other dulotic behaviors. Their simultaneous occurrence in all known instances of dulosis suggests that this relationship is more than fortuitous. Alloway addresses this problem by suggesting that brood transport during budding and emigrations in such societies may be preadaptations for brood transport during raiding. However, the fact that the entire sequence of raiding behaviors can be seen in territorial interactions with brood predation makes this supposition appear unnecessary. There is a conspicous lack of knowledge about colony founding in most of the relevant species.

These hypothesis postulate broadly defined behaviors (brood predation, territoriality, and non-independent colony founding) as evolutionary preadaptations for dulosis. However, each of these "behaviors" is really a complex sequence of behaviors, and these labels describe functional outcomes rather than the behaviors themselves. Environmental and physiological mechanisms controlling these complex sequences of behavior may be as diverse as the species groups in which these behaviors are found. This broad focus of previous theories may impede identification of evolutionary preadaptations by being overly inclusive. Therefore, I have explored the evolutionary origins of dulosis by seeking proximate mechanisms that regulate more narrowly defined behaviors of both slave-makers and their slaves.

ed to experience no aggression from the workers. That she still had some quality that was distinguished as "different" by her new nestmates was shown by occasional observations, over the two month period, of her being held or pulled by a tarsus or antenna lluminating aspects of the evolution of dulosis. I chose the slave-makers of the genus Polyergus as study organisms. The most advanced form of dulosis within the Formicinae is represented by this genus, which contains several species of obligatory slave-raiders. All are characterized by workers highly specialized for raiding, with long, sickle-shaped mandibles capable of piercing the bodies of their foes. Among the North American species P. lucidus and P. breviceps, some variation is shown in colony size and frequency of raiding, but descriptions of raiding behavior are very much the same (Wheeler, 1910, 1916; Talbot, 1967, 1968; Harmon, 1968; Marlin, 1969, 1971; Cool-Kwait & Topoff, 1984; Topoff et al., 1984). Although slaves are active throughout the day, activity of the Polyergus workers is usually confined to the afternoon and early evening, with the first workers emerging shortly after the temperature has passed its peak for the day. These workers, labeled "scouts", go out from the home nest and locate nests of the slave species. After locating an appropriate nest, the scout returns to the home nest, recruits her nestmates, and leads them in an orderly column directly to the slave-species nest (Cool-Kwait & Topoff, 1984; Topoff et al., 1984). Slaves do not participate in the raids.

In 1911, Carlo Emery performed the first investigation into colony founding in the genus Polyergus (described in Wheeler, 1916). A dealate queen, placed near a laboratory colony of the slave species F. fusca, usually managed to win acceptance by the F. fusca workers without a battle. Once securely established in the nest she would kill the resident queen. Since these experiments were conducted, it has been accepted that Polyergus queens found colonies by this method of adoption. Dealate queens are often found in the vicinity of Polyergus nests, following raiding columns, and hovering near the nests of slave species nests that have just been raided (Talbot, 1968; Marlin, 1971; Topoff et al., 1984). Marlin (1968) also reported instances of budding, in which a number of raiders join a queen in occupying a recently raided nest.

The system I chose to work with is the slave-making ant Polyergus lucidus Mayr and its slave species. P. lucidus is a North American species found in the east and midwestern United States. The population I studied is in the pine barrens of eastern Long Island. This habitat is unique in that it contains at least four different *Formica* species that are used as slaves, although only one species of slave is found in each *Polyergus* nest. The four are *F. schaufussi*, *F. nitidiventris*, *F. incerta*, and *F. fuscata*, all of which belong to the subgenus *Neoformica*. Considerable variation in characters occurs not only within each slave species, but within single colonies, and there is considerable overlap of characters between species, making this group particularly frustrating for taxonomists. The most recent revision of this group is by Creighton (1950). It is inadequate for differentiating among these species and has left the actual number of species in doubt. (For an account of previous attempts at classification of this group, see Kwait, 1982). Following Kwaits's example, I used Emery's 1893 revision of Mayr's 1886 classification because it corresponds to distinctions which appear to be made by the different colonies of *P. lucidus* in this area (Kwait, 1982; Sanwald, unpublished data).

*F. schaufussi* is the largest among the slave species groups and workers are reddish in color. The *F. nitidiventris* worker is smaller and considerably darker. The *F. fuscata* worker is smaller and darker than that of *nitidiventris* and confusion between the two is possible.

Fortunately, *fuscata* is extremely rare.

A species intermediate between *F. schaufussi* and *F. nitidiventris* is *F. incerta* and this group is most easily confused with *F. schaufussi*. It is less common than *F. schaufussi* yet more common than *F. fuscata* and thus is the most troublesome of the four. Habitat preference among these species follows a continuum consistent with characters, with *F. schaufussi* preferring sunny, open areas in sandy soil, and *F. fuscata*, at the other extreme, preferring shade or woods, in heavier soil, with the other species falling appropriately in-between.

*F. schaufussi* and *F. nitidiventris* were selected as representative species for this study because (1) they are common and (2) they are not easily confused with each other. It is possible that in the laboratory experiments, *F. fuscata* is occasionally presented as *F. nitidiventris* and *F. incerta* substituted for *F. schaufussi*. To minimize the latter problem, only the largest and reddest of the colonies labelled *F. schaufussi* were used. The low incidence of *F. fuscata* in the area made this accidental substitution even less likely. Type specimens were taken in the field study and await further analysis following the eventual resolution of taxonomic uncertainties. The *Polyergus* mixed colonies are considered unambiguous as to the identity of their slaves since a broad spectrum of workers from many colonies was available in each to assist in such a judgement.

In this study I have addressed the following questions: (1) In a habitat where several potential host species are present, how much host specificity is shown by slave-raiders in their choice of colonies to raid, and by queens in their choice of host species for colony founding? (2) How do scouts choose a "suitable" raid target? (3) How is pupae retrieval by the raiders affected by the presence of alien host species workers? (4) What factors influence the acceptance of pupae from alien nests by workers of the slave species?.

## Experiment 1: QUEEN "CHOICE" OF HOST SPECIES

### INTRODUCTION

Host specificity in slave-making ants is exhibited on two levels. Workers may exhibit specificity on slave-raids during which they invade the nests of some but not other species. Newly mated queens also must choose among species during colony founding. In this experiment I examine host choice in colony founding by P. lucidus queens. Polyergus queens found colonies either by adoption, where a queen invades a slave species nest, killing the resident queen and appropriating workers and brood present (Wheeler, 1910), or by "budding", in which a queen invades or is accepted into a host species nest accompanied by raiders from her nest of origin (Marlin, 1968). I hypothesized that a newly-mated *Polyergus* queen would choose and/or be chosen by a colony of the same host species found in her nest of origin.

## METHOD

The site for the experiment was a two acre lot in Suffolk County, N.Y., on which vegetation is kept closely cropped and where an unusually high density of colonies of potential slave species of *Polyergus* has been maintained. Also on this site are several artificially implanted in-ground *Polyergus* colonies that use two of the available slave species present. In addition, one portable colony of *Polyergus*, encased in a plywood box with a removable cover, was used. This colony was kept indoors except during afternoon hours, when it was occasionally placed in a fixed location on the site to allow raids to occur, and during mating flights, so the activity of the reproductives could be monitored. Only colonies using the slave species *F. schaufussi* or *F. nitidiventris* were used.

In August, when mating flights of *Polyergus* were expected to begin, female alates were taken from their nests and labelled as to their colony of origin. Those from in-ground colonies were recovered in the morning under sun-warmed rocks placed over the nest entrances. All queens in the portable colony were either removed from inside the nest or recovered when they first emerged from the nest after it was placed outside. Each was briefly immobilized by cooling, labelled with a streak of enamel paint on her gaster to indicate her colony of origin, and immediately returned to her home nest. There were no observable behavioral effects of the labeling process once the subjects were returned to the ambient temperature.

On warm sunny days favorable for mating flights, the in-ground colonies were periodically inspected for activity, the portable colony was placed outside and constantly monitored, and a search was made for labelled and mated _Polyergus_ dealates on the prowl for appropriate host colonies. Recovered dealates were each placed in a test tube (16 x 150 mm) containing approximately 4 cm of water held in place with a cotton plug, to prevent dehydration, and were restrained with an additional cotton plug. They were then transferred to the laboratory and within 24 hours presented with a choice of two host species. Two potential host "colonies", one of _F. schaufussi_ and one of _F. nitidiventris_, were each housed in a plastic box (21.6 x 29.2 cm). Each colony consisted of 20 or 30 pupae, two or three callows, and two adult workers, in 16 x 150 mm test tube with 4 cm of water plugged with cotton. The test tubes were a preferred nesting place and once established inside, the small group always chose to remain there except for foraging, "exploratory" activity, and occasional aggressive encounters with workers of the alternate species or the Polyergus queen. Food of honey-water and Tenebrio larvae were provided. Callows were included in the host groups because experience had shown that queens in laboratory "adoptions" experience a high death rate possibly due to attacks by the adult slave-species workers, while callows appear to be more accepting and less hostile. The number of adults chosen was considered the absolute minimum required for an interaction, while the number of callows and pupae, equalized between the two species in each test box, was the result of the number available in laboratory colonies of the appropriate species during the course of the experiment.

Each queen, in her own test tube, was placed in the box between the parallel test tubes containing the two potential hosts, and the restraining plug was removed. Checks were made daily to see if an adoption had occurred. An adoption was considered complete when the queen was found living in a test tube with workers and brood, and being tended (groomed, fed, or provided with other frequent, non-hostile contact) by the workers, for at least 24 hours.

## RESULTS AND DISCUSSION

Overall mortality in the adoption test was 59%, but was disproportionately high for queens from Colony II. There was no obvious cause for this high death rate. Possibly the handling required in capture, labelling, and recapture, was a factor. Attacks by the potential "hosts" may have contributed, but there were only two adult workers in each to give battle, and the queens, with an advantage in size and weapons, might be expected to easily overcome them if they offered resistance. Of the 13 surviving queens from colonies containing _F. schaufussi_ slaves and the one surviving queen from the nest containing _F. nitidiventris_ slaves, _all_ were adopted into "colonies" of the slave species found in their nest of origin (binomial test, $p < .001$, $n = 14$). Over 70% of the adoptions occurred within the first 72 hours. The most prolonged interval preceding a successful adoption was 17 days.

Table I summarizes the labelling, recovery, and survival of _Polyergus_ queens. Recovery of the mated queens was facilitated by the fact that all labelled dealates were found within one meter of the nest of origin and all appeared to be attempting to return to their nest, except for two queens who were recovered from under the rock covering the entrance to the home nest itself.

In this experiment, it is not possible to know whether the ultimate residence of the queens was determined by their "choice" or by the selective action of the slave species workers. Clearly queens and potential hosts must be accepting of each other for an adoption to occur. Observations of the interactions between the queens and their potential hosts suggested that the initial response to a queen by adult workers of both slave species present is aggression or withdrawal. Aggression usually consisted of the _Formica_ workers pulling on her legs or antennae. For a queen to succeed in becoming adopted, she must persist in her attempts to contact the workers and become acceptable over time. Laboratory interactions between single conspecific slave species workers from different colonies usually result in the death of one or both workers, and the introduction of an alien conspecific into a slave species nest almost always results in the death of the newcomer (Goodloe, unpublished data). These findings suggest that a queen's association, in her home nest, with slave species workers of the same species as a potential host, probably does not give her any useful identifiable odor to diminish the initial aggression of this host.

The observation of large numbers of dealates returning to their home nest is consistent with findings of Marlin (1968). In the two recoveries of dealates found in the nest entrances of their original nest, queens were being attacked by resident slaves. We excavated a large colony of Polyergus in late August, 1984, and found five dealate and numerous alate queens. Since polygyny is not characteristic of Polyergus, and since mating flights had been occurring, we assumed that at least four of these had recently mated and returned to the nest. In the laboratory, the colony was divided into five parts. Each part contained one of the dealate queens and a mixture of slaves and raiders, and was placed in a plastic petri dish nest within a plastic arena with a sand floor. Four of the queens were usually found outside the nest dish, running along the sides of the arena, apparently attempting to escape. Only one queen was consistently found within the nest dish and tended by slaves. She was darker than the others and was assumed to be the founding queen. Other queens, both alate and dealate, were observed being attacked by slaves. All the dealates and most of the alates died within two months. However, the corpse of the putative founding queen was retained in the nest dish for several days after death while the bodies of the other four were all found far removed from the nest. These observations suggest that it is unlikely that female reproductives are able to spend their first winter in their home colony.

Two matings of labeled alates from the portable colony were observed in the field. Both occurred within one meter of the nest entrance and took place with the queens clinging to blades of grass. The first occurred with a male from the same colony while the second occurred with a male from a Polyergus colony using the same slave species, who had been introduced by the experimenters.

The adoption results indicate that at least one constraint exists on the interaction of Polyergus lucidus and potential slave species. The slave species found in a queen's nest of origin appears to determine the host species she will successfully invade, although the exact mechanism through which this occurs is unknown. Jaisson (1971) and Le Moli and Passeti (1977, 1978) have demonstrated that Formica callows of several species imprint to brood, of their own or different species, present during a critical period following eclosion. Possibly the queens imprint to the host species they encounter upon eclosing.

## Experiment 2: SCOUT CHOICE OF HOST SPECIES TO RAID

### INTRODUCTION

Although at least four potential host species of Formica are present in the surrounding environment, only one species is used as slaves in any one P. lucidus nest. Even though taxonomic difficulties make identification of Neoformica species difficult, there is a conspicuous difference between P. lucidus mixed nests and mixed nests of the related facultative slave-makers found in the same habitat. In the latter group, differences in the species of slaves present are obvious, whereas in a P. lucidus colony, the range of character variation in the slaves is clustered about a single modal type with no obvious discontinuities, indicating that only one slave species is present. This could come about if slave raids of a particular P. lucidus colony were directed towards only one slave species, or if raids were directed at more than one species followed by differential consumption of captured brood favoring the survival in the nest of a single slave species.

This experiment was designed to explore the hypothesis that each P. lucidus colony will raid only colonies of the slave species already present in the mixed nest. Since a scout has been shown to lead her sisters to a particular target nest (Cool-Kwait & Topoff, 1984; Topoff at al., 1984) this selective process must occur through the perceptions and actions of the scouts.

The hypothesis of differential brood consumption, by contrast, will be discussed in Experiment 5.

## METHOD

This experiment was conducted in the field using the same two acre site described in Experiment 1. Two colonies of P. lucidus were collected in late spring of 1985 near Rocky Point in Suffolk County, New York. One colony, P/S#1, contained slaves of the species F. schaufussi, and the other, P/N#10, contained only F. nitidiventris slaves. Each colony was placed in a portable artificial nest contained within a 61 x 43 x 10 cm plywood box as shown in Figure 1. The nest consisted of a plastic box filled with moistened dirt and having a single entrance/exit hole leading into the remaining open area of the plywood box. An entrance/exit hole from the plywood box to the outside was closed with a plastic plug which could be removed to allow raids to occur. The lid of the plywood box was removeable to allow examination of the nest and the activity of P. lucidus workers prior to raiding. A cardboard cover was placed over the plastic nest box to prevent disturbing the colony and was removed only when examination of the nest was necessary.

Experience has shown that the size of a colony correlates with the number of raids, with very large colonies able to make multiple raids in a single day. To maximize the frequency of raids for this experiment, each colony was expanded, prior to the onset of raiding, by the addition of raiders and slaves of the appropriate species. Raiders from incipient *P*. *lucidus* nests being nurtured on the two acre site were removed and added to the artificial mixed nest containing the same slave species as their colony of origin. For unknown reasons, such mixing of workers and raiders occurs with very little aggression, most easily in the spring and fall but also, with somewhat more aggression, during the summer months. Such mixes between any combination of slaves and raiders from colonies using different slave species have been so far impossible to achieve (R. Sanwald, personal communication).

Both colonies were expanded until they each included approximately 1300 raiders and 4000 slaves at the beginning of the raiding season. Initially, each colony contained a single *P*. *lucidus* queen. The artificial colonies were kept indoors and observed until larvae appeared in the nest, from which time the colonies were allowed periodic opportunities to raid.

A fixed location was chosen on the two-acre site from which raids were allowed to occur. Approximately equal numbers of *F* *schaufussi* and *F*. *nitidiventris* colonies were present within raiding distance of this location. On every day when weather was favorable for raiding, between 1300 and 1400 hr, one of the colonies was brought outside and placed at this location. The plug was removed to allow passage in and out of the artificial nest. To prevent slaves from immediately locating a more favorable nest site and initiating an emigration out of the artificial nest, all emerging slaves were retrieved by hand and kept apart until the end of the day when they were returned to the artificial nest. All _P_. _lucidus_ workers were allowed to pass in and out so that raids could occur.

Observations were made until the raiders had returned from any raids that occurred and activity of the raiders in and out of the nest ceased. The artificial nest was then replugged and returned inside until the next raiding opportunity. The two colonies were alternated at the fixed location within the constraints of difference in time of onset of raiding activity and the demands of Experiment 3 which was conducted simultaneously.

Samples of slave species workers were taken from each raided colony, when possible, for identification (see Appendix). Data was analyzed using the binomial test for large samples to determine if the choice of species raided was non-random.

# RESULTS AND DISCUSSION

The results of this experiment are summarized in Table 2. Between June 1 and August 3, 1985, Colony P/S#1, containing _F. schaufussi_ slaves, made 45 raids. Samples of raided workers were obtained for 44 of these raids. In the blind identification of samples, 43 were determined to be _F. schaufussi_ and one was labeled _F. nitidiventris_. A binomial test for large samples showed that choice of host species to raid was non-random, $p < .00003$, using an expected P of .5 ($n = 44$).

Colony P/N#10, containing _F. nitidiventris_ slaves, conducted a total of 32 raids. Worker specimens were obtained for 28 of the raided colonies. Of these, 25 were identified as _F. nitidiventris_, one as _F. schaufussi_, and two as indeterminate, possibly _F. incerta_. A binomial test using an expected P of .5 showed that the choice of host species to raid was non-random, with $p < .00003$ ($N = 28$). The raid of P/S#1 on the _F. nitidiventris_ nest on June 6 netted approximately 50 pupae. The raid of P/N#10 on the _F. schaufussi_ nest on July 23 netted 5 pupae. These numbers are unusually low but there is no evidence to determine whether the raiders were less interested in the pupae of these species or if the raided nests were already depleted of pupae.

Geographic distribution of raids is shown in Figure

2. There is no overlap between the two raiding colonies: none of the available slave-species nests were raided by more than one P. lucidus colony although several colonies were subjected to multiple raids by the same raiding colony. P/S#1 raided slave colony #1 four times, colony #11A three times, and colonies #5, #9, #11, #34, #50, and #61 twice. P/N#10 raided slave colony #41 three times, and colonies #19, #21, and #58 twice.

It is clear from this experiment that raids are almost exclusively conducted to nests of the same slave species found in the raiders' home nest. However this experiment addressed the question of whether preference would be shown given roughly equal availability of both species. Yet to be explored is the question of whether the raiders would choose alternative targets if the preferred slave species were absent.

## Experiment 3: PUPAE RETRIEVAL BY RAIDERS

### INTRODUCTION

During the course of laboratory raiding experiments some unexpected behaviors were observed. Often, scouts inspecting the target nest boxes would pick up pupae and carry them back to the home nest. This occurred most frequently when the scout had just engaged in an aggressive encounter with introduced adult slave workers. Also, when a raid occurred, if we removed the adults prior to the arrival of a raid swarm, often the raiders would walk all over the pupae, explore them with their antennae, but fail to retrieve them. In fact pupa retrieval under these conditions was the exception rather than the rule, although it did occur. This led to the hypothesis that retrieval of pupae by the raiders was in some way facilitated by the presence of alien slave species workers. Some preliminary manipulations of the raiders alone reinforced this view. In this experiment the effects of the presence or absence of host workers in the acceptance of pupae by slave-making workers were assessed.

## METHOD

Two portable colonies, described in the previous experiment on host choice by the raiders, were used. This and the previous experiment were run concurrently during July and August, 1985. P/S#1 was a P. lucidus colony with F. schaufussi slaves and P/N#10 was a P. lucidus colony with F. nitidiventris slaves.

The two conditions of the experiment were "pupae only" (P), and "workers plus pupae" (W+P). For each raiding colony, the species of workers and pupae used was the slave species found in the raiders' nest. Most came from laboratory colonies. Fifteen pupae were used in the P condition, and 15 pupae plus 20 workers were used in the W+P condition.

The procedure consisted of placing the pupae and workers directly onto the advancing column on its outward trip. We chose open areas in the raiders' path, where observations would be best. To maximize raider-slave contact, the pupae and workers were dropped into the center of the advancing column, between 10 and 20 centimeters behind the leading edge. This also allowed at least some of the raiders to continue to their original target. The first meter of the column was usually the most dense, with raiders towards the rear of the column being more dispersed.

Conditions of P and P+W were alternated to cancel any order effects. Drops were made anywhere from 3 to 50 meters from the nest. Average number of drops per single raid was 1.38. Multiple drops on a single raid were separated by at least 15 minutes.

The measure of pupa retrieval was the number of pupae returned by the raiders to their nest. Also recorded were the number of Polyergus on the raid estimated to the nearest 50, and in the P condition, the number of contacts with the pupa by the raiders to guarantee that lack of retrieval was not due to lack of contact. A "contact" was directly touching the pupa with either tarsi or antennae, and could be either a prolonged exploration by a worker or merely having her run on top of it.

The data was analysed using a Mann-Whitney U test.

## RESULTS AND DISCUSSION

The number of pupae retrieved and returned to the nest by the raiders for the two colonies under the two conditions, P and P+W, are shown in Table 3. No correlation was found between the number of pupae retrieved and the number of raiders on a particular raid for all four conditions (r's = .0015, .08367, .4492, and .5727, $p > .05$). There is a significant difference between the number of pupae retrieved in the two conditions for both colonies ($p < .005$, Mann-Whitney U test, one-tailed) indicating that the presence of foreign slave species workers facilitates the retrieval of pupae by the raiders. It is also clear from this table that this is not an "all or none" effect. One or more pupae were taken in 16 of the 24 pupae drops in the P condition. Although it is possible that free-living slave species workers may have encountered the raiders in the area of the drop, none were observed in any of these instances. Instead, the presence of slave species workers appears to increase the probability of pupa-carrying behavior.

Although pupa-carrying behavior in the P condition showed variability from day to day, responses on a given date always showed the same relationship between the two conditions, with numbers of pupae retrieved under the P condition consistently less than those retrieved under the P+W conditions. There were no exceptions in the ten days on which drops were made in the two conditions.

Behavioral observations following the P+W drops indicated that for some raiders, the presence of the alien workers was a cue for behavior more likely to be seen upon arrival at the nest to be raided. On an undisturbed raid, when reaching the target nest, the raiders become more excited, as indicated by a conspicuous increase in activity within a limited circular area where the column has come to a halt. This slowly expanding circle becomes increasingly dense as the remainder of the column arrives. Raiders at this point appear to be searching for the entrance to the nest, and occasionally excavating by the raiders is observed. Since the drops were always made several centimeters behind the head of the column, the leading workers were unaffected by the drop and continued to advance. Many of the following raiders, although contacting both dropped workers and pupae, continued on after their leaders. However, some of the raiders, who had also contacted the dropped workers, behaved as if the drop site was the target nest. They circled frantically in the area and on two occasions excavated and removed debris from small depressions in the ground. On July 8, approximately 40 minutes following a P+W drop, more than a dozen raiders were found still circling in the area and several were still excavating a small hole immediately under the drop site. One raider was still digging there an hour after the drop. To determine whether we had accidently dropped the workers and pupae on or near an active nest, we subsequently excavated the area and found nothing.

Perhaps most significant was the fact that this exploratory behavior occurred only under the P+W condition. Occasionally, raiders who stopped to retrieve pupae would then continue on with the advancing column for a short distance before turning and bringing their booty back to the home nest. Of the raiders who stopped to explore the area of the drop, some would eventually depart in the direction that the raiding column had taken while others returned to the home nest.

One surprising observation was the aggression that occurred when the raiders encountered the dropped workers. Under normal circumstances, when raiders invade a slave species nest, there is usually very little violence. Although some of the besieged workers attempt to flee with brood, and occasionally even try to pull brood away from departing raiders, they are usually ignored or brushed aside. Rarely do Formica workers attack the invaders directly, and fighting, resulting in injury or death, is seldom seen. However, when the workers and pupae were dropped on the raiders, fighting almost always occurred, and those slave species workers unable to escape were usually killed. Contact of workers with raiders was inevitable under conditions of the drop and often there was no possible escape for the workers. During a normal raid, slave species workers would most likely be encountered on the periphery of the column rather than in the middle of it, and avoidance, by either the raiders or the beseiged workers, would be possible. Dropped slave species workers would often defend the accompanying pupae even though the brood was usually from colonies other than their own. It was not possible to discern which of the two groups was escalating the aggression although the main impression was that both raiders and slave workers were behaving in an uncharacteristic fashion.

The lowest number of pupae retrieved in the P+W condition occurred with Colony P/S #1 on July 18. No pupae were retrieved on either of the two P trials on the same date. However, due to an unexpected shortage of F.

schaufussi workers, workers for these two drops were taken from a pile of pupae gathered from local free-living colonies of the same species. Although a few of the workers in this group had been gathered with the pupae, many were young workers that had eclosed during the month that the pile had been kept. A few were obviously callows. While the results from some P+W drops in which pupae retrieval had been low were suspected to be due to low number of worker-raider contacts because of worker scatter, in this instance, considerable contact occurred between workers and raiders. Young workers are known to be "acceptable" to alien conspecifics during a certain "critical period" after eclosion (Jaisson, 1971). Perhaps the contacts between raiders and these dropped slave workers were qualitatively different from the others in which all workers were mature. Aggression here was mixed. Some workers were killed while others survived many contacts. One possible reason for this variability may be age differences in very young workers, some still within the critical period of acceptance, and others just past it.

Following the first two drops on July 18, the raiders successfully raided a slave species nest and about half the raiders emerged from the nest with brood and headed for home. There was apparently no brood left for the remaining raiders and they began to trail the others home. Ten pupae were dropped on this latter group shortly after they left the raided nest. Several workers contacted the pupae but none were picked up. Although this one-shot attempt is hardly definitive, it suggests that the effect of factors facilitating pupae-retrieval during a raid are short-lived. This is consistent with the data showing that alternation of the two conditions, during a single afternoon throughout several raids and involving many of the same raiders repeatedly, produced such clear differences.

One other one-shot manipulation was attempted on July 27 when raiders from Colony P/N #10 reached a target nest and were swarming over the rock covering the nest entrance. Three pupae were dropped on top of the rock where many raiders repeatedly contacted them before two were finally picked up. Five minutes passed and the entrance was discovered before the third was finally retrieved. This suggests that generalized excitement or arousal, as manifested in rate of activity, is not sufficient to elicit pupa-retrieval in the raiders.

Although the results show that the presence of the slave species workers facilitates pupae retrieval by the raiders, it is not clear exactly how they do this. In spite of encounters with both the dropped workers and pupae, many raiders continue on their way and only a few respond by retrieving the pupae. In a normal raid, most of the invaded workers are trapped down in the nest when the raiders break in. What happens below is unknown, but if massive fighting were occurring (and it's hard to imagine how that could happen in such tight quarters) some evidence of the casualties should eventually appear at the surface and that doesn't happen. Perhaps the trapped and alarmed workers give off a particular alarm pheromone that in high concentrations is more likely to trigger the pupae-retrieval behavior.

Laboratory attempts to explore pupae retrieval were first made near the end of an artificial "raiding season", when no brood was left in the nest. During the course of this "season", many successful raids had occurred and pupae were retrieved by the raiders. However, at this time, nothing we did to the raiders, including dropping numbers of alien slave workers on them, could induce them to pick up a single pupa. This particular colony was also somewhat depleted in numbers of both slaves and raiders, so it is risky to generalize from this one experience, but it is possible that seasonal or cyclical constraints also affect pupae retrieval. Onset of raids in the spring has been correlated with the appearance of brood in the nest (Cool-Kwait & Topoff, 1984).

Initial field attempts to confront raiders with alien slave workers were less crude than the final procedure. We attempted to have the raiders "discover" the workers by placing a small clear plastic box containing the workers directly in the path of an outgoing raid. Workers were restrained by a small trap-door which could be lifted as the raiders arrived. Unsterilized dirt was used to create a pathway into the box. However, on arrival at the box, the raiding column would neatly divide and pass around, uniting again on the other side. Any raiders with sufficient momentum to pass into the entrance of the box would stop within one or two centimenters, show evidence of alarm, and quickly retreat before encountering any of the planted slaves. Blocking the pathways around the sides of the box led to a considerable traffic back-up of raiders, but they would not venture on their own into the box. The cues by which they identified a "foreign object" are unknown. Laboratory observations suggest that the presence of alarmed slave workers may be aversive to raiders. This would in part explain the lack of aggression between raiders and *Formica* workers on undisturbed raids. However, in this case, the box was avoided also when it contained only pupae.

## Experiment 4: PROXIMATE FACTORS IN RAIDING BEHAVIOR

### Experiment 4a: Effect of temperature on raid-related

behavior

# INTRODUCTION

Considerable evidence suggests that Polyergus raiders may be subject to more specific temperature constraints on their behavior than their slaves. Spring excavation of a mixed nest by Harman (1968) revealed hibernating raiders although the slaves had been active for a month. He concluded that to achieve their maximum activity, the raiders require soil temperatures higher than other species found in the same environment. This view has been supported by the findings of Cool-Kwait and Topoff (1984).

Within their diurnal cycle, Polyergus workers again appear to operate within a more narrow temperature range than their slaves or most other ants. Within a typical mixed colony of Polyergus, slaves are active outside the nest from early morning to dusk, while it is rare to see a raider emerge from the nest entrance before the sun has passed its peak for the day (Talbot, 1967; Harman, 1968; Topoff et al., 1985).

Wheeler (1916) observing P. breviceps, concluded that the time of raiding was determined by temperature and that a certain maximum temperature was required. Talbot (1967) noted that colonies more exposed to the sun and experiencing high temperatures for a longer time, started raids later in the afternoon than cooler colonies. This suggests that while the heat may arouse the raiders, there is an upper limit to the soil temperature that the raiders can tolerate. Harman (1968) equated exposure of a nest to the sun with successful raiding and noted that few mixed colonies survived in the shade.

Although other factors may be influencing this temporal pattern, temperature seemed a good candidate for exploration. Experiment 4a was conducted to elucidate the influence of temperature on raiding behavior.

## METHOD

Two colonies of Polyergus lucidus, P1 and P2, each with Formica schaufussi slaves, were dug in the summer of 1983 and housed in laboratory nests at the end of their summer reproductive cycle. The colonies were placed in a cooled environment (3 - 8 C) for two months to simulate winter conditions. On removal from the cooler, colonies were kept at ambient laboratory temperature (21 - 27 C) until eggs were seen in the nest, at which time they were placed in the experimental apparatus.

Colony P1, consisting of approximately 100 raiders and 300 slaves (the queen had died) was placed in a plastic arena (61 x 61 cm). The sides of the arena were coated with polytetrafluorethylene to prevent ants from escaping, and a 2 cm layer of sand covered the base. The sand both increased the ease of observing the ants and was an aid to their locomotion. Within the arena and embedded in the sand, two 150 mm plastic petri dishes, connected by a small plastic tube and with entrances at both ends, served as a nest dish. Each contained white dental stone of approximately 5 mm in depth. Dental stone retains moisture and an opening in the lid of one of the nest dishes allowed a humidity differential to be established between the two modules. In the other module, the dental stone was covered by a layer of dirt from the original nest since dirt is necessary for successful pupation of <u>Polyergus</u> brood. A cylindrical plastic tube (3.8 cm diameter x 184 cm in length) was connected from a hole in the side of the arena to a plastic box (21.6 x 29.2 cm) which was to serve as a potential raid site. Both the box and the tube contained a thin layer of sand.

Colony P2 , containing a <u>P</u>. <u>lucidus</u> queen with approximately 200 raiders and 500 slaves, was placed in a 91.4 x 91.4 cm plastic arena. The arena had a sand covered floor and a pair of nest dishes identical to those in P1. Two plastic tubes (3.8 cm diameter) were attached to each of two adjacent sides of the arena. On one side the tubes were 185.4 cm in length, and on the other, 125.7 cm. (see Fig. 1). Length of the tubes was determined by the availability of space. In both arenas the tubes provided an opportunity for raiding. A plastic box (21.6 x 29.2 cm) was attached to the end of each raiding tube. Both the tubes and the boxes contained a thin layer of sand.

Fluorescent light was provided from 0600 to 2000 hr to simulate summer hours. In addition, a 150 watt flood light was placed over the colony during the hours of observation to supply some reference point for visual orientation of raiders and scouts. An infra-red (250 watt) light was also placed over each arena for the purpose of heating the sand near the nest area and was employed on alternate days, to provide the HEAT condition. Absence of the heat lamp constituted the NO HEAT condition. Ambient air temperatures in the laboratory during the experiment fluctuated between 22 and 27 C. Since the nest areas were not placed under the heat lamp, their temperature varied with the ambient temperature (except for a slight heating by the 150 watt floodlight and peripheral effects of the heat lamp) and varied for both colonies from 23 to 29 C in the HEAT condition and from 21 to 28 C under the NO HEAT condition. Sand temperature approximately 20 cm away from the nest was affected by the heat lamp and varied from 38 to 41 C for P1, and from 43 to 46 C for P2 in the HEAT condition. On NO HEAT days, sand temperature was 2 to 3 degrees above the ambient room temperature, due to heat from the floodlight, except for one NO HEAT day when the P2 floodlight was bumped out of its normal position and the sand temperature rose to 34 C.

Colonies of *Formica schaufussi* were provided by attaching their plastic box homes (previously described) to the ends of the raiding tubes or by placing parts of colonies, including the nest dishes, into the empty plastic boxes provided at the ends of the tubes. Observations began at 1300 hr with the onset of heat and floodlamps and were made every 15 minutes for at least 3 hours. Two activity measures were taken. First, scouting activity was measured as the number of *Polyergus* workers reaching the target boxes (not including the slave raids). This measure was taken only for P2. For P1 and P2, the total number of scout trips per day to all available sites was noted.

The second measure, taken for both colonies, estimated the amount of "activity" for the two species in each nest and was determined by recording the number of *Formica* and *Polyergus* workers out of the nest every 15 minutes. Means for the HEAT and NO HEAT conditions were compared using a t-test for correlated groups.

## RESULTS AND DISCUSSION

During the period of observation, between 4/8 and 4/23/84, no raids occurred in either colony. Polyergus brood never attained the pupa stage in P1. A maximum of 11 pupae were present at the beginning of the observations in P2 and this number subsequently declined, apparently due to consumption of brood, with only four raiders eclosing over the entire period of observation. The production of eggs by the queen indicated that she responded to the brief "winter-spring" cycle to which the colony was subjected. Later findings led to the conclusion that poor brood development was probably due to unacceptable dirt in the nest. Although sterilized dirt from the original nest site was provided, little brood was produced in any laboratory P. lucidus colony until unsterilized dirt was used. Lack of brood in the nest was suspected as a possible factor in the absence of raids.

Despite the lack of raids, behavior of P. lucidus raiders was consistent with field observations that raider activity begins during the afternoon. It is not possible to discern from field or lab data whether this is a time or temperature related phenomenon. However the same pattern of activity, although at a lower level, occurred in the laboratory on NO HEAT days where the temperature was relatively constant, suggesting that raider activity is time regulated but probably constrained by temperature.

Results concerning the differences in activity under the two heat conditions are found in Tables 4, 5, and 6, and are significant for all comparisons, for both raiders and slaves. This is not, by itself, a remarkable finding, in that most ants show increased activity under increased heat within normal limits. However, these results do suggest a role of temperature in increasing certain activities of the raiders. Of particular interest was the effect of temperature on scouting activity. Mean activity level, as based on the number of individuals outside the nest, was a less than ideal measure of the behavior of the raiders. Under the NO HEAT condition, they emerged at the appropriate hour and spent much of the afternoon resting near the nest. Under the HEAT condition, in P2, those workers outside the nest were generally much more active, running around in the main arena and occasionally scouting available sites. This qualitative difference in activity is reflected only in the measure of the number of scouting trips, shown in Table 6. Since scouting activity is a prerequisite for raiding, this finding suggests that the probability of a raid occurring is strongly linked to temperature.

### Experiment 4b: Proximate cues for choice of raid site

## INTRODUCTION

Previous research has confirmed that nests of slave species are located prior to raiding by single _P. lucidus_ scouts that leave the slave-makers' nest well before raids begin (Talbot, 1967; Marlin, 1969; Cool-Kwait & Topoff, 1984; Topoff et al., 1984). Marlin (1969) noted that scouts frequently enter the slave species nests, and Kwait & Topoff (1984) concluded from laboratory studies that scout entrance into a host species nest is a minimal prerequisite for a raid. However the cues by which the scout determines the "suitability" of the site have not been identified. Thus two laboratory experiments were conducted to ascertain proximate factors influencing the raiding behavior of _P. lucidus_ workers. I focused on three potentially important cues: the presence of foreign workers of the slave species, the presence of pupae, and the presence of slave species nest odors. These were systematically presented to _P. lucidus_ scouts to determine which were correlated with subsequent raids.

## METHOD

This experiment consisted of two parts. The first part, Baseline Series, was run between June 28 and July

18, 1984, to determine variables of interest as possible proximate cues to the scouts. The following part, Test Series, run between July 19 and August 27, 1984, was undertaken to improve control of the variables explored in the Baseline series. A raiding colony (P3) of *P. lucidus* with *F. schaufussi* slaves, obtained in June, 1984, in Suffolk County, N.Y., was brought into the laboratory and established in a 91.4cm x 91.4cm plastic arena. A nest was made in two plastic petri dishes as described in the previous procedure. Four potential raiding sites were provided as also described in the previous procedure (Fig. 3). Distances of raiding sites were determined by available space.

Ambient laboratory temperature ranged between 23 and 29 C. Laboratory lighting was as previously described, with the addition of the heating lamp to encourage raids. The infra-red light was turned on at the beginning of observations, usually between 1130 and 1230 hr, except for five days in Condition 1 when observations began later in the afternoon.

## Baseline Series:

Potential raid sites consisted of 21.6 x 29.2 cm plastic boxes, most of which served as homes for slave species colonies. These were attached daily to the ends of the four available raiding tubes. Sand (1 cm deep) covered the bottom of each box and those containing slave colonies also contained a covered 150 mm plastic petri dish with a dental stone substrate which served as a nest. Boxes with colonies also contained a small dish of honey, a water-filled test tube plugged with cotton, and whatever food and garbage the colony had accumulated. A 3.8 cm diameter hole was drilled in the side of each box to allow attachment to the tubes. All manipulations of slave species colonies took place within an hour of the expected onset of scouting activity. The conditions used were the following:

1. Sand only: One cm of freshly mixed sand was placed in an empty target box.

2. Brood only: A box containing a slave species colony was emptied of queen and workers. Most of the brood of the colony, mainly pupae and some larvae, were left in the box.

3. Workers only: A box containing a slave species colony was emptied of queen, workers, and brood. Several workers (range 8 to 20) were returned to the box. The nest dish was covered with dark felt to encourage these workers to remain inside until the

arrival of the scouts. The felt was then removed to permit observation of worker-raider interactions.

4. Workers plus brood: A box containing a slave species colony was emptied of queen and workers. Several workers were then returned to the box while brood remained in the nest dish. This was covered with felt to encourage the workers to remain inside. The felt was removed to allow observation when the scouts arrived.

5. Empty nest. Queen, workers, and brood were removed from a slave species colony and the freshly vacant box, with nest, garbage, and partially eaten food was attached to the arena.

These conditions were rotated among the four available sites and were switched daily.

## Test Series:

Each potential raid site consisted of a 21.6 x 29.2 cm plastic box containing a thin layer of freshly mixed sand. Unlike the previous series, most of these boxes were not used to house intact colonies. Four conditions of information available to the scouts were randomly distributed among the four available locations, with the exceptions that no condition was repeated in the same location immediately following a raid, and that each condition was presented approximately the same number of times in each location.

The nest of P3 within the plastic arena was as described in the Baseline Series and the same arena was used (see Fig. 1). The conditions for the four potential targets were as follows:

1. Workers only. Five to 13 workers (mean = 8.67) from a colony of the slave species were placed in a covered 60 mm petri dish which contained approximately 5 mm of sand and had two entrances 180 degrees apart. A piece of moistened paper towel was also included in the dish to encourage the workers to remain inside, and a water-filled test tube was placed in the box to prevent dehydration of the subjects. The dish containing the workers was placed in a corner of the target box. Since scouts usually explore first along the edges of the box, this improved chances of contact. Planted slave species workers occasionally escaped down the connecting tubes into the arena where they were indistinguishable from the resident slaves. Since

contact with the planted slave species workers was a potential indication of a raid site, replacement slave species workers were provided in the box when necessary. In the absence of scouting activity, the tube exit from the target box was plugged to prevent escapes. The 60 mm dish was also covered with felt in the absence of scouts to encourage the planted workers to remain inside.

2. Workers plus pupae. Five to 15 workers (mean = 8.4) and 5 to 20 _F_. _schaufussi_ pupae (mean = 9.6) were placed in a 60 mm plastic petri dish in conditions identical to #1 above.

3. Pupae only. Five to 25 pupae (mean = 13.97), depending on availability, from an _F_. _schaufussi_ colony, were placed on the sand along the edge of a target box.

4. Empty nest. Here a target box was used which had been housing an intact slave species colony for at least four weeks. All live ants and brood were removed from the box within one hour of the expected onset of scouting.

## RESULTS AND DISCUSSION

The number of raids occurring per number of opportunities (or number of days that a condition was presented) are shown in Table 7. In the Baseline Series, raids were directed only to the condition of "workers plus pupae". Although no raids occurred to the other conditions, the procedure used in the Baseline Series left some doubt as to the purity of the target conditions. In the process of removing workers and brood from the slave species nests prior to attaching them to the raiding tubes, workers would scatter in all directions with brood of various sizes. Although an attempt was made to retrieve all brood and workers scattered about the box prior to attachment, occasionally larvae would later be found dropped along the edges of the nest or a worker would suddenly emerge from the garbage. The procedure developed for Test Series was designed to correct for this problem, except for the empty nest condition which posed the same risk. In retrospect the risk appears minimal since the raiders consistently failed to choose the empty nest condition in the 37 opportunities that were presented.

The results from Test Series show that raids occurred primarily to the "workers plus pupae" and the "workers only" conditions, indicating that encountering slave species workers by the scout provides the cue for the presence of a raidable nest. Daily records indicate that for all of these raids, scouts made physical contact with the slave species workers prior to the raid.

The recently evacuated "empty nest" obviously had no appeal to the scouts since they made no raids in 37 opportunities. The two raids to the "pupae only" condition, out of 36 presentations of the condition, seem likely to be laboratory examples of what we have labeled in the field "flops", where recruitment occurs and a column of raiders emerges and travels several meters in a particular direction only to come to a confused halt. After considerable milling around, the raiders gradually trickle back to their nest. Another possibility is that disorientation occurred (discussed below), since there was often confusion in the raiding column finding the entrance to the raiding tubes. In both "pupae only" raids, the raiders failed to pick up any pupae during the first 5 minutes after arriving at the target. They eventually retrieved 12 out of 15 pupae in the first instance, and 2 out of 16 in the second. In each of the 26 presentations of the "pupae only" target in Test Series, scouts made repeated contact with the pupae.

That more raids occurred to the "workers plus pupae" versus the "workers only" condition could be an artifact of the unnatural conditions of the experiment. Slave species workers placed with pupae usually remained in the small nest dish provided, tending the pupae, whereas workers without pupae were more likely to scatter and occasionally escape down the raiding tubes. (Possibly such escaped workers were responsible for the raids to the "pupae only" condition since it was impossible to distinguish them from resident slaves and monitor their whereabouts once they escaped.) Concentration of the workers tending the pupae facilitated their being discovered by scouts in the target box. During the Baseline Series, when no "workers only" target was raided, fewer workers were being used and only seven opportunities provided.

Some liberties were taken with the original procedure. Shortly after beginning the series, it became apparent that there were few scouts at work. This increased the value of those that were already active. Although a <u>Polyergus</u> worker is a formidable fighter, capable of taking on and destroying any number of slave species workers in series, if pinned by several workers at once, a scout can be seriously injured or killed. When a scout was grabbed by more than one worker in the target nest, the entangled ants would be gently separated by hand and the scout would be freed. All would then be returned to the target box. Also, when the target boxes were opened at the onset of scouting, foraging or garbage-dumping slaves from the raiders' nest would immediately begin to radiate into the boxes. Since they inevitably got into fights with the planted workers and thus disrupted our plans, we removed all slaves as they entered the target boxes. Since they invariably and immediately returned to the same box if we put them back in the main arena, we finally kept them apart until the end of observations for the day when they were returned to the arena.

As mentioned above, disorientation was a serious problem in this experimental set-up. One manifestation of this problem was the frequent failure of a raiding column to find the raiding tube. On several occasions recruitment was clearly occurring and the column began to move but instead of heading into a tube they would head directly into a corner of the arena. Although the scout leading the column was never identified, the efforts were persistent, sometimes up to half an hour, before they either gave up or located the tube several centimeters away. Observations of P. breviceps in the field (Topoff et al., 1984) indicate that the scout leading the column outward is using optical cues. Our finding is consistent with the absence of chemical cues at this stage of the raid. If a returning scout left a chemical trail back to the nest, this particular problem would not have repeatedly occurred.

Although the floodlight was added to provide a salient stimulus for optical orientation, it was clearly unsuccessful. Another consistent and puzzling problem occurred when scouts or raiders were in the tubes. Once they entered the tubes it took an inordinate amount of time and back and forth movement for them to pass through. Although scouts do travel a meandering path on their outward scouting trips, their direction initially is consistently away from the nest. Although the longest raiding tube was only 185.4cm, it occasionally took a scout more than 45 minutes to traverse the length of the tube and emerge from the other end. Also, although back and forth motion of individual raiders in a raiding column is their usual pattern, the column as a whole moves forward at a fairly brisk pace. Again, the raiding column could take as much as a half hour or more to travel through a tube. The most obvious disorientation occurred on the homeward trip of the raiders after a raid. In the field there is no back and forth movement on the return trip. The raiders head straight back to the nest, probably over a previously laid chemical trail (Topoff et al, 1984). However the same back and forth motion occurred on the return trip in the tube so that it could take 45 minutes to an hour for all the raiders to get back to the nest.

Apparently, once in the tube, the raiders were unable to determine in which direction they were heading, and the finding of the target (or, on the return trip, the nest) seemed the result of their chance emergence from the correct end of the tunnel. Even if a chemical trail is laid on the outward trip by the raiders, this by itself does not provide the information on which direction in the tube is the correct one. Various adjustments and positions of the floodlight were tried. In one attempt to overcome this problem a raiding tube was covered with dark tape over 180 degrees on one side for its entire length in an attempt to provide an unambigous light direction but nothing produced any change in this behavior.

Many pupae were provided to the raiders in the course of this experiment. We were unable to track their fate individually but it was clear that few were surviving in the nest to eclose. For example: between July 6 and July 8, 50 slave pupae disappeared from the raiders' nest although only seven slave callows appeared. It is assumed that the remainder were consumed. Kwait and Topoff (1984) estimated that 75% of captured brood was eaten. Our observations support the idea that captured brood is an important food source for P. lucidus colonies.

Another behavior apparently an artifact of the experimental situation was the high level of aggression between scouts and raiders, and the target slave species workers. Field observations of both _P_. _breviceps_ and _P_. _lucidus_ have shown, on typical raids, slave-makers seldom attack and kill the workers of the raided colony. A few of the besieged workers are usually seen fleeing the nest with whatever brood they have been able to salvage. Occasionally slave species workers will attempt to retrieve brood from the raiders as they emerge from the nest. These efforts are commonly unsuccessful but the would-be rescuers are brushed aside or ignored, not killed, by the homeward bound raiders. However, in the laboratory, when scouts discovered the planted workers, there was almost always serious fighting, resulting in the death of the slave species workers, and occasionally the scout as well. Raiders inevitably killed any surviving workers in the target box. As a result, once a raid had clearly been initiated and the raiders were in the tube, any remaining planted workers were removed from the box to avoid unnecessary slaughter.

In the field, scouts penetrating the entrance of a slave species nest have one direction to retreat when encountering a worker and, in the narrow passage, can probably make an unchallenged exit. Within the target box it was very easy for the scouted workers to grab the scout. Disorientation may have hindered the scouts ability to make an orderly retreat and thus led to repeated encounters with the same or other _Formica_ workers.

Another unusual behavior observed was the stealing of pupae by scouts. Although some Myrmicine slave-making scouts single-handedly penetrate the host nest and steal pupae when possible (Wesson, 1939; Alloway, 1979) a Polyergus scout has never been observed coming home with pupae in the field prior to a raid. It is unlikely that under natural conditions a lone scout would be able to penetrate a nest and obtain brood without being molested. Of particular interest was the fact that this behavior was much more likely to occur following encounters with slave species workers. In the "pupae only" condition, the pupae were walked over and examinined but seldom retrieved. Similarly, during raids, when we removed the surviving Formica workers prior to the arrival of the raiders, the pupae were often ignored. This is consistent with the results of Experiment 3 which show that pupa-carrying behavior of the raiders is facilitated by contact with the defending workers.

One particularly surprising finding was the occasional scout that managed to survive aggression of the planted workers and gradually become acceptable. When a scout was grabbed by tarsi or antenna by the planted workers, she would often "freeze", or remain immobile except for occasional attempts to contact the workers with her antennae. In several instances the scout became accepted by the workers, as determined by the gradually diminishing of aggression until she could move easily within the planted nest dish contacting workers and pupae. When this happened she usually positioned herself in the middle of workers and pupae. On one occasion, during the HEAT - NO HEAT trials, a scout remained in the nest with the planted workers and stayed with them when they were returned to their original colony. The scout lived with this colony for almost two months and most of the time seemed to experience no aggression from the workers. That she still had some quality that was distinguished as "different" by her new nestmates was shown by occasional observations, over the two month period, of her being held or pulled by a tarsus or antenna by one of the workers. However she was apparently never harmed and her accidental death two months after she moved in was unrelated to aggression from her nestmates.

This behavior is very similar to the way _P. lucidus_ queens observed in the lab became accepted by alien slave species workers. Although capable of killing any number of slave species workers at once due to their large size and oversize saber-like mandibles, when grabbed by the limbs, queens would usually remain passively immobile.

## Experiment 5: PUPAE ACCEPTANCE BY _P. LUCIDUS_ SLAVES

## Experiment 5a: Intraspecific Pupae Exchange (Free-living *Formica*)

### INTRODUCTION

*Formica* slaves within a *Polyergus* nest have to care for both the the *Polyergus* brood and the brood, primarily pupae, retrieved from various *Formica* nests by the raiders. Pupae are licked and moved to areas of best available temperature by the slaves. In the absence of attending workers, under laboratory conditions, pupae frequently became moldy. Also, because most *F. schaufussi* pupae and many *F. nitidiventris* pupae under normal conditions are enclosed in cocoons, they must be assisted in eclosing by the removal of the cocoon. Even naked pupae seem to need some assistance in the removal of the old larval skin, if present.

An encounter between two *Formica* workers from different nests, either free-living or enslaved, is almost always fiercely aggressive. Under laboratory conditions where mutual avoidance is impossible, injury or death usually result (Goodloe, unpublished data). The existence of colony recognition odors in Hymenoptera has been well documented (Wilson, 1971). One might suspect that these workers would be able to perceive colony specific differences in pupae from alien conspecific colonies as well.

In the evolution of dulosis, the capacity for the acceptance of alien conspecific pupae by the host species must either exist as a preadaptation or in some way be induced by the slave-making ant. Alloway (1982) has explored this relationship in the Myrmicine slave-maker Harpagoxenus americanus and three species of Leptothorax used as slaves. He compared the pupae-acceptance behavior of the three species of slaves under three conditions:

1) using workers from free-living slave species nests

2) using enslaved workers from mixed nests but without the presence of the slave-makers

3) using the combination of enslaved workers and the slave-makers from mixed nests

Harpagoxenus americanus differs from P. lucidus in that often more than one slave species may be found in a single nest. In this experiment, however, Alloway used only mixed colonies with one species of slave. His results showed that presence of the slave-makers enhances the pupae-acceptance behavior of the slave species involved. This change in behavior of the slaves was shown to continue over time since the enslaved workers which had been isolated from the slave-makers for two weeks also showed enhanced pupae acceptance, although to a lesser extent than those in the presence of their slave-makers.

To explore the relationship between enslavement and pupae acceptance in _P_. _lucidus_, an experiment was designed to determine parameters of pupae-acceptance of slave species in their free-living state.

## METHOD

Seven laboratory colonies of the slave species _F_. _schaufussi_ were collected near Rocky Point in Suffolk County, N.Y., during the summers of 1983 and 1984 and were subjected to cooling-induced repetitions of their reproductive cycle. Prior to this series of manipulations, all seven colonies had been placed in the cooler between late July and early September and removed from late September to early November. Although they were not synchronized exactly in their cooling cycles, these seven colonies were chosen in December, 1984 because most of the brood in each was in the pupal stage.

_Formica_ colonies in the laboratory were kept in 21.6 X 29.2 cm plastic boxes, with approximately 1 cm of fresh sand covering the bottom. Within this box a 150 mm plastic petri dish, containing a white hydroset substrate of approximately 5 mm in depth to retain moisture, served as a nest. Honey, water, _Tenebrio_ larvae, and occasional ground meat and chopped hard-boiled egg were provided *ad libitum*.

Prior to the experiment, the queen and most of the workers from each of the seven colonies were removed from the nest and placed in a fresh box with a fresh nest dish and fresh food and water. The experimental colonies ranged in size from 200 to 550 workers. After 24 hours, the queen and workers in each of the seven new nests were presented with 25 pupae, either from their own colony ("same"), or from one of the other experimental colonies ("different"). Four of the colonies received the "different" condition first, while the remaining three began with the "same" condition. After five days, the surviving pupae and callows were removed and counted. After another 24 hours to recover, each colony was presented with 25 pupae from the alternative condition to their first presentation, and a count was made five days later.

## RESULTS AND DISCUSSION

No significant difference was found between the mean number of pupae accepted from the "same" and "different" conditions using a t-test for correlated groups (df = 6, t = 1.247, $p > .05$). No order effects were seen. Data and colony sizes are provided in Table 8. Free-living *F.*

schaufussi workers appear to treat alien conspecific pupae as their own. No pupae were discarded intact from any nest and none became moldy from any of the colonies in either condition. During the course of the experiment, 18 pupae eclosed and all callows were accepted by the workers. However, surprisingly, as also shown on Table 8, most of the eclosed pupae were in the "same" category ($p < .025$, $df = 5$ using a t-test for correlated groups). Thus the workers, although seeming to care equally for pupae from their own and from alien conspecific nests, were able to discriminate between them and preferentially assisted their own pupae in eclosing.

Alloway (1980) conducted laboratory experiments with Leptothorax slave species in which intraspecific raids occurred and resulted in intraspecific slavery. Laboratory manipulations with F. schaufussi and F. nitidiventris have shown that two colonies of the same species, when placed in close proximity with no opportunity to emigrate, may fuse with relatively little violence among the workers, but with the resultant loss of one or both queens. Interspecific confrontations are more violent and no fusions have yet occurred in such combinations (Goodloe & Sanwald, unpublished data). It is not known if such interactions occur under natural conditions or if any other ways exist in which free-living slave species colonies come into possession of alien conspecific pupae. Although all conspecifics eclosing in a nest will serve the resident queen, the potential exists in all the workers for the production of males. The results of this experiment suggest selective pressure towards this differential treatment of conspecific pupae.

The number of pupae that disappeared (and were believed to have been consumed) was fairly constant in all groups despite considerable differences in colony size. Possibly the lost pupae are defective or diseased ones that are detected and consumed. This number might be expected to occur with a similar frequency in all groups.

This experiment has shown that behaviors of pupae acceptance and related brood care, essential for _F. schaufussi_ to function as a slave of a dulotic ant, exist intact in the slave species apart from association with the slave-makers, and may have been an important pre-adaptation in the evolution of this relationship.

## Experiment 5b: Intraspecific Pupae Exchange (Enslaved _Formica_)

### INTRODUCTION

The next question considered is whether association with the slave-makers causes a change in this pre-existing pupae-acceptance behavior of the slave species. Field studies by Talbot (1967), Cool-Kwait and Topoff (1984), and Topoff et al.(1984), suggest that much of the brood retrieved by the raiders is consumed. Since the raiders are dependent on their slaves for feeding, those eating the retrieved pupae are the slaves themselves. This conflicts with the previous experiment that shows pupae-acceptance and survival to be the normal condition for the slave species in the free-living state. However, ants recently brought into the laboratory from the field are usually much hungrier than those that have been kept for several weeks or more. Perhaps colonies in the previous experiment, which had been in the laboratory for months and were provided with food ad libitum were satiated and for this reason failed to consume the brood presented to them. If satiation of the slaves was the determining factor in brood consumption, then mixes of slaves and slave-makers, under the same laboratory conditions should show similar results.

## METHOD

Three laboratory colonies of P. lucidus with F. schaufussi slaves (P3, P4, and P5), and six queenright colonies of F. schaufussi, collected near Rocky Point in Suffolk County during the summers of 1983 and 1984 were used in this experiment, which was conducted in January and February of 1985. All colonies had been subjected to one or more periods of cooling previously described and, at the time of the experiment, all were at the end of a reproductive cycle. Only one of the slave species colony had any brood.

Each colony was handled as in the previous experiment, with queen and workers being placed in a new nest. P5, a large mixed colony, was divided into two parts: P5(1), with a ratio of slaves to raiders of approximately 10:1, and a total of 459 ants, and P5(2), with a ratio of 1:1 and a total of 200 ants. After a period of isolation of 5 days, 25 F. schaufussi pupae from different colonies were added to each. Five days later the surviving pupae and callows were counted.

## RESULTS AND DISCUSSION

The number of pupae surviving in each colony is shown in Table 9. The number of pupae surviving in the mixed nests was significantly less than the numbers surviving in the free-living Formica colonies. The presence of the raiders induced consumption of pupae by the slaves. Since all colonies were on the same diet and appeared to be satiated at the time of the experiment, these results suggest that pupae have a particular importance as a food source in the mixed colonies, perhaps providing some necessary nutrient needed by the raiders that is not provided by the typical slave species diet. The importance of pupae as a food source for P. lucidus has been noted by Talbot (1967) and Cool-Kwait and Topoff (1985). These findings are the reverse of the findings by Alloway (1982) and suggests that in the evolution of dulosis in Polyergus, the pupae may have originally of primary importance as a food source. The sample size is too small to determine if there is a correlation between size of the mixed colonies or the slave/raider ratio and the number of pupae consumed.

## Experiment 5c: Interspecific Pupae Exchange

### INTRODUCTION

As previously mentioned, the Long Island habitat of P. lucidus is unique in that it contains at least four slave species of Formica. Several species of the Raptiformica species group (see Creighton, 1950), examples of facultative dulosis, also exist in this habitat and enslave these same Neoformica species as well as at least one species belonging to the F. fusca group. In the nests of these facultative raiders, it is common to find more than one species enslaved. P. lucidus is found with only one slave species in a single nest despite the proximity of other potential hosts. Experiment 2 has shown that a _P_. _lucidus_ colony will preferentially raid colonies of the same slave species found in the home nest. If nests of other potential slave species are raided, another factor that might foster host-species exclusivity in a _Polyergus_ nest would be differential consumption of pupae. Thus if a species other than the slave species currently in residence is raided, brood from this raid might be more likely to be consumed. An experiment was designed to explore this possibility.

## METHOD

In March of 1985, five experimental colonies, none of which included a queen, were derived from a large _P_. _lucidus_ colony with _F_. _schaufussi_ slaves as follows:

P5A - 100 slaves and 26 raiders
P5B - 100 slaves and 12 raiders
P5C - 100 slaves and 25 raiders
P5D - 100 slaves and 50 raiders
P5E - 100 slaves and 100 raiders

In addition, a colony (S), consisting of 200 workers from a free-living _F_. _schaufussi_ colony, was used for comparison. As in the previous experiments, each group was placed in a fresh box with a fresh nest dish. No brood was present in P5 at the time the groups were removed. After 24 hours to recover from the move, all the experimental colonies except P5A were presented with 20 pupae from F. nitidiventris nests. P5A was presented with 20 pupae from F. schaufussi nests, to control for order effects. After five days, a count of surviving pupae was made in each group and all pupae were removed. Following a three day recovery period, 20 pupae of the opposite species were presented to each colony. After five days another count was made and again three days were allowed for recovery. This cycle was repeated two more times, using 15 rather than 20 pupae (due to a decreasing laboratory supply), so that each colony received six presentations, three of pupae from each species, for a total of 55 pupae from each.

## RESULTS AND DISCUSSION

All experimental colonies consumed significantly more F. nitidiventris pupae than F. schaufussi pupae, including the group containing no P. lucidus raiders, suggesting that this is characteristic of F. schaufussi workers whether free or enslaved. These results are shown in Table 10. No correlation was seen between the size of the colonies or the ratio of slaves to raiders and amount of pupae consumed although the variability in both size and the ratio of workers to slaves under natural conditions is much greater than that represented here. Experiment 2 showed that a raid on a F. nitidiventris nest by a P. lucidus raiding colony with F. schaufussi slaves is a rare event. Kwait (1982) estimated that 75% of the brood retreived by the raiders is consumed. Considering the differential consumption of pupae by species shown in this experiment, it seems unlikely that any F. nitidiventris pupae would survive to eclose in a P. lucidus nest with F. schaufussi slaves.

## GENERAL DISCUSSION

Of the three hypotheses concerning the evolution of dulosis described in the introduction, two focused on the slave-makers' raiding behavior. The first suggested the origin of this behavior in brood predation (Darwin, 1859), while the second emphasized territoriality combined with opportunistic brood predation as a potential preadaptation for raiding behaviors (Wilson, 1971; Holldobler, 1976; Alloway, 1980; Stuart & Alloway, 1982). The third hypothesis, which focused on the non-independent colony founding behavior of dulotic queens, is less parsimonious. Although a functional link between this colony founding behavior and dulosis must still be accounted for by any complete theory of the evolution of dulotic behavior, experiments reported in this thesis pertain only to the first two hypotheses.

Host-specificity, both in colony-founding and in raiding, found in Experiments 1 and 2, results in exclusive use of a single host species by each _P. lucidus_ colony. This distinguishes _P. lucidus_ from both Myrmicine raiders (Alloway, 1980) and facultative Formicine raiders, where more than one slave species in a single nest is common. This species selectivity, requiring both a selective recognition mechanism and a unique behavioral response to a single species, is most commonly found in territorial interactions between conspecific or closely related colonies.

A preadaptation toward brood predation alone would not require this narrow focus. Experiment 5 demonstrated that slave species colonies accept alien conspecific pupae without consuming them, suggesting that this is the primitive condition and that consumption of captured brood in the presence of raiders is a modification of this basic condition. This might also be assumed to be true for the facultative Formicine raiders who share some slave species with _P. lucidus_. However, this host species specificity is not shown in this group.

"Territoriality" is usually defined as "an aggressive interaction between neighboring colonies of conspecifics or close relatives." The most logical place to begin the search for territorial origins of raiding behavior would be among colonies of the slave species who, according to Emery's Rule, would probably be the closest relatives of the slave-makers. Alloway (1980), looking at interactions among the slave species of Harpagoxenus americanus and Leptothorax duloticus, found both interspecific and intraspecific slavery as a result of aggressive interactions between colonies of these species. Descriptions of the interactions indicated that contacts between workers from different colonies were usually hostile, that fighting occurred continuously, and casualties were high. These results provided a convincing argument for territoriality as a preadaptation for slave raiding behavior in this group.

Preparing for the portable colony of raiders used in Experiment 2, it was noted that, starting with a single colony of P. lucidus and a given slave species, large numbers of both slave-makers and slaves from different slave-maker colonies and from free-living colonies of the same slave species could be added with little aggression occurring. Subsequent laboratory manipulations with the slave species F. schaufussi and F. nitidiventris, using workers from two different colonies, demonstrated that the greater the number of workers placed together, the lower the level of aggression and the higher the proportion of surviving workers (Goodloe, unpublished data). A one-on-one encounter between two alien conspecific workers usually resulted in the death of both. However, adding 8 workers from one colony to a group of 8 workers from another usually resulted in the survival of all workers.

Further manipulations, which consisted of linking pairs of colonies together, showed that initial encounters between workers of different colonies exhibited high levels of aggression, but that where frequent contact was occurring, the level of aggression usually dropped rapidly. Within several days there was no difference between the contacts of residents of each nest with their original nestmates and their contacts with aliens, and the workers gradually fused into a single group. From this point on, the only aggression observed was directed against the queens, who were in three instances observed to be killed by the "alien" workers within their own nest. In this lab set up, confrontation with the alien workers was inevitable and it was impossible for either colony to emigrate. Therefore it is not safe to generalize from this to natural conditions, but certainly the outcome of this encounter resembles the outcome of Alloway's work in which intra-specific slavery was demonstrated. Those workers who lose their queen become the slaves of the surviving queen and workers.

Except for the initial encounters, aggression is difficult to identify in the worker-worker interactions and nothing resembling raid behavior occurs. There is not even a ritualized aggression such as that found by Hölldobler (1976) in *Myrmecocyctus mimecus*. Rather, aggression becomes rapidly muted by repeated encounters. That recognition of differences continues is suggested by the later efforts of workers from each colony to eliminate the "other" queen. The diminished aggression in worker-worker interactions leaves each queen virtually undefended.

These results suggest that the familiar terms "territoriality" and "aggression" may be too general to be of value in attempting to illuminate origins of raiding behavior in this group. What is needed is both a more detailed description of this behavior and an understanding of the diverse mechanisms which regulate it. One possibility is a selective rapid habituation to conspecifics (interspecific pairing of colonies resulted in continuous high levels of aggression). Another possibility is suppression of aggression by some other means, possibly chemical. Many newly mated *P. lucidus* queens somehow manage to become acceptable to host species workers although they initially encounter aggression. Similarly, as described in Experiments 4a and 4b, a few scouts became acceptable to the alien slave species workers to which they were exposed, after initially encountering aggressive responses. _P_. _lucidus_ slave raids seldom involve any visible aggression between raiders and workers of the invaded colony. Hölldobler (1984) has described a pygidial gland found in _P_. _lucidus_ queens and males, the function of which is unknown, and suggests that perhaps this provides some attractant or a "disorganizing alarm substance" (p. 234) to facilitate acceptance.

Clearly these findings concerning ease of penetration into alien conspecific colonies have implications for the evolution of scouting, raiding, and colony founding behaviors. Although aggression is usually absent when _P_. _lucidus_ workers invade a slave species nest, in Experiment 3, when workers were dropped with pupae on the advancing column of raiders, almost all the slave species workers were killed. Whatever may be muting aggression under normal raid circumstances was absent here. Similarly, in Experiments 4a and 4b, all planted slave workers in the raid target boxes were usually killed, either by the scouts or later by the raiders. Thus to gain understanding of the role of "aggression" in the evolution of dulosis, it will be necessary to understand the factors modulating its presence or absence in different contexts.

Possibly related to this curious diminution of aggression in intraspecific encounters is the facility with which pupae from different conspecific colonies can be switched, as shown in Experiment 5, even when evidence of differing rates of eclosion suggests that colony differences are still recognized. Conspecific alien brood has little or no importance as a food source to free-living slave workers, at least under laboratory conditions. However, this relationship is dramatically altered by the presence of the slave-makers. This finding emphasizes the importance of the captured brood to the slave-makers as a food source, and the laboratory and field estimates of the rate of brood consumption (Cool-Kwait & Topoff, 1984; Talbot, 1967; and see Experiment 4) support this view. It seems most reasonable that conspecific pupae acceptance rather than consumption is the primitive characteristic and the use of alien brood as a food source is derived. Still unanswered is the important question of how the alien brood was first introduced into a conspecific nest. More detailed knowledge is needed of foraging patterns, recruitment, and intraspecific interactions described above, among the Neoformica and also in the _F_. _fusca_ species group that also contains many enslaved species. No obvious pattern of behavior, such as the more conspicuous territorial interactions found by Alloway among Myrmicine slave species, is apparent in this group.

Host specificity in raiding and colony founding also may have important implications for the process of speciation in the genus *Polyergus*. One possible mechanism for slave species identification by the slave-makers is imprinting, but this is yet to be demonstrated. If *Polyergus* queens seeking mates are as selective as mated queens in choosing host colonies, and raiders in choosing a raid target, then they might be expected to mate only with males from a *Polyergus* colony using the same slave species. If exclusivity exists on all three levels, then reproductive isolation is present and *Polyergus* colonies using different slave species would be themselves different species. Thus sympatric speciation would be possible due to imprinting of eclosing *Polyergus* brood in a slave-maker colony to the host species present. Territorial interactions between *Polyergus* colonies using the same slave species have been described (Topoff et al., 1984), yet two *P. lucidus* colonies, one using *F. incerta* as slaves and the other using *F. nitidiventris*, were found near Rocky Point, N.Y., living approximately three feet apart. Perhaps this cohabitation was possible because of limited competition due to species differences. More needs to be learned about the mating behavior in *P. lucidus* colonies and the mechanisms of host species identification by the slave-makers. Also a search should be made for potential character or biochemical differences that would support the hypothesis that sympatric speciation is occurring here.

# Appendix

## Identification of Slave Species

Specimens obtained from raided nests in Experiment 2 were identified, without knowledge of the slave species of the raiders, by Stefan Cover of the Department of Ecology and Evolution, Division of Biological Sciences, State University of New York at Stony Brook, according to the following criteria:

1) Specimens reddish to yellowish brown on the head and alitrunk (=thorax). Gaster usually darker. Head and alitrunk usually concolorous, head sometimes slightly darker, never as dark as gaster. Body size typically large. (= *F. schaufussi*).

2) Specimens distinctly brown. In mature workers head and gaster shiny medium brown, alitrunk sometimes concolorous, sometimes lighter with brown infuscation on dorsal surface. Younger workers usually have a lighter alitrunk: yellowish brown with brown infuscation on the dorsal surface. Body size usually small to medium. (= *F. nitidiventris*).

3) Specimens fitting into neither of the above

categories. These ants have the dark heads and gasters characteristic of _F_. _incerta_ but there are too few workers in the samples to provide confidence in this label. The alitrunk color is yellowish or reddish yellow but dark infuscation appears on some specimens.

## Table 1

## SUMMARY OF LABELLING, RECOVERY, AND ADOPTIONS OF *POLYERGUS* QUEENS

| COLONY OF ORIGIN | II | IV | V | X | TOTAL |
|---|---|---|---|---|---|
| Host species* | FN | FS | FS | FS | |
| Labelled | 21 | 12 | 26 | 61 | 120 |
| Recovered | 12 | 1 | 5 | 16 | 34 |
| Laboratory adoptions | 1 | 1 | 1 | 11 | 14 |

*FN - *Formica pallidelfulva nitidiventris*
FS - *Formica schaufussi*

## TABLE 2

## SLAVE SPECIES RAIDED BY P. LUCIDUS COLONIES

| | SAME | DIFFERENT | INTERMEDIATE*** | TOTAL |
|---|---|---|---|---|
| P/S#1* | 43 | 1 | 0 | 44 |
| P/N#10** | 25 | 1 | 2 | 28 |

---

* P. lucidus colony with F. schaufussi slaves
** P. lucidus colony with F. nitidiventris slaves
*** intermediate in characters between the two slave species

## TABLE 3

## PUPAE RETRIEVED

| | P/S #1 | | | P/N #10 | |
|---|---|---|---|---|---|
| DATE | P | P+W | DATE | P | P+W |
| 7/8 | 5 | 12 | 7/4 | 0 | 7 |
| 7/8 | - | 9 | 7/4 | 1 | 10 |
| 7/16 | 5 | 15 | 7/4 | - | 7 |
| 7/16 | 0 | - | 7/10 | 0 | 6 |
| 7/18 | 0 | 2 | 7/10 | 1 | 12 |
| 7/18 | 0 | 2 | 7/17 | 0 | 5 |
| 7/22 | 1 | 4 | 7/17 | 0 | 14 |
| 7/22 | 2 | 6 | 7/17 | - | 7 |
| 7/29 | 4 | 14 | 7/24 | 7 | 13 |
| 7/29 | 4 | 14 | 7/27 | 0 | 9 |
| 8/2 | 3 | 9 | 7/27 | 3 | 8 |
| 8/2 | 2 | 15 | 7/27 | 5 | 7 |
| 8/9 | 1 | 5 | 7/27 | 4 | 8 |
| | | | 7/27 | 3 | - |
| MEDIAN | 2 | 9 | | 1 | 8 |

## TABLE 4

## EFFECTS OF HEAT ON ACTIVITY

### P1
### MEAN ACTIVITY LEVELS*

| | POLYERGUS | | FORMICA | |
|---|---|---|---|---|
| DATE | HEAT | NO HEAT | HEAT | NO HEAT |
| 4/9/84 | 2.53 | | 1.50 | |
| 4/10/84 | | 2.71 | | 1.29 |
| 4/12/84 | 1.44 | | 2.22 | |
| 4/13/84 | | .80 | | .73 |
| 4/14/84 | 3.23 | | 2.69 | |
| 4/15/84 | | .07 | | .33 |
| 4/16/84 | 2.76 | | .77 | |
| 4/17/84 | | 3.23 | | 1.85 |
| 4/18/84 | 3.83 | | 3.17 | |
| 4/19/84 | | .08 | | .31 |
| 4/20/84 | 3.07 | | 2.31 | |
| 4/22/84 | | .38 | | 1.46 |
| 4/23/84 | 3.77 | | 2.46 | |
| MEAN | 2.95 | 1.21 | 2.16 | .995 |
| t | 2.79269<br>p < .01 | | 2.87114<br>p < .01 | |

* activity level: number of workers out of the nest

## TABLE 5

## EFFECTS OF HEAT ON ACTIVITY

### P2
### MEAN ACTIVITY LEVEL*

| DATE | POLYERGUS<br>HEAT | NO HEAT | FORMICA<br>HEAT | NO HEAT |
|---|---|---|---|---|
| 4/8/84 | 8.70 | | 6.30 | |
| 4/9/84 | | 2.00 | | .73 |
| 4/10/84 | 7.60 | | 2.60 | |
| 4/13/84 | 9.40 | | 7.40 | |
| 4/14/84 | | 3.54 | | 3.46 |
| 4/15/84 | 10.47 | | 4.60 | |
| 4/16/84 | | 6.79 | | 1.42 |
| 4/17/84 | 11.00 | | 4.80 | |
| 4/18/84 | | 11.17 | | 4.50 |
| 4/19/84 | 8.23 | | 2.62 | |
| 4/20/84 | | 4.15 | | 1.92 |
| 4/22/84 | 11.79 | | 5.77 | |
| 4/23/84 | | 4.15 | | 3.15 |
| MEAN | 9.60 | 5.30 | 4.87 | 2.53 |
| t | 3.11892<br>p < .005 | | 2.56735<br>p < .025 | |

* activity level: number of workers out of the nest

## TABLE 6

## EFFECTS OF HEAT ON ACTIVITY

### P2
### NUMBER OF SCOUTING TRIPS

<u>POLYERGUS</u>

| DATE | HEAT | NO HEAT |
|---|---|---|
| 4/8/84 | 15 | |
| 4/9/84 | | 1 |
| 4/10/84 | 9 | |
| 4/13/84 | 7 | |
| 4/14/84 | | 0 |
| 4/15/84 | 10 | |
| 4/16/84 | | 4 |
| 4/17/84 | 25 | |
| 4/18/84 | | 5 |
| 4/19/84 | 2 | |
| 4/20/84 | | 0 |
| 4/22/84 | 11 | |
| 4/23/84 | | 0 |
| MEAN | 11.29 | 1.67 |

t 3.11456
$p < .005$

## TABLE 7

### NUMBER OF RAIDS
### PER OPPORTUNITIES GIVEN

| | BASELINE | TEST* | COMBINED |
|---|---|---|---|
| SAND | 0/18 | ---- | ---- |
| PUPAE ONLY | 0/10 | 2/26 | 2/36 |
| EMPTY NEST | 0/11 | 0/26 | 0/37 |
| WORKERS ONLY | 0/7 | 6/26 | 6/33 |
| WORKERS + PUPAE | 11/19 | 9/26 | 20/45 |

* Test Series

Chi square = 11.471
df = 3
$p < .05$

## Table 8

## PUPAE ACCEPTANCE BY SLAVE SPECIES WORKERS

| COLONY | #WORKERS | SURVIVING PUPAE SAME | SURVIVING PUPAE DIFFERENT | PUPAE ECLOSED SAME | PUPAE ECLOSED DIFFERENT |
|---|---|---|---|---|---|
| F23 | 250 | 25 | 24 | 0 | 0 |
| F28 | 550 | 25 | 23 | 7 | 1 |
| F16 | 200 | 22 | 25 | 3 | 0 |
| F24 | 214 | 24 | 21 | 1 | 0 |
| F36 | 354 | 25 | 25 | 2 | 0 |
| F57 | 208 | 25 | 24 | 0 | 0 |
| F55 | 300 | 25 | 22 | 3 | 1 |
| MEAN | | 24.43 | 23.71 | 2.29 | .29 |
| | | t = 1.247<br>df = 6<br>NOT SIGNIFICANT | | t = 2.54<br>df = 6<br>p < .025 | |

## Table 9

## PUPAE ACCEPTANCE: FREE VS. PARASITIZED SLAVE SPECIES COLONY

| FREE LIVING | | | ENSLAVED | | |
|---|---|---|---|---|---|
| COLONY | #WORKERS | #SURVIVING PUPAE | COLONY | #WORKERS | #SURVIVING PUPAE |
| F18 | 94 | 21 | P3 | 150F*<br>120P** | 5 |
| F56D | 82 | 23 | P4 | 140F<br>40P | 15 |
| F76 | 110 | 24 | P5(1) | 415F<br>44P | 9 |
| F15 | 643 | 22 | P5(2) | 100F<br>100P | 8 |
| F32 | 781 | 23 | | | |
| F70 | 100 | 21 | | | |
| MEAN | | 22.333 | | | 9.25 |

$t = 7.3957$
$df = 8$
$p < .0005$

* F = <u>F</u>. <u>schaufussi</u> workers
** P = <u>P</u>. <u>lucidus</u> workers

## Table 10

## SURVIVAL OF PUPAE: SAME VS. ALIEN SLAVE SPECIES

| COLONY | #SLAVES | #RAIDERS | TOTAL F.N.* PUPAE CONSUMED | TOTAL F.S.** PUPAE CONSUMED |
|---|---|---|---|---|
| P5A | 100 | 25 | 15 | 3 |
| P5B | 100 | 12 | 33 | 0 |
| P5C | 100 | 25 | 32 | 2 |
| P5D | 100 | 50 | 12 | 0 |
| P5E | 100 | 100 | 35 | 3 |
| S | 200 | 0 | 33 | 2 |
| MEAN | | | 26.667 | 1.667 |

$t = 5.2380$
$df = 5$
$p < .005$

* of 55 _F._ _nitidiventris_ pupae
** of 55 _F._ _schaufussi_ pupae

## Figure Captions

Figure 1. Portable nest for _P_. _lucidus_ colony.

Figure 2. Distribution of raids by colonies P/S#1 and P/N#10.

Figure 3. Laboratory raiding apparatus.

Fig. 1

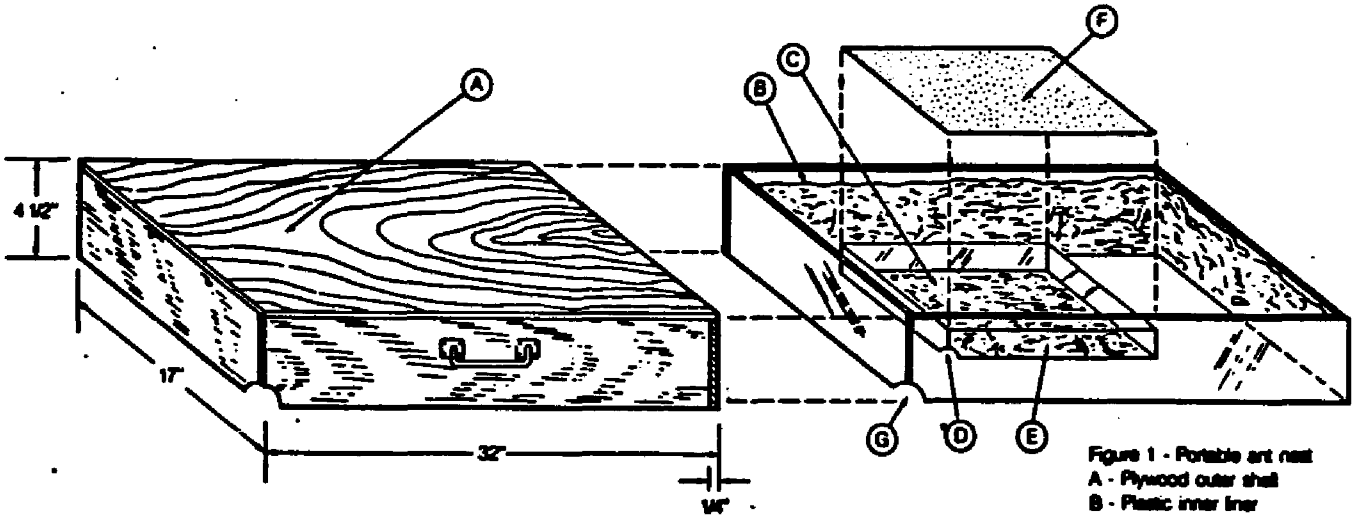


Figure 1 - Portable ant nest
A - Plywood outer shell
B - Plastic inner liner
C - Inner brood chamber
D - Inner entrance
E - Moistened soil
F - Cardboard cover
G - Outer entrance

Figure 2 - Map of *P. lucidus* raids

○ Raided by P/S #1
□ Raided by P/N #10
▲ *P. lucidus* Portable nest

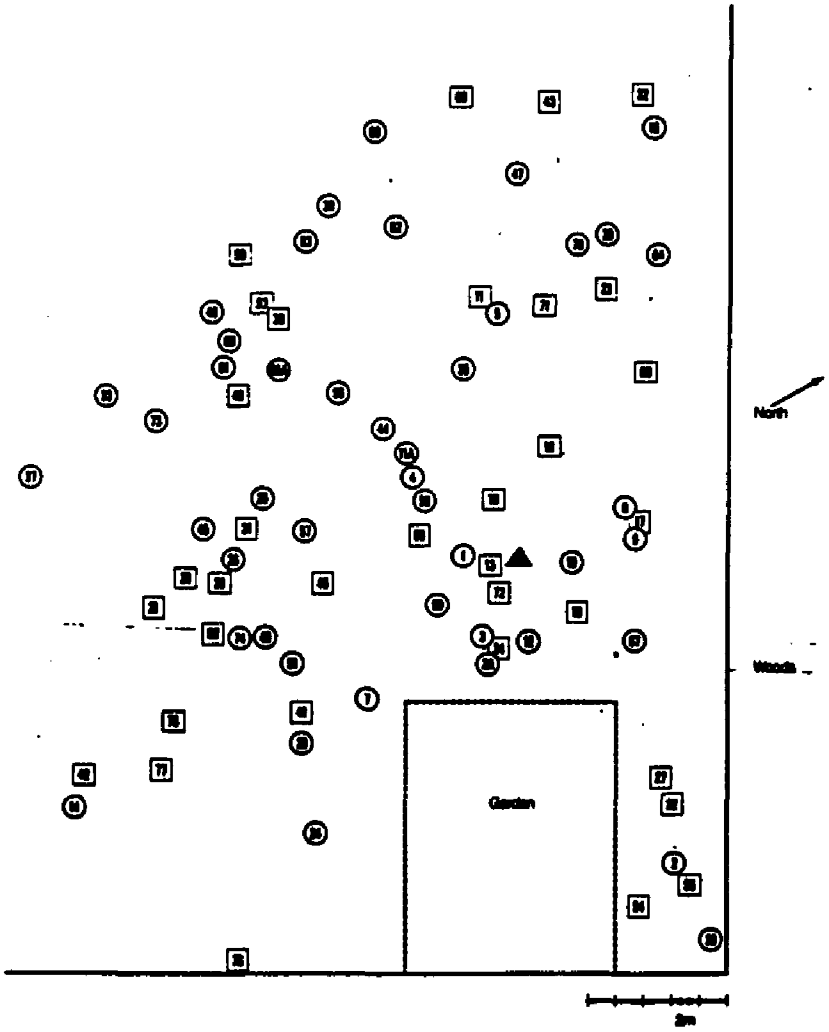
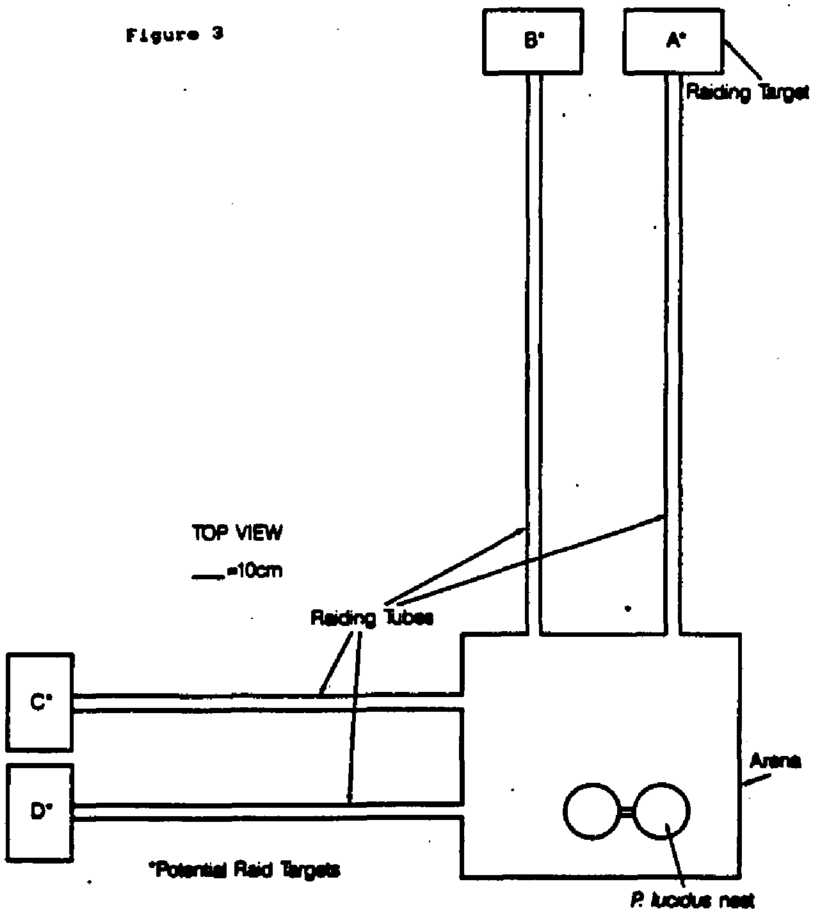


Figure 3


B*
A*
Raiding Target
TOP VIEW
___=10cm
Raiding Tubes
C*
D*
Arena
*Potential Raid Targets
P. lucidus nest

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