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MECHANISMS OF DEPENDENT COLONY FOUNDING IN THE SLAVE-MAKER ANT, *POLYERGUS BREVICEPS* EMERY (HYMENOPTERA: FORMICIDAE)

by

Christine Andrea Johnson

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

Mechanisms of dependent colony founding in the slave-maker ant, ***Polyergus breviceps*** Emery (Hymenoptera: Formicidae)

by

Christine Andrea Johnson

Advisors: Dr. James M. Carpenter, American Museum of Natural History
Dr. Howard Topoff, Hunter College

An essential component in the evolution and maintenance of exclusive insect societies is the ability of individuals to "accept" other members of the society and to reject non-members. The enigma surrounding social parasites is their ability to circumvent being detected as a non-nestmate in order to benefit from the behaviors of another species. The five species that make up the genus *Polyergus* are all slave-makers - social parasites that raid nests of *Formica* for pupae to consume and to "convert" into slaves. The *Formica* workers that develop from the "kidnapped" pupae accept their captors and perform all colonial duties as if they were in their home nest. Unlike many non-parasite (free-living) ant species, newly mated *Polyergus* queens are incapable of establishing their own nest without the help of host workers. Newly mated *Polyergus* queens sequester a supply of host workers and usurp the role of reproductive by invading a *Formica* nest and killing the resident queen.

Polyergus workers and newly mated queens utilize different strategies to deceive host workers, although the catalyst for slave-raiding and nest invasion by queens may be the same. The experiments reported in this thesis examine the processes involved in the integration of newly mated *Polyergus breviceps* Emery queens and their offspring in nests of *Formica* during colony founding. First, the role immature and adult *Formica* offspring play in stimulating *P. breviceps* queens to attack *Formica gnava* Buckley queens is investigated. Newly mated *F. gnava* queens that were provided with foreign eggs, larvae, pupae, and workers did not stimulate *P. breviceps* queens to attack them. Tests conducted every two weeks with maturing *F. gnava* queens revealed that *F. gnava* queens do not "stimulate" *P. breviceps* queens to attack until 28 weeks after *F. gnava* queens had mated, at which point *F. gnava* queens had produced between 14 - 22 workers in the laboratory. Analysis of cuticular hydrocarbons revealed no differences between newly mated *F. gnava* queens that did not induce attacks and established *F. gnava* queens that did induce attacks. However, it seems likely that chemicals are responsible for triggering attacks and that these chemicals are other non-polar and/or polar compounds.

Second, an analysis of cuticular hydrocarbon profiles of newly mated *P. breviceps* queens before and after killing a *Formica* queen revealed that the profile changes after the attack and that the changes are specific to the species of *Formica* queen attacked (*Formica gnava* Buckley or *Formica occulta* Francoeur). Killers developed a hydrocarbon profile that was virtually identical to the species profile of their victim, suggesting that *P. breviceps* queens may acquire cuticular hydrocarbons during attack. These findings are in accord with the hypothesis that *Polyergus* queens use chemical camouflage to avoid being detected as an outsider.

Third, the tendency of *Polyergus*-naïve *Formica* workers to rear *P. breviceps* eggs and pupae as compared to conspecific alien immatures and to heterospecific *Formica* immatures is investigated. The rejection of *P. breviceps* eggs for the first five months after being enslaved indicates that *Formica* are not naturally inclined to rear *P. breviceps* eggs either because *P. breviceps* eggs are naturally attractive or chemically similar.

Instead, some time-dependent modification of the *Formica* nestmate-recognition template or of the chemical signature of *P. breviceps* eggs is probably responsible for the eventual rearing of *P. breviceps* offspring. The increased tendency of *Formica* to adopt *P. breviceps* pupae that are from nests containing a host species that is the same species as test workers suggests that pupae may be tainted with odors of tending *Formica* workers and indicates that pupae may not be suitable for such "brood-attraction" tests. Patterns of hydrocarbons in profiles of eggs and in profiles of pupae did not correlate well with rearing tendencies, indicating that other non-polar and polar compounds are likely to mediate worker recognition of immatures as nestmate.

Finally, a review and in depth analysis of the four hypotheses that attempt to account for the evolution of slave-maker behavior are presented, as well as an alternative conjecture on the evolution of non-independent colony founding. While it seems that raiding for slaves might have evolved out of a response to territorial intruders, invasion by newly mated *Polyergus* queens of host nests being raided by nestmates may have evolved from queen exposure to raiding recruitment signals upon attempting to be readopted by natal colonies after mating.

This thesis is dedicated to my Onkel Edgar,
who died well before his time on September 1, 1999.
His love of nature, his wealth of knowledge, and
his commanding presence have been inspirational.

...recognition of a nestmate seems outwardly a casual matter, usually no more than a pause and sweep of the antennae over the other's body.

Wilson 1971, *The Insect Societies*

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I would also like to express appreciation to the following individuals: Jim Carpenter for wading through my manuscripts; Howard Topoff for introducing me to *Polyergus* and the Chiricahuas; David Grimaldi for bringing me where I wanted to go (thesis completion, AMNH, & Viet Nam); Bob Vander Meer for saying, "Yes, come on down, I will help you with *Polyergus* chemistry", and then doing so; Tam Nguyen for his friendship and unselfish willingness to indulge my needs as a last year doctoral student; Johannes Foufopolous, Tony Frankino, Diane Wagner for their friendship, intellectual and emotional support, and faith in me; Amy Berkov for inspiration and her love of bugs; Michele Hosack for her friendship and unpatronizing help with gas chromatography; Toby Schuh for bearing with me as his scientific assistant while I struggled to finish my thesis and for his daily conversation; Peter Moller for manuscript review and encouragement; Robin Stuart for manuscript review; Barry Lavine for his expertise in chemometrics; Songul Kiyat for taking care of my ants whenever I was away; the staff and volunteers over the years at the Southwestern Research Station; Jean Willi, Christine Weber, Julie Horton, and Hyon mi Chang for emotional support and lots of laughter; Tom Terleph, Katya Ilina, and Daniel Eslava for assistance in the field; Jim Gordon for my bike, which allowed me to escape during my rides to and from school and work; and last, but not least, Bruni and Robert Johnson and Betty de Groh for their patience and for never giving up on me.

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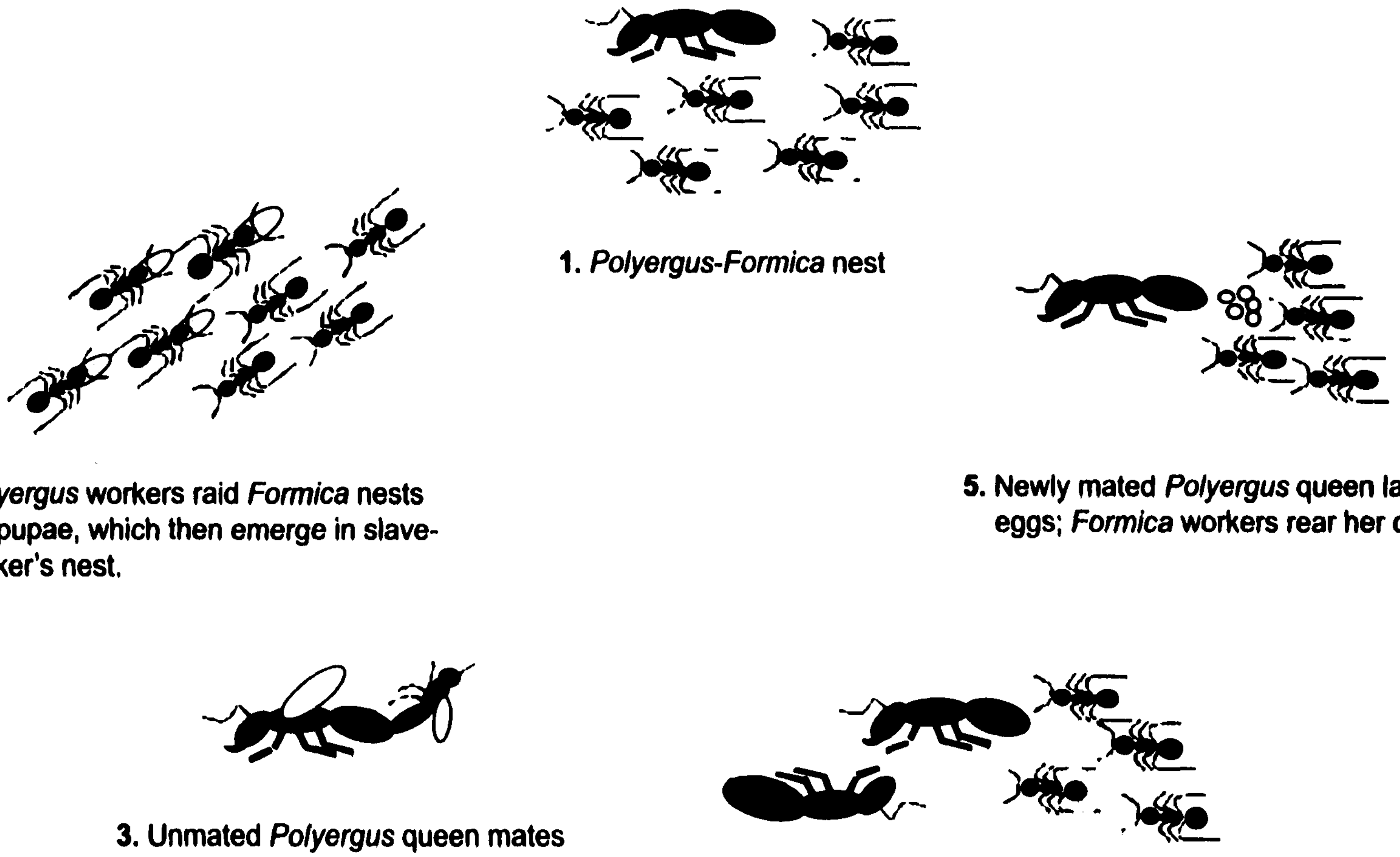
INTRODUCTION

Six Years of Digging for Ants and for Answers

The genus *Polyergus* is one of just a few ant genera that belong to the slave-making club. The five species that make up the genus are distributed across the Northern Hemisphere, in Europe (*Polyergus rufescens* Latreille), Japan and Eastern Siberia (*Polyergus samurai* Yano), North America (*Polyergus breviceps* Emery, *Polyergus lucidus* Mayr) and the former Soviet Union (*Polyergus nigerrimus* Marikovsky) (See Table 5.1 for host species and other species-specific characteristics). All *Polyergus* species rely on species of *Formica* to forage for them, feed and groom them, and rear their offspring. Without these services, *Polyergus* would perish. *Polyergus* acquire their *Formica* hosts, servants, or slaves in two ways depending on the stage in the colony life cycle. A newly inseminated *Polyergus* queen requires a court of slaves to establish her own colony of *Polyergus*. She acquires her slave supply by invading a *Formica* nest and killing the resident queen, whereby the *Formica* workers then treat her as the colony queen. Having effectively usurped the reproductive role, once the number of her offspring (which are reared by the newly enslaved *Formica*) reaches a point in which they can take on a nest of *Formica*, *Polyergus* workers replenish the servant supply by raiding other *Formica* nests for their brood (Fig. I.1). Daily, during the early to late afternoons of the summer months, *Polyergus* workers begin to surface above ground and run around the nest entrance in an excited state. Individual workers, or "scouts"', leave this milling group and "search" for a *Formica* nest. Upon the discovery of one, the scout returns to the home nest and recruits the nestmates gathered near the nest entrance, which follow the leader to the target *Formica* nest. The *Polyergus* storm the *Formica* nest, snatch the pupae, and carry them back to the *Polyergus* home nest. Some of the pillaged brood is eaten, and some mature and become active members of the *Polyergus-Formica* colony.



1. *Polyergus-Formica* nest

2. *Polyergus* workers raid *Formica* nests for pupae, which then emerge in slave-maker's nest.

3. Unmated *Polyergus* queen mates while running along with raiders.

4. Newly mated *Polyergus* queen enters nest being raided, kills resident queen, and usurps workers.

5. Newly mated *Polyergus* queen lays eggs; *Formica* workers rear her offspring.

Figure I.1. Life cycle of *Polyergus* .

The evolution of eusociality has undoubtedly involved the ability to form altruistic behavioral bonds with related individuals and to exclude those which do not share a proportion of the genetic makeup, making the relationship that *Polyergus* has with its host species a paradoxical one. How is *Polyergus* able to penetrate nestmate recognition insulation, especially during colony founding when a *Polyergus* queen and her offspring need to avoid being rejected by adult *Formica*? I eventually came to study some of the mechanisms involved in answering this question via a circuitous route. As an undergraduate student, I was intent on studying sensory perception in sociopaths at King's County Psychiatric Hospital with my then advisor. An elective biology course for nursing students and a Saturday field biology course during my final year as a psychology major completely changed my life. Choosing to give an oral presentation on beetles, I was approached by my biology laboratory professor, who studies Tabanidae, and encouraged to pursue my entomological interest (as most entomologists enjoy doing). The idea of working in wild nature rather than in the minds of wild men was certainly appealing. I had never imagined that one could have a profession working outdoors and with insects. But how was I going to bridge psychology (heavily laden with therapy techniques) and entomology? His reply was simple, animal (insect) behavior.

I graduated, quit my job as an accounting assistant in the bursar's office at New York University (where I maintained thousands of student records by hand), took a job at the Karen Horney Therapeutic Nursery working with emotionally disturbed children, and searched for a program and an advisor that would guide me into the world of insects. One year later, I found myself on Howard Topoff's doorstep - not his New York City doorstep but his Chiricahua Mountains doorstep - one of the most beautiful doorsteps anyone could find themselves. Unbelievably short of students not wanting to conduct laboratory experiments with animals that have been ordered through a catalog, the Biopsychology program had funding available for me to spend my first summer becoming familiar with *Polyergus* at the Southwestern Research Station (SWRS) of the American Museum Natural History (AMNH). I was very excited at the prospect of fieldwork, but I was admittedly somewhat anxious about working in the dry, hot southwest having been raised in a temperate climate. How was I going to survive without water? Without green grass and luscious trees? And, never having done any work with ants (except for occasionally determining how long it would take an ant to burn under a magnifying glass as a child), how would I react to having them crawl all over me? It took only the drive up highway 80 from Douglas, Arizona to SWRS in the station van to fall in love with the desert and the Chiricahua Mountains. As for the six-hour ant nest excavations with *Formica* bombarding me with formic acid - digging puts me into a meditative state and I actually like the smell of formic acid. I still, however, have not acquired affection for carrying large buckets of dirt.

Chiricahua Mountains

The Chiricahua Mountains are just one home to *Polyergus breviceps* Emery, the western representative of two *Polyergus* species found in North America; *Polyergus lucidus* Mayr, the other species, is restricted primarily to the eastern half of United States. *Polyergus breviceps* is also found from Ontario and British Columbia south to California, New Mexico, and Arizona. The Chiricahuas reach about 3048 meters and are particularly species rich as the range serves as a shelter from the surrounding deserts for northern ranging southern species (of plants and animals) and southern ranging northern species. The species diversity is also similar to that which is commonly associated with climatic changes in varying elevational habitats. Mountain ranges that are separated from other mountain ranges by desert often are biotically distinct islands, as they are isolated from similar plants and animals. Weldon F. Heald, from whom AMNH purchased the Painted Canyon ranch and much of the land that now constitutes the research station in 1955, appropriately referred to the Chiricahua Mountains as "Sky Island". Moving up from the desert base to the mountaintop, one can experience the five climatically-distinct Life-zones of the western United States: the desert of the Lower Sonoran, the pinyon-juniper belt or Upper Sonoran, the ponderosa pine-Gambel oak forest of the Transition, the Douglas-fir-aspen forest of the Canadian, and the spruce-fir forest of the Hudsonian. Luckily (for me) SWRS is also found in the Chiricahuas.

Southwestern Research Station

At 1646 meters in elevation, 15.5 kilometers west of the New Mexico boarder, and 46.6 kilometers north of the Mexico border, SWRS is located in the Upper Sonoran Life-zone, where much of the dominant vegetation is alligator juniper (*Juniperus deppeana*), scrub oak (*Quercus turbinella*), and pinyon pine (*Pinus edulis*). A significant portion of my work on *P. breviceps* and *Formica* behavior was completed at SWRS, (from 1992 to 1998) where researchers are provided with laboratory space, access to a variety of supplies for experimental chamber construction, and access to a well-equipped technical equipment laboratory (TEL). Communal living quarters and designated mealtimes foster useful discussions among graduate students, research assistants, volunteers and established scientists. Evening seminars allow students to present their work to a knowledgeable though (sometimes) not overly critical audience. In turn, students are exposed to research that, in some cases, has been ongoing for 20 years or more. Most importantly, there is generally an abundance of eager beavers who want to go into the field and experience fieldwork. These individuals tend to be bright and energetic undergraduates from all over the world that are willing to volunteer their dishwashing, food-preparing, rock-pile moving talents for the opportunity to experience nature and work with researchers. The volunteers have provided me with an invaluable service, even if most decline a second day of turning over large rocks in search of *Formica* nests and then digging them up.

Research Objectives

The purpose of my studies was to elucidate processes underlying three stages of colony founding in *Polyergus breviceps* (Fig. I.2). Alate *P. breviceps* queens are recruited along with their non-reproductive nestmates to a target *Formica* nest. The young queens mate *en route* and enter the raided *Formica* nest soon after arriving. Presumably, in nature as in laboratory tests, the newly mated *P. breviceps* queens subsequently find the resident *Formica* queen and kill her. The objective of my first experiment (Chapter 1) was to ascertain whether *P. breviceps* queens modify their behavior towards *Formica gnava* queens on the basis of *F. gnava* queen offspring, since newly mated *F. gnava* queens do not elicit aggression and have no offspring (Fig. I.2A). When provided with a supply of immature and mature offspring, however, newly mated *F. gnava* queens still did not induce aggressive behaviors in *P. breviceps*. Examination of cuticular hydrocarbon profiles of newly mated and mature *F. gnava* queens revealed no qualitative differences in hydrocarbon constituents or in relative proportions and are unlikely to produce this kairomonal effect.

Once the invading *P. breviceps* queen ceases attacking the resident *Formica* queen, resident *Formica* workers begin grooming the *P. breviceps* queen. My second investigation (Chapter 2) examined whether the *P. breviceps* queen acquires some *Formica* queen chemicals that are critical to nestmate recognition and queen attractiveness during these aggressive interactions (Fig. I.2B). Profiles of cuticular hydrocarbons changed significantly after a *P. breviceps* queen had killed a *Formica* queen, and the change was specific to the species of *Formica* queen killed.

The third and fourth experiments (Chapters 3 & 4) were designed to ascertain whether *Formica* are inclined to rear immature *P. breviceps* pupae and eggs respectively, and whether patterns of surface hydrocarbons could be correlated with the rearing

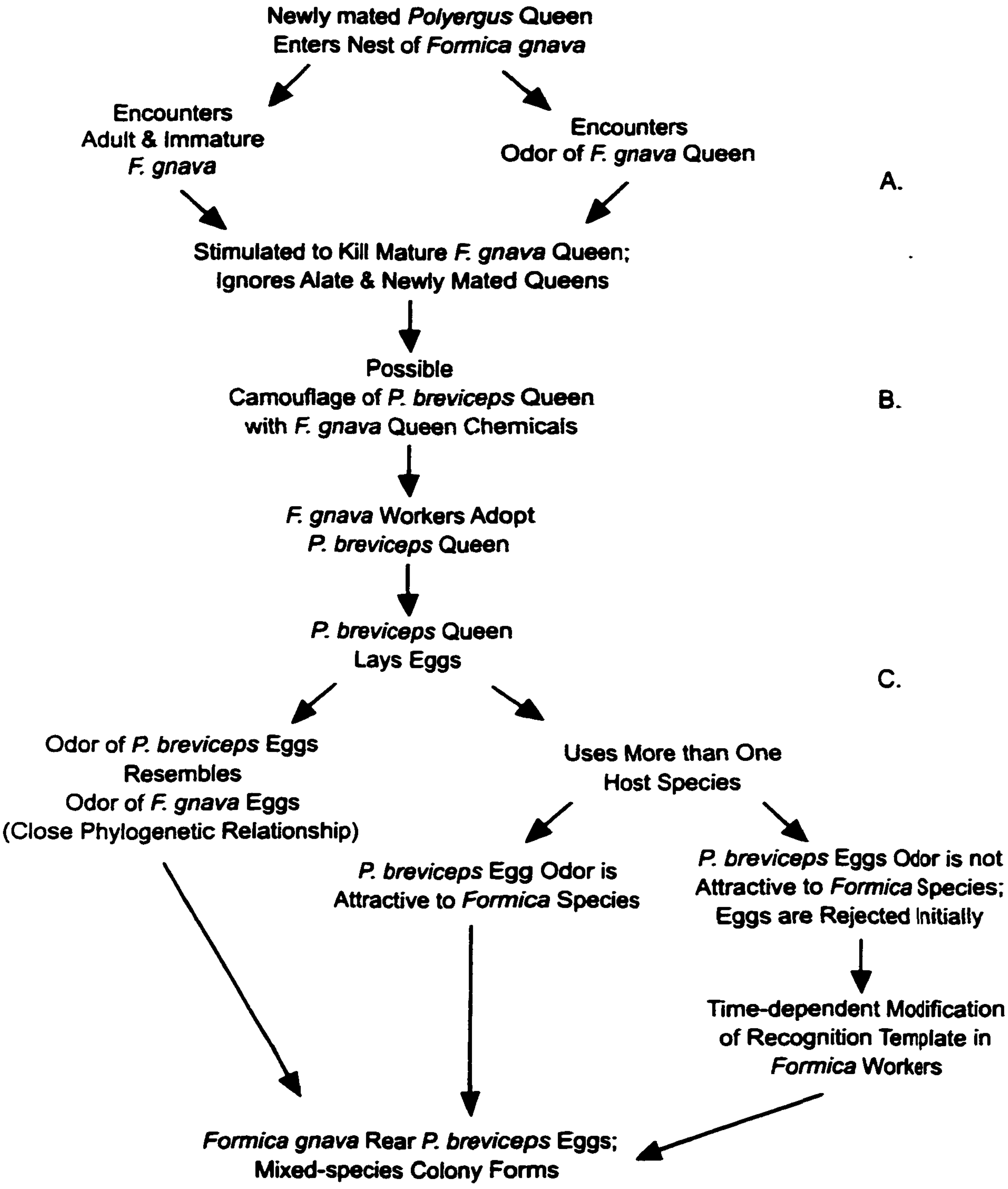


Figure I.2. Diagram illustrating the possible processes involved in colony take over by *Polyergus breviceps* queens, and the three stages (A, B, C) that were investigated in this study. See text for discussion on experiments.

tendencies of *Formica* (Fig. I.2C). Because *Polyergus* evolved from *Formica* or both evolved from a common ancestor, it is often presumed that *Polyergus* shares chemical characteristics with *Formica* and may thereby avoid being rejected by *Formica*. Aggression on the part of *Formica* against invading *Polyergus* queens and between workers of both genera indicates that for adults this is not true. Past research has indicated that *Formica* have a natural tendency to care for *P. breviceps* pupae. However, if post-pharyngeal chemicals, which are considered a source of nestmate recognition cues, are transferred among individuals through allogrooming, the tendency of *Formica* to rear *P. breviceps* pupae may reflect a response to conspecific cues. By observing *Formica* given *Polyergus* pupae from nests with different slave species, I concluded that *Formica* were not necessarily inclined to rear *P. breviceps* pupae and that using pupae in adoption tests was not necessarily reflecting the mechanism underlying the integration of *P. breviceps* offspring during colony takeover by a *P. breviceps* queen. When *Formica* were provided with *P. breviceps* or heterospecific *Formica* eggs, surprisingly they rejected almost all of them. Less than 3% of all heterospecific eggs were reared to adulthood, including those that were presented to newly enslaved *Formica*. And, newly enslaved *Formica* did not begin rearing *P. breviceps* eggs in laboratory tests until 5 – 6 months after having been taken as slaves, suggesting that a time-dependent modification in *Formica*, the *P. breviceps* queen (and her eggs), or both is involved in adopting *P. breviceps* offspring.

The prominent hypotheses regarding the evolution of slave-making behavior are reviewed and discussed in Chapter 5, and will therefore not be discussed here. I also present an alternative hypothesis and data not included in Chapters 1 - 4.

Polyergus: *General Biology & Collecting Methods*

Polyergus breviceps are found on the station grounds down in elevation to the base of the mountains just shy of the desert of the Lower Sonoran Life-zone, where they enslave *Formica gnava* Buckley, and up in elevation to the Canadian Life-zone, where they enslave *Formica occulta* Francoeur. *Polyergus breviceps* begin their slave-raiding forays sometime in May, and by June they are raiding daily. SWRS populations begin raiding between 15:00 - 19:00 hours. Raids by the Barfoot populations (el. 2750 m) begin as early as noon and cease around 16:00 hours. Alate females first appear at nest entrances and in raiding columns at the end of June, and become increasingly abundant once the monsoon season begins in early July. Reproductives continue to be produced throughout July and some colonies may produce alates as late as August 10th. *Polyergus* is protandric: males begin appearing at nest entrances slightly earlier in season and in time than female reproductives. Mating of *P. breviceps* reproductives on slave raids makes collecting newly mated *P. breviceps* queens very easy, and sometimes 25 newly mated queens were collected at a raided nest on a single day. Collecting newly mated *F. gnava* queens is much more unpredictable. There are no known reports of their mating swarms and during my six years in the field I have never observed one. The majority of newly mated *F. gnava* queens collected during 1995 - 1998, were found on one day of each summer, between the hours of 13:00 to 15:30 after several days of medium rainfall or one day of very heavy rainfall of the seasonal rains. Newly mated *F. occulta* queens were similarly found by searching the ground once the monsoon rains began.

Polyergus breviceps alates for the most part mate on raids. However, when conditions are extremely dry, raids cease and alates take flight. This was the case in 1994 when 25,000 acres of the Chiricahuas burned. Drier than normal, a small fire started bringing swaths of smoke from miles away down into the canyon enclave where SWRS is located. Soon pines could be seen and heard exploding into flames up on Snowshed

Peak. The station, often a lure for animals, was even more so frequented by black bears, deer, and peccaries desperate for water, escape, or both. The hummingbirds that normally come in droves to the feeders in front of the main office disappeared. Bats were found lying dead on Cave Creek Road. Smoke was at times so intense that the only refuge for us was the air-conditioned TEL.

Volunteer firefighters, of which the most prized for their swift action, strength, and endurance were the Mexican and Indian women, were bused in from Idaho, Nebraska, and Wyoming. Eventually the station director, Wade Sherbrook, announced that all the fire lines had been jumped and the fire, only a mile a way, would force us to evacuate the station the following day. The community center in Rodeo, New Mexico, where the July 4th barbecue and dance are held, was to provide us with temporary shelter. That evening, camped out under the stars on a hill that provided a birds-eye view of the fire, I felt the first rain drops. Within moments, wind, lightning, thunder, and rain roared up the canyon. The firefighters, sacked-out in their cots on the station volleyball court, ran into the main house. By morning the rain had put out the fire. Unfortunately, this was not an indication that fieldwork could resume immediately. Some field sites had been completely burned or were off limits. For many whose study sites were unscathed, the study animals just did not want to participate. The *Polyergus* on station grounds were not raiding, and most alates had flown; the Barfoot area, my second field site, had been closed off.

Departing earlier from SWRS than I had planned, I had hoped to catch the mating flights of either *P. breviceps* in Allerton State Park near Champaign-Urbana, Illinois, or *P. lucidus* in the Pine Barrens of Long Island, New York. In Allerton, where John Marlin, a fellow myrmecologist, had acquired a permit for me to work in the state-park, I was able to find one colony of what appeared to be *P. breviceps*. But, by August 7th, alates appeared to be long gone. I still, however, had *P. lucidus* to look forward to on Long Island. The night prior to my scheduled visit with Raymond Sanwald, who would show me where I could find *P. lucidus* in the Pine Barrens, I received a call from him reporting that the area was on fire. Sure enough, I turned on the television and watched in amazement as, once again, the pines of a potential study site turned bright orange and red. My 1994 field season was chalked up as a zero, and a year would pass before I could increase my data points.

Nesting Sites

Mixed nests of *P. breviceps-F. gnava* and of *P. breviceps-F. occulta* are relatively uncommon and their nesting sites are somewhat non-descript, found under logs, large rocks, or just a bunch of grass. Disturbance of a mixed-species nest generally produces a defense swarm of only *Formica*; the *Polyergus* remain below, deep within the ground. *Polyergus* nests are more frequently found by stumbling across a slave-raid. Over the years members (immatures, workers, reproductives, slave-makers, slaves) from ten colonies of *P. breviceps-F. gnava* and from four colonies of *P. breviceps-F. occulta* were collected for use in experiments in addition to new colonies I created in the laboratory and the 50 or so *F. gnava* and *F. occulta* nests I excavated. *Formica gnava* and *F. occulta* nests are much more common and generally easier to find than *Polyergus* nests, particularly the larger, polygynous nests, which are often disclosed by the *Formica* activity surrounding the nest. Smaller nests are less obvious, and raiding *Polyergus* often reveal their whereabouts. Providing a forager with food and following it back to its nest is also a useful method for finding nests. Using both methods, I was able to determine the general density of *F. gnava* nests in 100 X 100-meter area around a large *P. breviceps* nest (Fig. I.3).

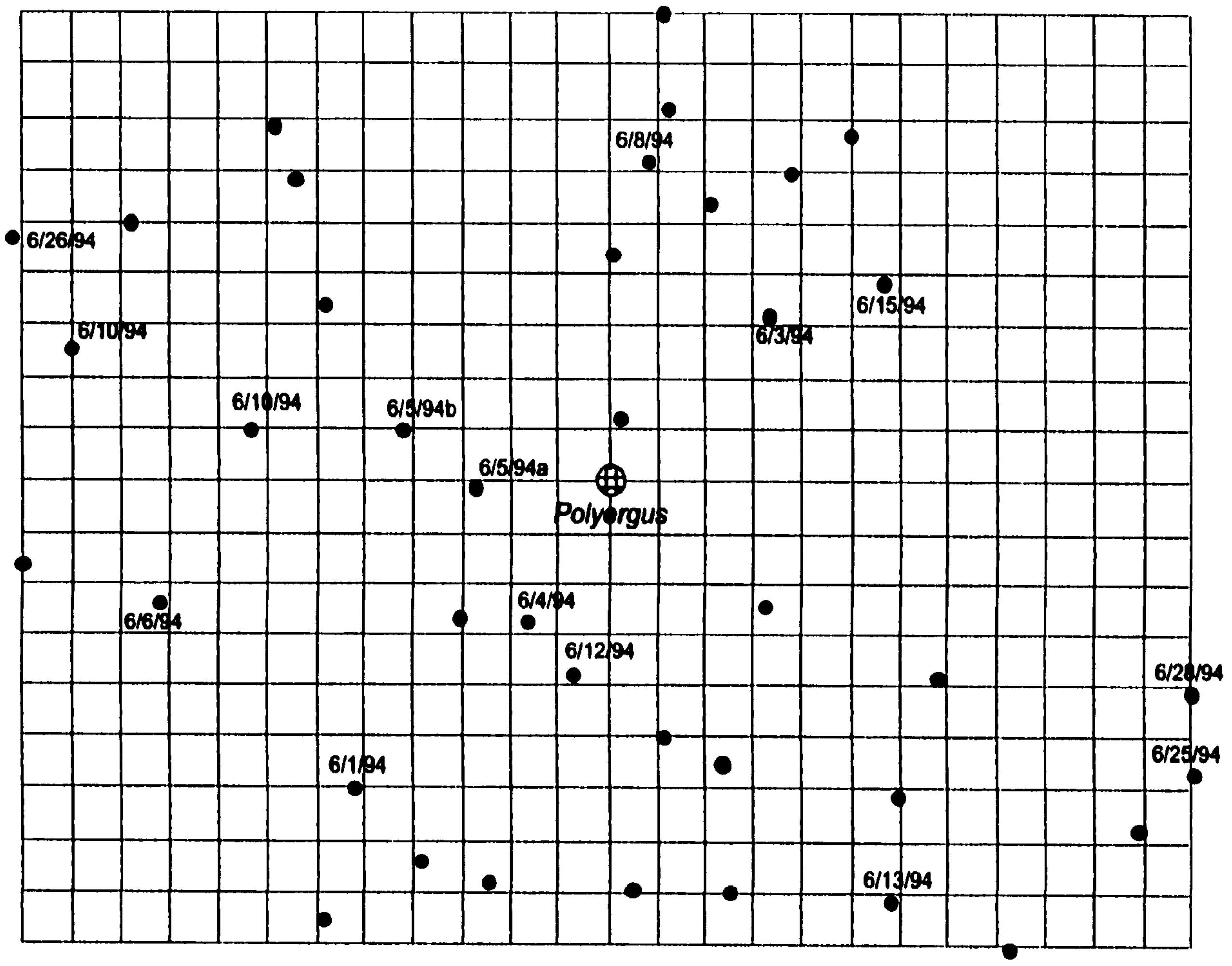


Figure I.3. Density of *Formica gnava* (●) colonies around a single *Polyergus breviceps* (⊕) colony during June of 1994. Dates are given for *F. gnava* colonies that were raided by *P. breviceps* during censusing.

Rhus radicans *(Poison ivy)*

Sometimes, good-sized nests can be found among poison ivy (*Rhus radicans*). However, even if you appear not susceptible to poison ivy, cutting away the vines and digging up the nest among the cut vines for several hours is not advisable. In desperation to collect a large colony of *F. gnava*, this was my precise method. As the effects of poison ivy did not surface immediately, I delayed treatment for exposure to poison ivy and spread the virulent oils in my sleeping bag and my clothing. After two weeks oozing lesions had spread to the base of my eyes and I was so uncomfortable I placed a 7:30 a. m. call to Howard Topoff begging that he drive me 75 miles to Douglas to see a doctor. Swollen and covered from head to toe with weeping, itching sores, one volunteer looked at me that morning and remarked that if she looked that bad, she wouldn't come out of her room. It was difficult for me to leave my room as I was passed out from drinking copious amounts of Benedryl in addition to taking the steroid, Prednisone.

Traveling Ants

Many of my tests required observations to be made over many months, so not all work could be completed at SWRS and my ant colonies were brought to Hunter College, and later to the AMNH. The first few summers, for lack of funds to rent a car, my ants were transported by plane. Intent on keeping them alive, I refused to put them on the plane as luggage and quite naïvely explained to the woman at the ticket counter that my carry on luggage contained the ants I was studying for my dissertation. That (ants on a plane) was an even bigger no no, against FAA regulations. I insisted on talking to the manager, whom she summoned for me with great leisure even though my plane was leaving in 5 minutes. The manager was so interested and excited about the ants and the project, he had the departure delayed (only briefly) and personally walked me to the plane, fending off other attendants who kept insisting I could not bring these boxes onto the plane. The next leg of the trip, from Chicago to New York, was by Greyhound. Crates of equipment, books, papers, and ants in tow, I loaded myself onto the bus that would drive for 19 hours. Ants again went in the overhead bin. This time, however, they started to escape. My seatmate, a rather large girl who was wearing eau de McDonald's, made this discovery and turned to me and whispered, "This is so gross; there are ants on this bus! I just found one crawling on me while I was sleeping". And lo and behold, there were my ants crawling around on the seats. I just prayed that there would not be a mass exodus out of the box(es) and that no one would discover were they were coming from. (At least on this bus trip my seatmate did not repeat the ENTIRE time that the ants were the work of the devil, much like rain and lightning obviously are.)

Fortunately, only a few trips to and from the field involved such bus transport. In the years following, I avoided the plane/bus hassle and succumbed to at first renting a car, and then buying one (with a generous donation from the Mom and Dad Johnson Fund). I now had the means to expand critically my fieldwork to the population of *P. breviceps* at higher elevation where *F. occulta* serves as the host. My own car, however, also meant driving 6,000 miles roundtrip alone. But, at least the limitations on the numbers of colonies that could be brought back to New York lessened substantially. And each year about 250 lbs of ants and dirt were sifted into gallon-sized freezer bags, packed into Styrofoam coolers with Blue Ice® ice packs, and piled to the ceiling in my car. Amazingly most of the ants survived the long, often very hot, journey to New York (and sometimes back again to Arizona!). Unfortunately, mites inhabiting the soil adapted to the New York City climate better than the ants. After two weeks in the laboratory, all my colonies were inundated with mites of several types, one of which latched themselves onto the ants. Within days, the mites blanketed some of the ants so much so that the ants could barely walk. The ants had to be aspirated out of the soil and placed in new containers that were soil free. The mites migrated from the ants to the only source of moisture in these containers, a small wad of moist paper towel. The towel was replaced daily, and eventually the mites disappeared. After approximately 5 weeks, nest soil that had been baked for 1/2 an hour to destroy any remaining mites was added back to the containers to provide some substrate for the ants. If there is a way to insure getting formicosis (formic acid in the lungs), one week of intense aspirating does it. In fact, working at all with the soil in the southwest almost guarantees Valley Fever. My incessant coughing and regular swelling almost shut of my throat during the summers led some at SWRS to conclude that I had Valley Fever. I, of course, thought this was a joke, particularly when Jay Cole remarked that people who smell strange smells in their cars have Valley Fever (I kept smelling burnt rubber). But Valley Fever does exist and about 80% of the people in the southwest have this fungus that is found in the wind-blown dust in their lungs.

Fire Ant Laboratory

The results of my behavioral tests as well as the experiments of others were indicative of the chemical relationship between *P. breviceps* and its two host species. However, only examination of the cuticular chemicals, or the patterns of chemicals that make up the cuticular profile, could verify this. Hydrocarbons have been implicated in mediating nestmate recognition processes due to their proportionately large contribution to cuticular chemical profiles and because hydrocarbon profiles are species, colony, and caste specific. Therefore, I chose to concentrate my analyses on this class of compounds. Knowing nothing about what was entailed, I sought the help of Dr. Robert K. Vander Meer to help me accomplish this task. He sent solvent and Pasteur pipettes to SWRS along with a set of directions on how to extract cuticular chemicals. Once my summer field season was completed, I would then travel to his laboratory at the United States Department of Agriculture Agricultural Research Service in Gainesville, Florida, to conduct the chemical analyses. As behavioral experiments would have it - my newly mated *Polyergus* queens were not producing the eggs that I had anticipated - I had to delay my trip to Gainesville for nine months. Finally, ants in tow once again Gainesville was the first, stop on my way to my final field season at SWRS.

Cuticular Hydrocarbons

The laboratory in Gainesville allowed me to usurp storage space, equipment, supplies, and, most importantly, technicians during the month I resided there. Here analysis of my cuticular extracts would reveal the mystery underlying the behavioral results, well at least to some degree. As hydrocarbon compounds began eluting from the gas chromatography column, patterns emerged that were so distinct I could identify not only the different species, but whether the individual was an adult or immature, and in the case of *P. breviceps*, whether the adult was a newly mated queen. Visual examination of gas chromatographic data, however, is insufficient for analysis because chemical data sets tend to have large numbers of interrelated measurement variables. Therefore to ascertain real differences or similarities in hydrocarbon profiles, principal component analysis (PCA) was used. PCA reduces the "dimensionality" of chemical data by transforming original measurement variables into new, uncorrelated variables called principal components, and then finds directions of greatest variance in the data. Each gas chromatogram is represented by a data vector (or a point in an n-dimensional Euclidean space) and reflects a period of standardized retention times that correspond to hydrocarbon peaks. A set of chromatograms, therefore, is represented by a set of points in an n-dimensional space. The points (eigenvectors) that represent chromatograms from one class (e.g. *F. gnava* queens) cluster in a limited region of a measurement space distance from the points that correspond to another class (e.g. *F. occulta* queens) when they are mapped. Because these data projections are made without information on the class of the samples, the separation (or cluster) of data points is a strong indication of real differences (or no differences) in the patterns of cuticular hydrocarbons that are reflected in the gas chromatographic profiles.

CHAPTER 1

Queens ripe for the killing: When the slave-maker queen, *Polyergus breviceps*, kills a host queen

SUMMARY

Queens of the slave-maker ant, *Polyergus breviceps*, require the foraging, feeding and brood care services of host workers to establish a colony. *Polyergus breviceps* queens acquire their first servants by invading a nest of *Formica* and killing the resident queen. *Formica* queens that have recently mated, however, are not targets for attack. To determine whether *P. breviceps* queens select their victims on the basis of worker and/or immature presence, eggs, larvae, pupae, and workers from mature *Formica gnava* queens were placed with newly mated *F. gnava* queens and the responses of introduced *P. breviceps* queens were observed. *Polyergus breviceps* queens did not attack the newly mated queens, even with foreign offspring, therefore pairs of *P. breviceps* and newly mated *F. gnava* queens were tested every two weeks until aggression ensued. Testing on day 204, 29 weeks after *F. gnava* queens had mated, resulted in *P. breviceps* queens attacking the *F. gnava* queens in all four tests conducted. This suggests that, while offspring may be the benefit that results from attacking an established *F. gnava* queen, *P. breviceps* queens seem to detect mature queens using another time-dependent feature, one that might be a reliable indicator of reproductive status. Ovarian development and dominant queen status have been correlated with cuticular hydrocarbon profiles. In this study, however, hydrocarbon profiles of newly mated and established *F. gnava* queens were indistinguishable. Nonetheless, my findings suggest that *P. breviceps* queens avoid attacking *F. gnava* queens that are unable to supply a work force immediately.

INTRODUCTION

Social parasites rely on other species to fulfill tasks that enable a colony to survive, such as foraging, feeding, and brood care (Wilson 1971). Queens of socially-parasitic ant species are typically incapable of founding new colonies independently, often lacking the fat stores found in most non-parasitic ants that are needed to survive the period of isolation before the first brood matures (Keller & Passera 1989). Occasionally these queens form new colonies by departing from their natal nests with a supply of host workers (Marlin 1968, 1969; Wilson 1971; Buschinger *et al.* 1980; personal observation 1997). More commonly, they invade other colonies and become accepted as members. Inquiline queens coexist with both queen and workers of their host species, and it is not uncommon for new parasite queens to reproduce within the nest. Queens of many dulotic and temporary parasitic species, on the other hand, are initiated into allospecific societies by killing the resident queen of their host species. Slaves of the temporary parasite fulfill the colony duties but are not replaced by new slaves once they have died. Eventually the colony functions independently with offspring of the slave-maker queen taking over the chores. Dulotics, or slave-makers, on the other hand, continue their dependent relationship and restock the slave force by stealing immatures from other nests (Buschinger 1986; Hölldobler & Wilson 1990; Topoff *et al.* 1984; Topoff *et al.* 1985).

Polyergus is probably the most formidable of the dulotic taxa, replenishing their slave supply by regularly invading and stealing brood from nests of *Formica*. *Polyergus* queens frequently use these raids to locate a *Formica* nest to usurp. In taking over a *Formica* nest to establish her own colony, a *Polyergus* queen fatally attacks the resident queen (Emery 1908; Wheeler 1906, 1916; Topoff *et al.* 1985). *Formica* workers, which typically assault invading an *Polyergus* queen, immediately begin grooming her after she ceases attacking their queen.

Tendencies of *Polyergus* queens to fatally attack *Formica* queens seem to be commonplace and necessary for taking over a nest. The percentage of *Formica* colonies that adopt a *Polyergus* queen is greater if the *Polyergus* queen had killed a queen of the same *Formica* species as the adopting workers (79% - Topoff *et al.* 1988; 54% - Topoff *et al.* 1990; Topoff & Zimmerli 1993; Zaayer 1967) than if the *Polyergus* queen had attacked a queen of another *Formica* species or had not attacked a *Formica* queen at all (12% - Topoff *et al.* 1988; 38% Topoff & Zimmerli 1993). When denied the opportunity of killing a host queen, *Polyergus* queens tend to be killed by host workers (e.g., Topoff *et al.* 1988; D'Ettorre *et al.* 1997).

Not all types of *Formica* queens of the respective host species are attacked, however. Laboratory tests have shown that *Formica gnava* Buckley queens that have recently mated (Topoff & Zimmerli 1993; Zimmerli & Topoff 1994) and alate *Formica* queens do not induce attack behavior in *Polyergus breviceps* Emery queens. This indicates that newly mated and established *F. gnava* queens differ somehow and that there has been selection on *Polyergus* queens to be able to detect and respond to these differences.

What are *P. breviceps* queens responding to when they attack an established *F. gnava* queen? Because a slave force is crucial to the survival of founding *P. breviceps* queens, the most obvious stimulus inducing attack behaviors would be the presence of offspring. Contact with workers might reliably "inform" an invading queen that she would gain slaves, while contact with immature offspring might "communicate" future slaves. It is thus conceivable that host species offspring, mature or immature, may induce *P. breviceps* queens to attack *F. gnava* queens. Alternatively, established *F. gnava* queens may differ in chemistry from their spared counterparts. Parasites and parasitoids commonly use chemical products of their hosts to locate them. Sometimes host chemicals trigger the parasite to oviposit into or to feed on the host[1] (Lewis *et al.* 1976; Conti *et al.* 1996). A number of parasitoid species seem to choose their hosts using the surface hydrocarbons of the host as well as of the plants on which the hosts feed (Espelie *et al.* 1991).

Among social parasites, cuticular hydrocarbons may similarly mediate aggressive behavior against host queens. In some ant species, up to 90% of the cuticular lipids are non-polar compounds (Jackson & Blomquist 1976) making these compounds likely targets for use in trying to find a host. Quality, quantity, and relative proportions of cuticular hydrocarbons are frequently characteristic of a species or colony (Gamboa *et al.* 1986; Bonavita-Cougourdan *et al.* 1987, 1989, 1993; Vander Meer *et al.* 1989; Nowbahari *et al.* 1990; Dahbi *et al.* 1996) and would allow a social parasite to become specialized on a particular species. Furthermore, cuticular hydrocarbon profiles of ants differ consistently between immatures and adults (Bonavita-Cougourdan *et al.* 1988, 1990), among immatures at different stages of development (Bagnères & Morgan 1991; Fénéron & Jaisson 1995; see Chapter 4), and among adults of different castes of the same species (Bonavita-Cougourdan 1990; Wagner *et al.* 1999). Thus, parasites could further specialize on a particular caste or stage of development by using the differences in cuticular hydrocarbons patterns.

Unique patterns of hydrocarbons in established *F. gnava* queens could hypothetically provide an inadvertent signal that elicits attack behaviors from *P. breviceps* queens. As relatively non-volatile compounds, hydrocarbons are less likely to saturate sensory receptors, even in the spatially restricted nest chambers of many social insects

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[1] The term kairomone is often used to denote a chemical product that elicits a behavior detrimental to the emitter. However, the use of the term is under debate because it implies that kairomonal products are independent of the chemicals considered pheromones and allomones and not useful to the emitter, only detrimental (Blum 1977). Kairomonal effect has been proposed as an alternative and will be adopted for the purpose of this paper.

(Blomquist & Dillwith 1985). Other more volatile compounds might inhibit sensory reception after saturation, and if they were involved in the attacks on *F. gnava* queens by *P. breviceps* queens, attacks would probably not be sustained for 20 min or more.

In this paper, I tested whether *Formica* offspring serves as a stimulus for aggression in *P. breviceps* queens by providing newly mated *Formica gnava* queens with eggs, larvae, pupae, and workers from another established *F. gnava* queen and observing *P. breviceps* queen responses. Whereas lone established *F. gnava* queens were attacked, newly mated *F. gnava* queens supplied with immatures and callows were not attacked. Pairs of newly mated *F. gnava* queens and *P. breviceps* queens were therefore tested every two weeks until aggression ensued. Hydrocarbons of established *F. gnava* queens and newly mated *F. gnava* queens were analyzed to determine whether the queens could be distinguished on the basis of their principal components.

METHODS

Ant Collections

During June and July (1995 - 1998), I collected queenright colonies of *Formica gnava* from the Arizona oak-alligator juniper woodland grounds of the Southwestern Research Station of the American Museum of Natural History in the Chiricahua Mountains of southeastern Arizona (el. 1646 m). The colonies were all monogynous to insure that the "established" *F. gnava* queens used in this study were not uninseminated dealate queens that are sometimes found in large polygynous colonies. Colonies were kept in large Fluon®-coated Tupperware® boxes (20.5 x 45 x 20 cm) with original nest soil. Newly mated *F. gnava* queens (8 in 1995, 2 in 1996, 3 in 1997, and 32 in 1998) were found by searching the ground during the beginning of July when the "monsoon" season begins.

Newly mated *P. breviceps* queens were collected as they approached the *F. gnava* nest being raided by their nestmates during June, July, and August of each year.

Housing & Food

All queens were kept in individual plastic vials with a small moist cotton ball until testing time. After testing, *F. gnava* queens were left in their testing box. *Polyergus breviceps* queens were either 1) placed back into their individual vials, if tested with a *F. gnava* queen that did not induce aggression, or, 2) placed with the workers of the *F. gnava* queen that induced aggression and which the *P. breviceps* queen subsequently killed. Queens were provided with a drop of 1:1 honey/water solution on the cotton ball when in individual vials. Otherwise, colonies were provided local insects when in Arizona or with crickets (Fluker Farms) when in New York, the Bhatkar & Whitcomb (1970) diet, 1:1 honey/water solution, and water when needed.

Behavior Tests

All *P. breviceps* queens were tested with a newly mated *F. gnava* queen alone or with a full complement of brood (5 eggs, 5 larvae, 5 pupae, 5 callows) from another *F. gnava* queen (Table 1.1). However, only those *P. breviceps* queens that had killed an established *F. gnava* queen were tested in the remaining conditions (Table 1.1). Only these "experienced" queens were adopted by *F. gnava* workers and survived long enough for further testing. Queens without host workers died within 10-15 days after collection. I was also restricted to using experienced *P. breviceps* queens because production of new *P. breviceps* queens ceases early August, and aggression towards newly mated *F. gnava* queens did not occur until February. The order in which *P. breviceps* queens were first tested with *F. gnava* queens was counterbalanced: some were paired with an established *F. gnava* queen on their first trial, and others were paired with newly mated *F. gnava* queens alone or with brood for their first trial.

Table 1.1

Percentage of trials in which *Polyergus breviceps* queens attacked established *Formica gnava* queens, and newly mated *F. gnava* queens with respect to the number of offspring produced and the number of days since mating

	Newly-mated *Formica gnava* Queen								Established *Formica gnava* Queen
TESTING CONDITION	**Alone**	**Alien Eggs, Larvae, Pupae, Workers**	**Own Eggs**	**Own Eggs, Larvae**	**Own Eggs, Larvae, Pupae**	**Own Eggs, Larvae, Pupae, Workers**			**Alone**
PERCENT* AGGRESSION	**0%** N = 20	**0%** N = 12	**0%** N = 10	**0%** N = 10	**0%** N = 10	**0%** N = 10	**0%** N = 10	**100%** N = 4	**100%** N = 15
MEAN # OF OFFSPRING (Foreign or Own) **AT TEST TIME**	None	E = 5 L = 5 P = 5 C = 5	E = 13	E = 5.46 L = 8.19	E = 8.43 L = 2.17 P = 8.61	E = 6.95 L = 5.23 P = 3.86 W = 8.23	E = 1.8 L = 3.9 P = 5.6 W = 11.4	E = 3.5 L = 4 P = 1.5 W = 14.5	Unknown
DAYS SINCE MATING	1 - 3	1 - 3	4 - 13	14 - 30	31 - 50	51 - 65	190	191 - 204	Unknown

N = number of queens tested, NA = not applicable, E = eggs, L = larvae, P = pupae, W = workers.

* The first worker emerged on day 51 and by day 65, all incipient *F. gnava* colonies had at least one new worker. From this time on, pairs were tested every two weeks until aggression ensued.

All tests were conducted between the hours of 3:00 p.m. and 7:00 p.m., the period during which raiding and colony takeover normally occurs. Preliminary tests with established *F. gnava* queens at other times of the day were frequently unsuccessful; *P. breviceps* queens did not attack established *F. gnava* queens. Re-testing during typical raiding hours with the pairing of the same individuals resulted in attacks in 100% of the observations. This suggests that there may be a circadian component in reception and response to the cue(s) which stimulates attack, much like the daily rhythms of milling, scouting, and being activated to raid (Topoff *et al.* 1984, 1985).

Trials were conducted weekly for the first two months and bimonthly until attacks on newly mated *F. gnava* queens occurred. The number of offspring produced by the newly mated *F. gnava* queen and the number of days from the date of collection (likely to be day of mating) were recorded. Periodically, *P. breviceps* queens were tested with an established *F. gnava* queen to verify that they would still attack a *F. gnava* queen long after having mated and usurped a small colony, which they did.

Thirty minutes prior to the introduction of the *P. breviceps* queen, *F. gnava* queens were placed in individual Fluon®-coated Tupperware® boxes (20.5 x 35 x 9 cm) lined with soil. When the condition included brood, alien or own, they were placed along with the *Formica* queen into the testing box. If the condition included workers and the workers attacked the *P. breviceps* queen, the trial was terminated, the *P. breviceps* queen was removed, and the trial was repeated the following day without workers in the testing chamber.

Observations of queen interactions were 20 minutes unless a *F. gnava* queen was being attacked, at which point the observation continued until the *P. breviceps* queen stopped and began wiping her antennae. The latency to attack, and number and duration of attacks were recorded. Attacks were defined as a strike (an attempt to bite) or a sustained bite. T-tests were used to compare latencies to attack between established *F. gnava* queens and newly mated *F. gnava* queens that induced aggression, and to compare the total duration of the attacks. T-tests were also used to compare the number of offspring produced by newly mated *F. gnava* queens at the time they induced aggression with the number of offspring produced 15 days prior to inducing aggression.

Chemical Analysis

Cuticular components were extracted from individual newly mated *F. gnava* queens and established *F. gnava* queens by immersing them in 2 ml of high purity hexane (B & J, GC^2 Grade) for 10 min in 7 ml scintillation vials. *Formica gnava* queens attacked by *P. breviceps* queens were immersed in hexane immediately after the *P. breviceps* queen ceased her attack but before death of the *F. gnava* queen. Solvent extracts were transferred from the sample with a fresh Pasteur pipette to a 2 ml scintillation vial, allowed to evaporate, and transported to the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida where chemical analyses were performed. Specimens were preserved for voucher in ethyl alcohol (70%).

Evaporated solvent extracts were reconstituted with 0.2 ml hexane, vortexed for 1-2 sec, and then applied to a small silicic acid (70-230 mesh, 60Å, Aldrich Chemical Co., Inc.) Pasteur Pipette column. Hydrocarbons were isolated from other lipids by eluting the column with hexane. The elutant, containing purified hydrocarbons, was evaporated to ca. 10 µl under a stream of nitrogen. Samples were analyzed by gas chromatography (Varian 3700 equipped with a split-splitless injector, a capillary column [J & W, DB – 1 30 m] and flame ionization detector). The injector and detector were 300° C; the oven was programmed from 190° to 290° C at 5° C/min, and then held at 290° C for 5 min. Hydrogen was the carrier gas and nitrogen was the make-up gas. Standards (C16, C18, C20, C22, N–Parafin mix, 500 mg/5 ml in hexane stock, #NP-MIX-H, Alltech Associates, Inc.) were injected at regular intervals during sample analysis and used to calculate KI values. Retention times were converted to Kovat Indices (KI) using the linear regression formula:

$$Y = 1.340x - 23.131$$

Each "peak" was labeled with a KI value and was considered an individual variable before adjusting for redundancies when analyzing principal components. The data were analyzed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer Corp., 1998).

Principal Components Analysis

Analyses of principal components were conducted on 45 normalized variables. One alate *F. gnava* queen and one newly mated *F. gnava* queen were deleted from the data set because the generalized distance test determined them to be outliers at the 0.01 probability level.

The relative proportions of cuticular hydrocarbons were calculated by dividing the percent area given for each cuticular hydrocarbon by the total percent area of that profile. Singular value decomposition was used to implement principal component analysis. A correlation matrix using the data set was computed:

$$C = (1/N-1)X^{T} * X$$

where X = the autoscaled data matrix, X^T is the transpose of the autoscaled data matrix, and N = the number of hydrocarbon components which make up X. An eigenanalysis was then performed on the correlation matrix. The direction(s) of greatest variance in the data are represented by an eigenvector or principal component, which corresponds to the largest eigenvalues. Multivariate analysis was performed using Pirouette software (Infometrix, Woodinville, WA).

RESULTS

Behavior Tests

Newly mated *F. gnava* queens alone (n = 10) or with a full complement of foreign immature brood and a small number of workers (n = 10) did not trigger any aggression from *P. breviceps* queens (Table 1.1). *Polyergus breviceps* queens spent most of the 20 min trial running around the periphery of the testing box or attempting to climb up the Fluon®-coated side. If the *P. breviceps* queen encountered the *F. gnava* queen during her meandering, there was brief antennal contact (1 - 2 sec) and an immediate retreat on the part of the *P. breviceps* queen. If the *P. breviceps* queen encountered the *F. gnava* queen near her brood, the *F. gnava* queen would sometimes strike with her mandibles at the *P. breviceps* queen. These strikes never resulted in attack by the *P. breviceps* queen, only in retreat. These results clearly indicate that *F. gnava* offspring, immature or adult, alone is not the stimulus that triggers the aggressive behaviors in a *P. breviceps* queen that are directed against a *F. gnava* queen during colony take-over. This is further supported by the fatal attacks against lone, established *F. gnava* queens in 100% of the trials (n = 15). In these trials, *F. gnava* queens did not strike at *P. breviceps* queens. They either ran away from an approaching *P. breviceps* queen or wiggled beneath the attacking *P. breviceps* queen, sometimes flexing the gaster.

For 190 days after mating, all newly mated *F. gnava* queens tested still failed to elicit aggression from *P. breviceps* queens, even though offspring were being produced (Table 1.1). However, on the next test date, which fell on the 204th day after mating, 100% of the newly mated *F. gnava* queens that were tested were attacked. The difficulty in maintaining *P. breviceps* in the laboratory without diapause left me with only three *P. breviceps* queens on this day. Therefore, only four newly mated *F. gnava* queens were tested, with one *P. breviceps* queen having been used in two of the four tests. Nonetheless, all newly mated *F. gnava* queens elicited aggression, and this difference was significant (Fisher's Exact Probability > 0.9999).

In all cases, attacks on *F. gnava* queens (established or 204 day post mating) were dramatic and sustained until completion. There was no difference in the duration of attacks on newly mated *F. gnava* queens as compared to the duration of attacks on established *F. gnava* queens ($t_{17} = 1.723$, $p = 0.10$). The latency to attacking established *F. gnava* queens, however, was significantly shorter than the latency to attacking newly mated *F. gnava* queens ($t_{17} = 3.156$, $p < 0.006$) (see Table 1.2 for means). A statistical comparison of the number of eggs, larvae, pupae, and workers produced by *F. gnava* queens on days 190 and 204 did not reveal any significant difference although workers had eclosed from existing pupae during this time. There were fewer larvae present on day 204 presumably having metamorphosed into pupae (see Table 1.3 for means and Table 1.4 for *t*-test values).

Chemical Analysis

Cuticular hydrocarbon profiles of alate, newly mated, and established *F. gnava* queens appear very similar in quality and relative proportional quantity (Fig. 1.1). Multivariate analysis, however, separated alate *F. gnava* queens from newly mated and established *F. gnava* queens, which clustered together, on the basis of their first and second principal components, which accounted for 57% of the total cumulative variance (Fig. 1.2).

Table 1.2

Summary of aggressive attacks on *Formica gnava* queens by *Polyergus breviceps* queens

Status of *Formica gnava* Queens	**Mean Latency to Attack (min)**	**Mean Total Attack Time (min)**
Established	0.51 (0.45) n = 15	19.2 (4.1) n = 15
Newly-Mated (at 204 days)	2.64 (2.7) n = 4	24.25 (8.8) n = 4

Averages in minutes are given for the time until *Polyergus breviceps* queens began attacking established *Formica gnava* queens and newly mated *F. gnava* queens that were attacked, and for the duration of these attacks. Standard deviations are in parentheses. **Latency to attack:** Established *F. gnava* queens were attacked significantly sooner than newly mated *F. gnava* queens that were attacked by *P. breviceps* queens (t_{17} = 3.156, $p < 0.006$). **Total attack time:** There was no statistically significant difference in the time *P. breviceps* queens spent attacking newly mated *F. gnava* queens as compared to established *F. gnava* queens (t_{17} = 1.723, $p = 0.10$).

Table 1.3

Total number of offspring in incipient nests of *Formica gnava* on day 190 and on day 204

		Stage & Number of Offspring	
***Formica gnava* Queen #**	***Polyergus breviceps* Queen #**	**Day 190 (No Attack)**	**Day 204 (Attack)**
NMFG#4-98	SP#7-98	Workers: 14	Workers: 16
		Pupae: 2	Pupae: 0
		Larvae: 7	Larvae: 4
		Eggs: 0	Eggs: 12
NMFG#24-98	SP#1-98	Workers: 11	Workers: 20
		Pupae: 1	Pupae: 5
		Larvae: 10	Larvae: 2
		Eggs: 0	Eggs: 0
NMFG#25-98	SP#2-98	Workers: 1	Workers: 1
		Pupae: 0	Pupae: 0
		Larvae: 1	Larvae: 0
		Eggs: 5	Eggs: 2
NMFG#27-98	SP#2-98	Workers: 17	Workers: 21
		Pupae: 9	Pupae: 11
		Larvae: 12	Larvae: 0
		Eggs: 10	Eggs: 0

Table 1.4

Statistical comparison of offspring produced by newly mated Formica *gnava* queens on day 190 and on day 204

Developmental Stage Compared	Mean Difference	DF	*t*-Value	*P*-Value
Eggs	-0.667	2	-0.105	0.93
Larvae	7.667	2	2.95	0.10
Pupae	-1.33	2	-0.76	0.53
Workers	-5.0	2	-2.4	0.14

Values from t-tests comparing the number of offspring at each developmental stage in nests of newly-mated *Formica gnava* queens 15 days prior to attack with the number of offspring at each developmental stage on the day of attack are given. No significant differences between the number of immatures of any stage at days 190 and 204 were found*. See Table 1.3 for immature totals for each queen tested.

*NMFG#25-98 was excluded from analysis because all but one worker escaped prior to test dates.

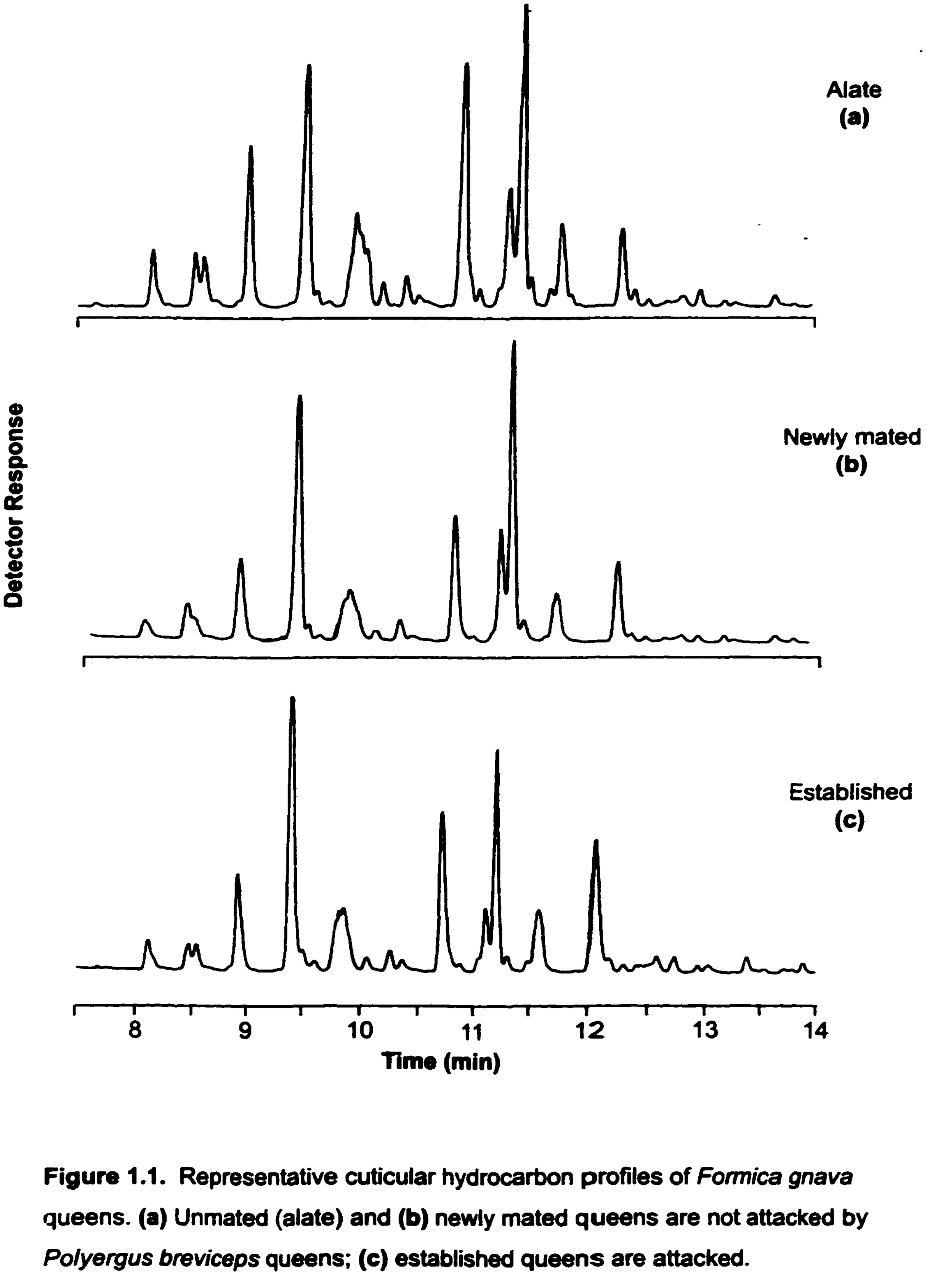

Figure 1.1. Representative cuticular hydrocarbon profiles of *Formica gnava* queens. (a) Unmated (alate) and (b) newly mated queens are not attacked by *Polyergus breviceps* queens; (c) established queens are attacked.

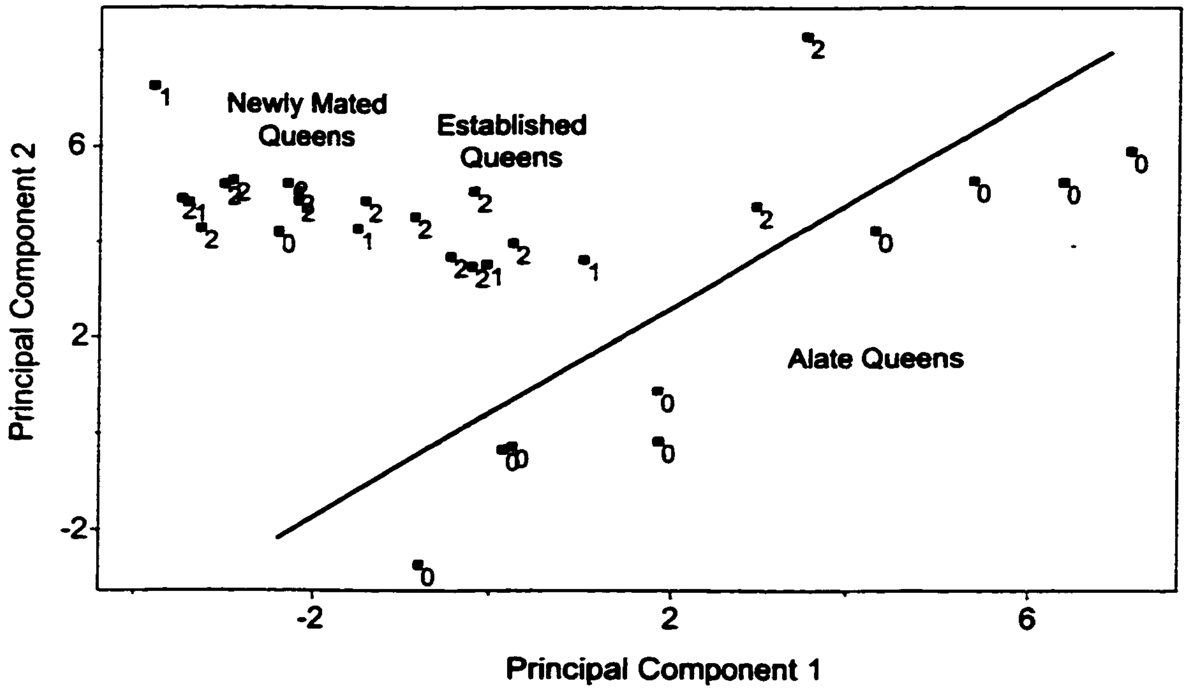

Figure 1.2. Principal component map of cuticular hydrocarbons from alate (unmated), newly mated, and established *F. gnava* queens. On the basis of the first and second principal components, which accounted for 57% of the total cummulative variance, alate *F. gnava* queens separated from both newly mated and established *F. gnava* queens, which clustered together. 0 = alate (unmated) *F. gnava* queens, 1 = newly mated *F. gnava* queens, 2 = established *F. gnava* queens.

DISCUSSION

The results of this study demonstrate that *P. breviceps* queens were not stimulated to attack *F. gnava* queens by the presence of or encountering non-reproductive offspring. Newly mated *F. gnava* queens that were supplied with a full complement of immatures and a small number of workers did not induce aggressive behavior in *P. breviceps* queens. Lone, fully matured *F. gnava* queens, however, were fatally attacked in 100% of the observations. Taken together, these results suggest that the stimulus triggering attacks may involve chemicals associated with established *F. gnava* queens. Cuticular hydrocarbons from newly mated and established *F. gnava* queens, however, did not differ qualitatively or in relative proportions, and clustered together on the first and second principal components. This indicates that if *P. breviceps* queens were using chemicals to detect an established *F. gnava* queen, they must have been responding to chemicals other than or in addition to cuticular hydrocarbons. Nonetheless, whatever the signal was, this signal, and the ability of a *P. breviceps* queen to detect this signal, allowed the queen to avoid attacking host queens that were unable to immediately supply at least a minimal work force. By the time *P. breviceps* queens first attacked newly mated *F. gnava* queens (day 204), there were on average 17.89 workers in each of all the newly mated colonies. While the size of this work force may be sufficient to support a newly mated queen, this cannot be verified from the study reported here because *P. breviceps* queens were placed back with the colony they first usurped.

Why Not Target Newly Mated Formica Gnava *Queens?*

Newly mated *P. breviceps* queens typically "find" established *Formica* queens by following raiding nestmates to a *Formica* nest (Talbot 1968; Marlin 1971; Kwait & Topoff 1984; Topoff & Greenberg 1988; Topoff *et al.* 1988). Occasionally, however, they are found wandering the ground alone, having either been unsuccessful in entering a raided nest or, when environmental conditions dampen raiding activity, taken flight without accompanying a raiding party. Although nothing is known of *F. gnava* mating flights (Topoff personal communication), newly mated *F. gnava* queens also appear to establish new colonies during this time. Certainly *F. gnava* alates (male and female) were abundant in *F. gnava* nests during June, and these numbers decreased throughout July (personal observation) when newly mated *F. gnava* queens were found (although with great difficulty) wandering the ground. Thus, newly mated *P. breviceps* might conceivably encounter newly mated *F. gnava* queens in the field (Topoff & Zimmerli 1993; Zimmerli & Topoff 1994). If a newly mated *P. breviceps* queen were to attack the newly mated *F. gnava* queen, she would expend substantial energy without gaining a worker force and might even handicap a future attempt at usurping a *F. gnava* nest.

Polyergus breviceps queens appease (Topoff *et al.* 1988) or repell (Vander Meer, personal communication) attacking workers by discharging contents of their Dufour's gland during attempts to kill *F. gnava* queens. Preliminary comparisons showed that one week after mating, the Dufour's gland is significantly smaller than before mating (Topoff *et al.* 1988), indicating that much of the contents was depleted. Attacking a newly mated *F. gnava* queen in the field would leave a *P. breviceps* queen still in need of a work force and having already exhausted one significant line of defense against attacking workers once a *F. gnava* nest was eventually found. Her chances at taking over a nest would be greatly reduced, thus selecting for prudent attacks (but see Chapter 5, *Power Seat*).

Chemical Signature and Ovarian Development

Production of cuticular hydrocarbons appears to be under hormonal control in several insect taxa (Blomquist *et al.* 1984; Schal *et al.* 1991), as is ovarian development (Röseler *et al.* 1980; Ross & Gamboa 1982). The composition of cuticular hydrocarbon profiles has been strongly correlated with the degree of ovarian development in several species of wasps and ants. Several cuticular hydrocarbons (C_{31}-C_{35}) from subordinate *Polistes* *dominulus* Christ, whose ovaries were not fully developed, differed in relative proportions from the typical profile of dominant reproductives (Bonavita-Cougourdan *et al.* 1991). In the queenless ant *Dinoponera quadriceps* Kempf, cuticular profiles of alpha females were characterized by greater quantities in consistent relative proportions of the long-chain hydrocarbon 9-hentriacontene (9-C_{31}:1) (Monnin *et al.* 1998; Peters *et al.* 1999). If the alpha female was removed from the colony, within six to eight weeks, the ovaries of the beta female became fully developed and 9-C_{31}:1 increased to alpha levels.

The lack of aggression prior to 191 days and the full-blown attacks observed on day 204 suggest that the releaser stimulus, which appeared to be all or none, could also be a product of host queen maturation as defined by ovarian development. But my findings indicate this was probably not the case. First, hydrocarbon profiles were not distinguishable between newly mated and established *F. gnava* queens. Hence, the profile did not change from the point of mating until the time of being attacked and thus, hydrocarbons were probably not responsible for stimulating attacks. Second, although ovaries of *F. gnava* queens used in this experiment were not measured, offspring born to newly mated *F. gnava* queens indicate that the ovaries of these queens were developed and functional long before aggression was observed. These two factors indicate that hydrocarbon profiles of newly mated *F. gnava* queens did not change with time or with ovarian development, and that whatever the stimulus was, it also was not correlated with ovarian development unless ovaries needed to be fully developed to produce sufficient amounts to elicit attack. Measurement of ovaries and interval sampling of other chemicals after mating might clarify any relationship between ovarian development and production of chemicals that might serve as a releaser stimulus. Bioassays using purified extracts of the chemicals correlated with ovarian development (if there is a correlation) are then needed to determine conclusively the property that produces this kairomonal effect.

Why Do Formica Gnava *Queens Differ?*

Polyergus breviceps queens have evidently evolved the ability to detect differences between newly mated and established queens of *F. gnava*, and to respond in a manner that is likely to increase their chances of establishing a colony. However, this raises an additional question: Why do newly mated *F. gnava* queens differ from established *F. gnava* queens at all? I propose two, not mutually exclusive, hypotheses. First, reproductive queens that draw most of worker attention towards themselves (or at least bring non-reproductives in to closer proximity relative to other individuals) may achieve greater reproductive success, as they have a greater chance of being fed and groomed. In many ant species, pheromone release is dictated by fecundity. Dominant or alpha queens, which contribute a significant proportion of the viable eggs in a colony, maintain a chemical relationship with non-reproductive members that is distinct from typical nestmate recognition (Hölldobler & Wilson 1983). Others tend to cluster around them, and they are groomed more frequently and at higher rates than non-reproductives, unmated reproductives and subordinate reproductives (e.g., Heinze *et al.* 1992). In choice tests, 70% of *Solenopsis* workers aggregated around the *Solenopsis* queen (Chen & Vinson 1999). Similarly, newly mated *F. gnava* queens may differ from established queens because they are still subordinate in a hierarchical polygynous colony.

Second, queens that maintain an immature chemical suit may easily be adopted into other conspecific colonies shortly after mating. Numerous ant species utilize different strategies to disperse and establish "new" nests in response to different environmental conditions and according to different genetic tendencies (see Chapter 5, *Variation in Dispersal, and Founding Strategies & Differential Adoption*). In many monogynous species, female reproductives that have taken flight and mated are banished from their birth nest and forced to excavate a new home. If newly mated queens of monogynous species return and attempt to re-enter their nest, they are aggressively rejected. This suggests that the chemical adornment of newly mated queens in these species is sufficiently dissimilar to warrant rejection. Polygynous species on the other hand attain multiple queen status through intranidal mating, adoption of newly mated, conspecific queens, or pleometrosis (Hölldobler & Wilson 1990).

Nests of *Formica gnava* are either monogynous or polygynous, suggesting that some queens establish nests independently while others mate within the nest or seek adoption after mating flights. For those that mate intranidally or seek adoption, maintaining an innocuous signal, that of a virgin, non-egg laying reproductive, would lessen their chances for rejection. The lack of aggression towards newly mated *F. gnava* queens in my study suggests that mating does not produce an immediate change in the chemical profile of *F. gnava* reproductive females, at least not a change that results in a profile that is similar to mature queens. Instead the chemical profile of a "mature" queen appears to develop over time. If *F. gnava* queens hide their mated status, they may be accepted back into their colony. As they mature, so might their chemical signature. Recent work has demonstrated that the dynamic chemical nature of ant colonies necessitates an ability to form new associations (Vander Meer 1989; Vander Meer & Morel 1998). Thus, if *F. gnava* workers are able to habituate to modifications in the developing chemical signal of newly mated nestmates, these queens may eventually be treated like dominant reproductives. On the other hand, once the signature is fully matured, queens may be forced to found a colony alone or to bud with a group of workers (see Recognition Cue Hypothesis [Stuart *et* al. 1993]). Such is the case for queens of some *Leptothorax* species and other *Formica* species. Queens of these species forego independent colony founding and opt for adoption in the fall. In the spring, many of these queens leave the nest with a group of workers presumably because of conflict within the polygynous nest (Alloway *et al.* 1982; Stuart 1985; Herbers & Tucker 1986; Heinze & Buschinger 1988).

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CHAPTER 2

Evidence of Murder: Cuticular Hydrocarbon Patterns of the Slave-maker Queen, *Polyergus breviceps*

SUMMARY

Queens of the slave-maker, *Polyergus breviceps*, take over nests of their *Formica* host species by fatally attacking the resident queen. Once attacks cease, workers of the invaded nest begin grooming the killer queen, suggesting that the slave-maker may acquire chemicals that are involved in nestmate recognition. In this study, *Polyergus breviceps* queens that had killed a *Formica* queen could be identified on the basis of their cuticular hydrocarbon profiles, as could the species of *Formica* queen that the *P. breviceps* queen had killed. Killer queens developed a hydrocarbon profile that was virtually identical to the queen profile of species killed, suggesting that a transfer of chemicals does take place and that *P. breviceps* queens may use their new hydrocarbon profile to avoid being detected as an outsider. Preliminary investigation of cuticular hydrocarbons from *Formica wheeleri* queens, a slave-maker species that takes over a nest by banishing adults and annexing the brood, accurately reflects the absent behavior of killing a *Formica* queen. Although *F. wheeleri*'s profile also changed over time, it did not resemble the profiles of the queens from the enslaved species (*Formica occulta*). Surprisingly, profiles of *P. breviceps* queens that had not killed a *Formica* queen but were from nests with different slave species (and different elevations) were also distinguishable. The particular pattern of hydrocarbons in their profiles suggests that the two populations of *P. breviceps* examined in this study may be two host races or, perhaps, different species.

INTRODUCTION

The slave-making ant, *Polyergus*, is unable to support itself without the foraging, feeding, and brood care behaviors of their *Formica* hosts. Queens of *Polyergus* also depend on the behaviors of their slaves and are unable to establish their colonies without them. To secure a slave force, a *Polyergus* queen that has just mated takes over a colony of adult *Formica* by fatally attacking the queen of that nest (Wheeler 1906; Topoff *et al.* 1988). Workers at first attack the invading queen but are appeased or repelled with secretions from the Dufour's gland of the *Polyergus* queen (at least in *Polyergus breviceps*) during her attack on the resident queen (Topoff *et al.* 1988). Once the *P. breviceps* queen has ceased attacking the *Formica* queen, workers begin grooming the slave-maker queen. This redirection of worker attention towards the slave-maker queen may be due to the death of the resident queen. Laboratory experiments, however, indicate that interactions between *Polyergus* and *Formica* queens are involved in behavior modification. *Polyergus* queens are generally less successful in their attempts to take over queenless *Formica* nests than when a *Formica* queen is present and the *Polyergus* queens kills her. Worker attacks in these queenless colonies are often relentless, and usually result in the death of the *P. breviceps* queen (Topoff *et al.* 1988; Topoff & Zimmerli 1993; Topoff *et al.* 1990; Zaayer 1967). Furthermore, the associated change in worker behavior occurs immediately after the *Polyergus* queens stops attacking the *Formica* queen, unlike the delayed and gradual decrease in worker aggression that sometimes takes place when the resident queen is experimentally removed.

Workers may instead adopt a *Polyergus* queen because she is camouflaged with chemicals of the *Formica* queen she has attacked (Topoff *et al.* 1988; Topoff *et al.* 1990; Topoff & Zimmerli 1993; Zimmerli & Topoff 1994). The lipid layer of the insect cuticle is prone to absorbing other lipid soluble compounds (Soroker *et al.* 1994, 1995; Vienne *et al.* 1995), and chemicals can be spread among individuals, even across higher taxa, through social contact (see Vander Meer & Wojcik 1982). During attacks on the *Formica* queen, *Polyergus* queens may absorb chemicals that are involved in nestmate recognition from the *Formica* queen. Recently, Errard & D'Ettorre (1998) supported the Chemical Transfer Hypothesis (Topoff *et al.* 1988; Topoff *et al.* 1990; Topoff & Zimmerli 1993; Zimmerli & Topoff 1994) by demonstrating that, after killing a *Formica cunicularia* Latreille queen, cuticular hydrocarbon profiles of *Polyergus rufescens* Latreille queens resemble the hydrocarbon profiles of *F. cunicularia* queens.

In this study, I examined whether newly mated *Polyergus breviceps* Emery queens undergo similar changes in hydrocarbon profile after killing a queen of their host species. In southeastern Arizona, *Polyergus breviceps* enslaves either *Formica gnava* Buckley or *Formica occulta* Francoeur. Thus, I was also able to ascertain whether changes in hydrocarbon profiles were specific to the species of *Formica* queen killed.

METHODS

Ant Collections & Housing

Ant colonies were collected during June and July 1997 and 1998 in the Chiricahua Mountains of southeastern Arizona. From the Arizona oak - alligator juniper woodlands of the Southwestern Research Station (SWRS) of the American Museum of Natural History (el. 1646 m), I collected 15 queenright colonies of *Formica gnava*. From an area just east of the Barfoot Peak trailhead (el. 2750 m) in Coronado National Forest populated with ponderosa pine, I collected 13 queenright colonies of *Formica occulta*. Colonies were brought into the laboratory at SWRS and kept in large Tupperware® boxes lined with Fluon® (Northern Products) to prevent escape. Dealate *P. breviceps* queens from nests with *F. gnava* slaves (n = 49) and from nests with *F. occulta* slaves (n = 12) were collected individually in 4 dram vials containing a moistened ball of cotton, as they approached the *Formica* nest being raided by their non-reproductive nestmates.

Twelve established *F. gnava* queens and ten established *F. occulta* queens were removed from their nests and placed in individual Tupperware boxes (20.5 x 45 x 3.5 cm) lined with a thin layer of soil. A single newly mated *P. breviceps* queen was introduced into the box and allowed to attack the *Formica* queen. Immediately after each *P. breviceps* queen ceased her attack on the *Formica* queen, both queens were individually immersed in 2 ml of high purity hexane (B & J GC^2 Grade) for 10 min in 7 ml scintillation vials to extract the cuticular components (see Table 2.1). Solvent extracts were transferred from the sample with a fresh Pasteur pipette to a 2 ml scintillation vial and allowed to evaporate. In addition, newly mated *P. breviceps* queens from nests containing *F. gnava* slaves and from nests containing *F. occulta* slaves, and *F. gnava* and *F. occulta* queens that had not been attacked by *P. breviceps* queens were immersed individually in hexane and treated using the protocol presented above (see Table 2.1). For preliminary comparisons, cuticular chemicals were also extracted from one newly mated *Formica wheeleri* Creighton queen and from two *F. wheeleri* queens that had reared a supply of *F. occulta* pupae and lived with them for one year. Specimens were preserved for voucher in 70% ethyl alcohol.

Chemical analysis

The evaporated solvent extracts were transported to the United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida where chemical analyses were performed. Evaporated solvent extracts were reconstituted in 0.2 ml hexane, vortexed for 1 - 2 sec, and applied to a small silicic acid (70 - 230 mesh 60 Å) Pasteur pipette column.

Table 2.1

Species and conditions of queens from which cuticular hydrcarbons were analyzed

Species	Condition	Host Species in Nest	Number
Polyergus breviceps	Newly mated	*F. gnava*	42
		F. occulta	5
	Killed *Formica* Queen	*F. gnava*	6
		F. occulta	6
Formica gnava	Killed by *Polyergus**	-	12
	No interactions with *Polyergus**	-	3
Formica occulta	Killed by *Polyergus**	-	10
	No interactions with *Polyergus**	-	3
Formica wheeleri	Newly mated	*F. occulta*	1
	1 year with *occulta* workers	*F. occulta*	2

* *Formica* queens that had no interactions with *P. breviceps* queens clustered with conspecific queens that had been attacked by *P. breviceps* queens. These data were therefore pooled to increase sample size.

Hydrocarbons were isolated from other lipids by eluting the column with hexane. The elutant, containing purified hydrocarbons, was evaporated to ca. 10 µl under a stream of nitrogen. Samples were analyzed by gas chromatography (Varian 3700 equipped with a split-splitless injector, a capillary column [J & W DB - 1 30 m] and flame ionization detector). The injection and detector were set at 300° C; the oven was programmed from 190° to 290° C at 5°/min, and then held at 290° C for 5 min. Hydrogen was the carrier gas and nitrogen was the makeup gas. The data were analyzed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer Corp., 1998).

Data were analyzed as two separate groups based on the host species present in the nests of newly mated *P. breviceps* queens: Group 1 compared newly mated *P. breviceps* queens from nests containing *F. gnava* slaves, *P. breviceps* queens that had killed a *F. gnava* queen, *F. gnava* queens, and *F. occulta* queens. Group 2 compared newly mated *P. breviceps* queens from nests containing *F. occulta* slaves, *P. breviceps* queens that had killed an *F. occulta* queen, *F. occulta* queens, and *F. gnava* queens.

Analyses of Principal Components were conducted on 45 normalized variables from Group 1, or on 48 normalized variables from Group 2. In dataset 1, one newly mated *P. breviceps* sample and one *F. occulta* sample were deleted because the generalized distance test determined them to be outliers at the 0.01 probability level. In dataset 2, one newly mated *P. breviceps* sample, one *F. occulta* sample, and one *F. gnava* sample were deleted because of outlier determination at the 0.01 probability level.

The relative proportions of cuticular hydrocarbons were calculated by dividing the percent area given for each cuticular hydrocarbon by the total percent area of that profile, and autoscaled to provide each peak with equal weight in the analysis. Singular value decomposition was used to implement principal component analysis. Pirouette software (Infometrix, Woodinvill, WA) was used to perform the multivariate analyses.

Hydrocarbon profiles of *F. wheeleri* were analyzed by visually comparing peaks with the same retention times. Peaks in all profiles presented here have been labeled with letters to facilitate comparisons. Several "peaks", however, appear to consist of more than one hydrocarbon component (e.g., Peak F in Fig. 2.1) but are treated as one peak to simplify comparison.

RESULTS

Comparisons of Queen Cuticular Profiles:
Polyergus breviceps, Formica gnava, & Formica occulta

Relative to the number of hydrocarbons present on the cuticle of killer *P. breviceps* and host *Formica* queens, few hydrocarbons were present on the cuticle of *P. breviceps* queens that had just mated but had not killed a *Formica* queen (Figs. 2.1 & 2.2). Assuming that chemical peaks with the same retention times correspond to the same hydrocarbon components, *P. breviceps* queens that had killed an *F. gnava* queen retained only four of the major components from their pre-killing state (in similar proportions) (Fig. 2.1 a & b, Peaks D, G, I, K). They shared, however, twelve peaks with *F. gnava* queens (in similar proportions) (Fig. 2.1 b & c, Peaks A – I, K - M, O). *Polyergus breviceps* queens that had killed a *F. occulta* queen also retained four of the major components from their pre-killing state in relatively similar proportions (Fig. 2.2 a & b, Peaks A, D, G, K). They shared at least twenty-one of the major components with *F. occulta* queens (Fig. 2.2 b & c, Peaks A - P).

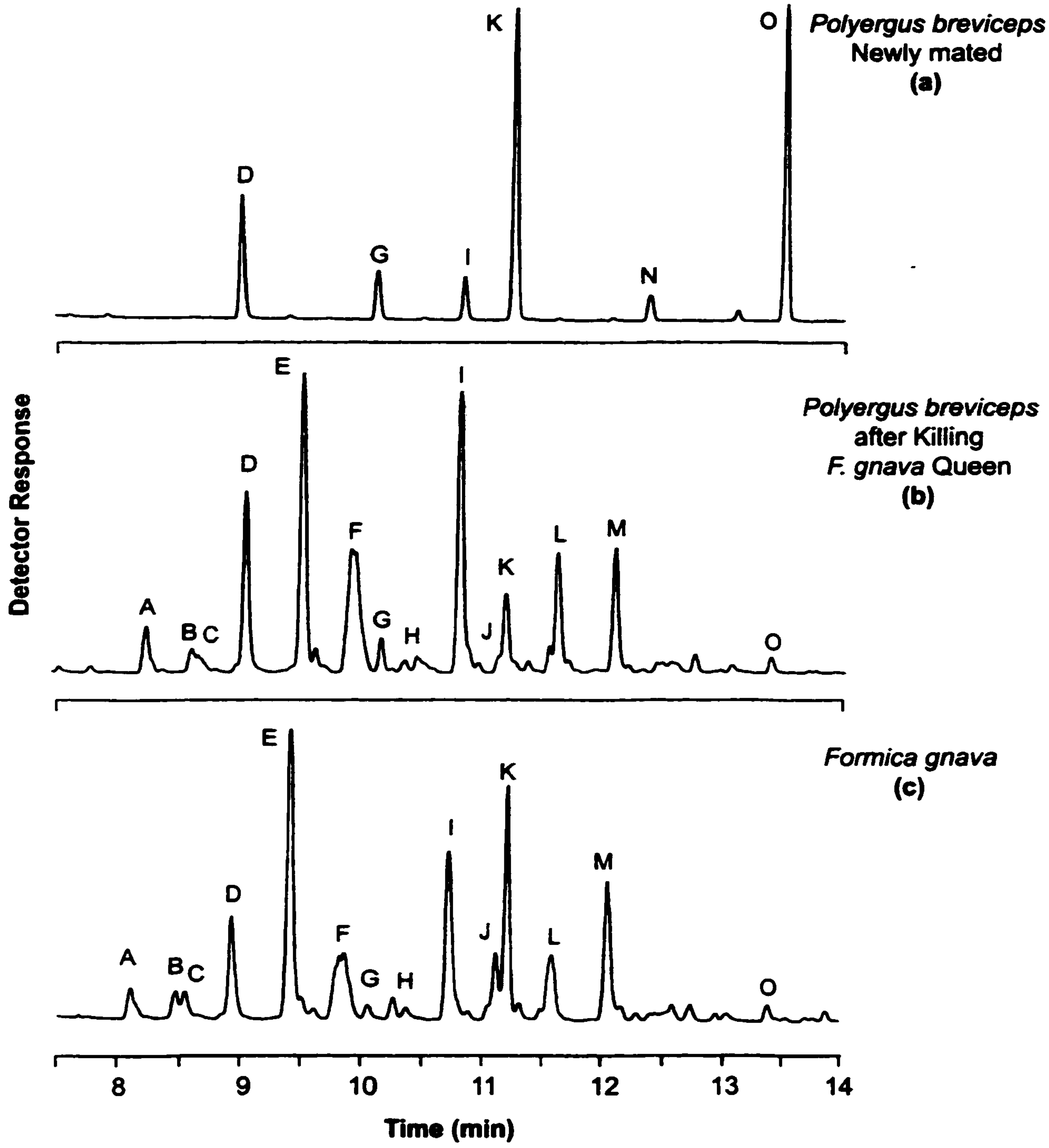

Figure 2.1. Representative profiles of cuticular hydrocarbons from *P. breviceps* and *F. gnava* queens. **(a)** Newly mated *P. breviceps* queen from a nest containing *F. gnava* slaves. **(b)** *Polyergus breviceps* queen after killing a *F. gnava* queen. Note the emergence of chemical peaks that correspond to peaks in the profile of an established *F. gnava* queen **(c)**. Compare with Fig. 2.2.

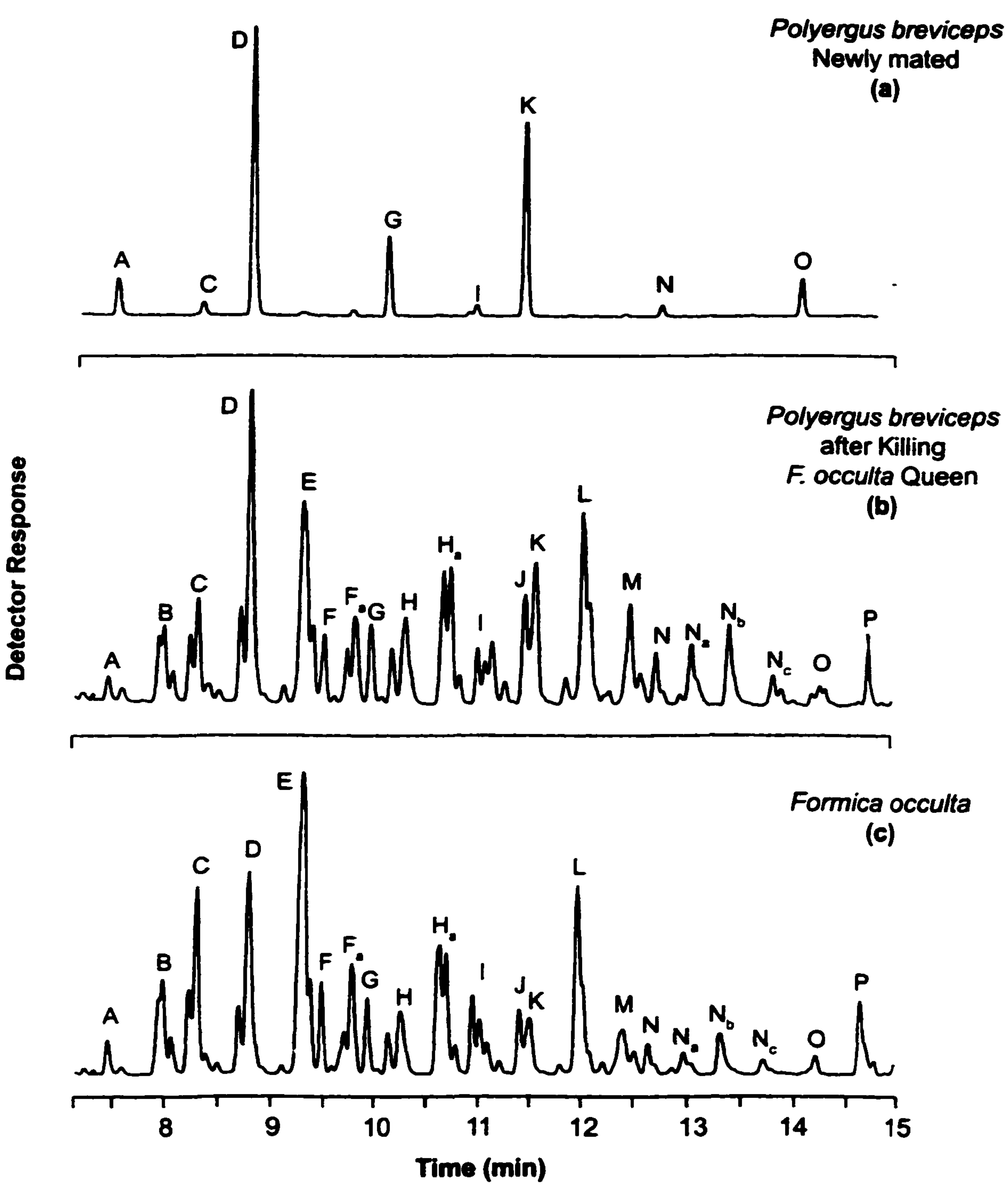

Figure 2.2. Representative profiles of cuticular hydrocarbons from *P. breviceps* and *F. occulta* queens. **(a)** Newly mated *P. breviceps* queen from a nest containing *F. occulta* slaves. **(b)** *Polyergus breviceps* queen after killing a *F. occulta* queen. Note the emergence of chemical peaks that correspond to peaks in the profile of an established *F. occulta* queen **(c)**. Compare with Fig. 2.1.

In Group 1 (*P. breviceps* associated with *F. gnava*), newly mated *P. breviceps* queens separated from killer *P. breviceps* queens and *F. gnava* queens on the first principal component and from *F. occulta* queens on the second principal component (first and second components equal 58% of the total cumulative variance). *Polyergus breviceps* queens that had killed a *F. gnava* queen, however, clustered among *F. gnava* queens. *Formica gnava* queens that had no contact with *P. breviceps* queens also clustered with *F. gnava* queens killed by *P. breviceps* (identified by arrows, Fig. 2.3a), an indication that there was no reciprocal transfer of hydrocarbons from *P. breviceps* to *F. gnava*.

In Group 2 (*P. breviceps* associated with *F. occulta*), newly mated *P. breviceps* separated from killer *P. breviceps* queens, *F. occulta* queens, or *F. gnava* queens on the basis of the first principal component (61% of the total cumulative variance). *Polyergus breviceps* queens that killed a *F. occulta* queen clustered among *F. occulta* queens. *Formica occulta* queens having no contact with *P. breviceps* also clustered with *F. occulta* queens that were killed by *P. breviceps* (indicated by arrows, Fig. 2.3b), again indicating that hydrocarbons were not transferred from *P. breviceps* to the *Formica* queen killed.

Comparisons of Queen Cuticular Profiles:
Formica wheeleri & Formica occulta

The hydrocarbon profile of the newly mated *F. wheeleri* queen extracted consisted of relatively few major components, all of which were still present in similar proportions after one year of living with *F. occulta* workers (Fig. 2.4 a & b, Peaks A, D, G, K, N, O). Of the two *F. wheeleri* queens sampled after living for one year with *F. occulta* workers, one queen's profile became noticeably more complex (Fig. 2.4b) while the other queen's profile became only marginally more complex (not pictured here). The emergent

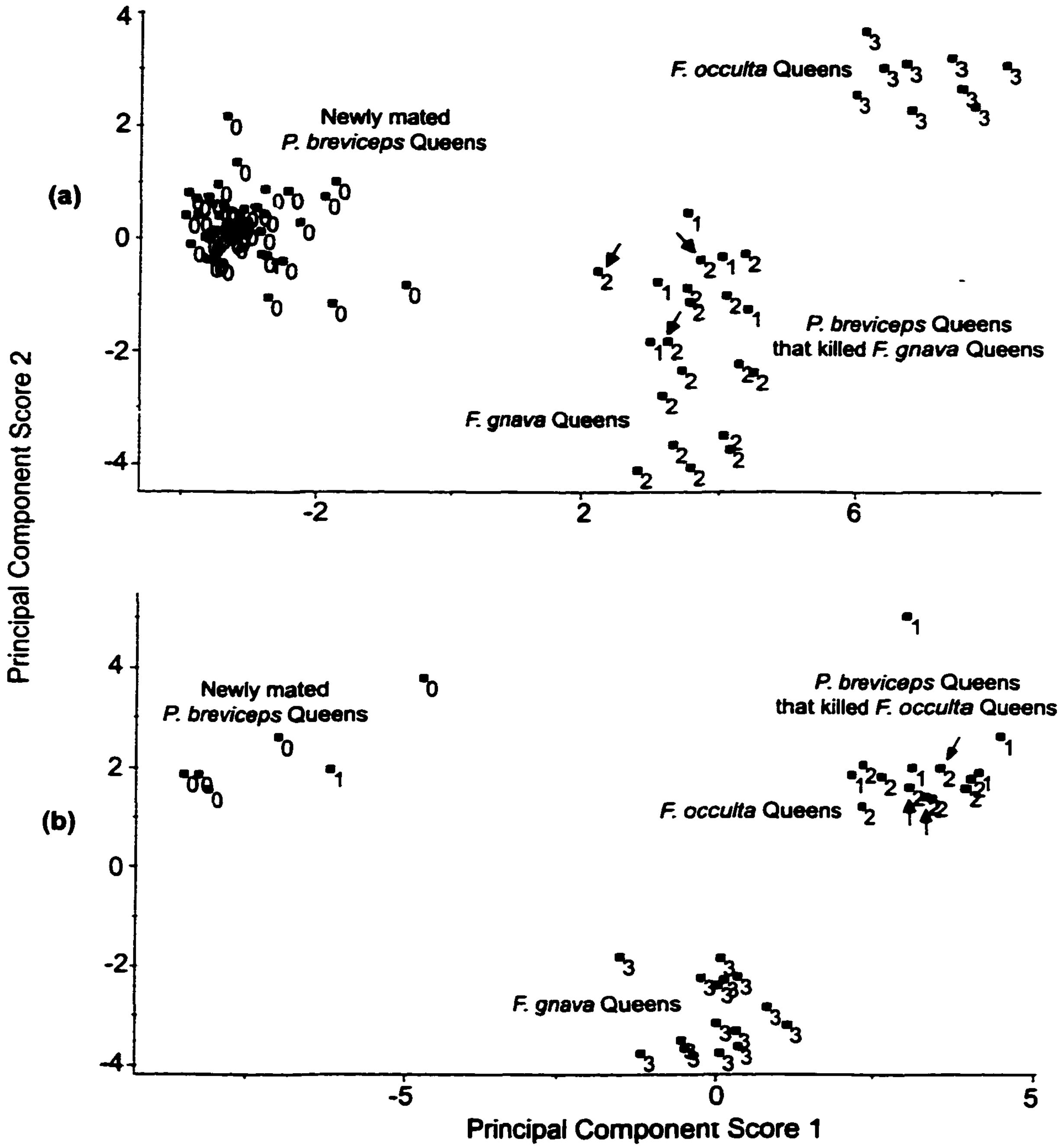

Figure 2.3. Principal component maps of cuticular hydrocarbons from *P. breviceps*, *F. gnava*, and *F. occulta* queens. **(a)** 0 = newly mated *P. breviceps* queens from nests with *gnava* slaves, 1 = killer newly mated *P. breviceps* queens from nests with *F. gnava* slaves, 2 = *F. gnava* queens, 3 = *F. occulta* queens. **(b)** 0 = newly mated *P. breviceps* queens from nests with *F. occulta* slaves, 1 = killer newly mated *P. breviceps* queens from nests with *F. occulta* slaves, 2 = *F. occulta* queens, 3 = *F. gnava* queens. Arrows indicate extracts from *Formica* queens that had no interactions with *P. breviceps* queens.

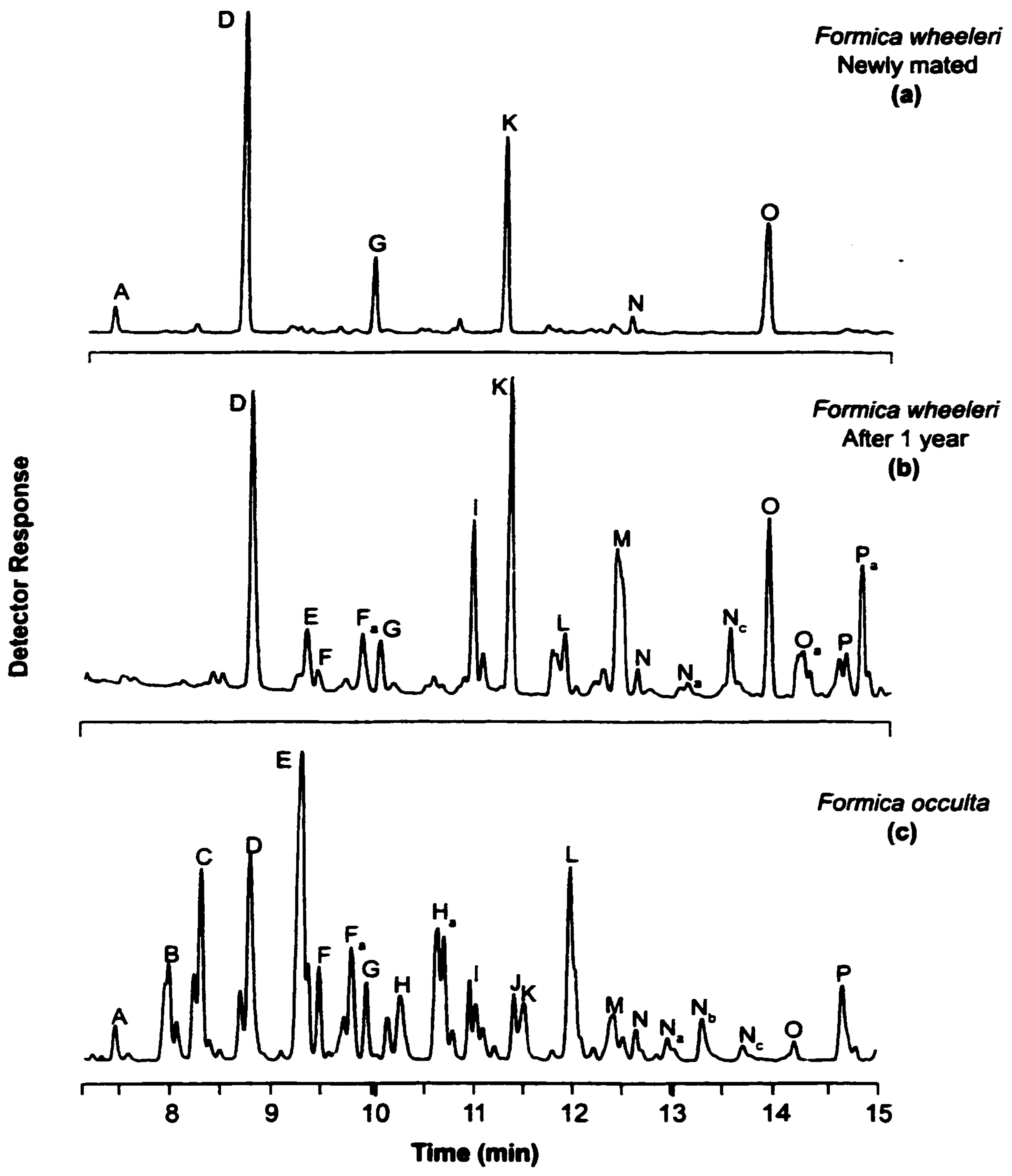

Figure 2.4. Cuticular hydrocarbon profiles of *F. wheeleri* and *F. occulta* queens. **(a)** Newly mated *F. wheeleri* queen. Profile simplicity is reminiscent of *P. breviceps* queen profiles (see Figs. 2.1 & 2.2). **(b)** *Formica wheeleri* profile of queen after living with *F. occulta* for one year is more complex, but few peaks are shared with *F. occulta* queens **(c)**.

components in the profiles of both queens, however, were generally not constituents found in profiles of *F. occulta* queens, the species of which it enslaves (Fig. 2.4c). Any shared components were not similar in their relative proportions with respect to the other queen. In effect, the *F. wheeleri* queen profile was distinguishable from the *F. occulta* queen profile (Fig. 2.4 b & c).

DISCUSSION

Errard and D'Ettorre (1998) found that the pattern of cuticular hydrocarbons in newly mated *Polyergus rufescens* queens changed after having killed a *Formica cunicularia* queen, and that the pattern resembled the profiles of *F. cunicularia* queens. The findings reported here are similar. Cuticular hydrocarbon profiles of newly mated *P. breviceps* queens changed significantly after the queens had killed a queen of either *Formica* host species. The number of components comprising a *P. breviceps* profile increased and, assuming that peaks with the same retention time correspond to the same chemical products, most of these components were found in profiles of the species of *Formica* queen that had been attacked in similar proportions. Several of the components found in newly mated *P. breviceps* profiles either decreased or disappeared altogether in the post-killing profiles. Hydrocarbons from the *P. breviceps* queen appear not to have been transferred to the *Formica* queens, or if there was a transfer of chemicals, it was not detectable. Although the sample size of *Formica* queens that were not killed by a *P. breviceps* queen was small, all of these samples clustered with *Formica* queens that had been killed by a *P. breviceps* queen. None clustered with newly mated *P. breviceps* queens.

Most *P. breviceps* queens used in this study were extracted almost immediately after they had killed a *Formica* queen, indicating a relatively immediate change occured in hydrocarbon profiles of *P. breviceps* queens. This suggests that the emergence of new hydrocarbon components, at least initially, was unlikely due to a change in hydrocarbon biosynthesis triggered by the aggressive interaction, as has been proposed for other social parasites (Bagnères *et al.* 1996), which would presumably entail some duration of time. Thus, it appears as though *P. breviceps* queens acquired cuticular chemicals from *Formica* queens during attack, although only experiments tracing the movement of labeled hydrocarbons can conclusively determine whether such a transfer occurs. It should also be mentioned that the profiles of two *P. breviceps* queens that had killed a *Formica* queen (one *F. gnava* and one *F. occulta*) did not cluster with the respective *Formica* species, but clustered with newly mated *P. breviceps.* The reasons for this are unclear. Perhaps these two queens did not sustain attacks sufficiently for a transfer of chemicals to occur. Alternatively, these data points may merely suggest that the system of chemical transfer is imperfect.

Preliminary data on cuticular hydrocarbons of *F. wheeleri* queens, a facultative slave-maker, is consistent with the chemical transfer hypothesis for *P. breviceps*. Because *F. wheeleri* is both a congener and a social parasite of *F. occulta*, we might expect the *F. wheeleri* profile more so than the *P. breviceps* profile to resemble the *F. occulta* profile. Instead, the hydrocarbon profiles of the two *F. wheeleri* queens extracted did not resemble the profiles of *F. occulta* queens (unlike the *P. breviceps* queens that had killed a *F. occulta* queen), even after having lived for one year with the *F. occulta* workers they had reared from pupae. Moreover, newly mated queens of *F. wheeleri* and *P. breviceps* seemed to start out on the same hydrocarbon profile footing. The hydrocarbon profiles of each were qualitatively similar, containing relatively few components, and unlike the complex hydrocarbon profiles of newly mated *F. occulta* queens (unpublished data). Hence, the profile similarity of a *P. breviceps* queen to its particular host species is likely to be the result of a derived ability to adopt the profile of a species the *P. breviceps* kills.

This discrepancy in hydrocarbon profiles of the two species of slave-maker queens may be the result of different selective pressures related to the respective methods of colony usurpation. *Formica wheeleri* queens "force" *F. occulta* adults to abandon their nest and then appropriate the orphaned brood. Because emerging *F. occulta* workers can form their recognition template using odors of the *F. wheeleri* queen (Morel 1983; Errard 1984; Morel *et al.* 1988), there seems to be little need for *F. wheeleri* queens to be chemically similar to host queens. A *Polyergus breviceps* queen takes over nests of adult *Formica*. Thus, she needs an alternative mechanism to bypass the security check by workers that have already formed an odor code of what is acceptable. The initial simplicity of the hydrocarbon pattern in newly mated queens of both slave-maker species probably serves slightly different functions. In *P. breviceps*, a simple hydrocarbon profile may facilitate this apparent transfer of chemicals from the *Formica* queen being attacked. In *F. wheeleri*, a simple hydrocarbon pattern may reduce the chances for "revolt" by newly emerged *F. occulta* workers since the workers will probably incorporate odors of worker nestmates as well as the queen nestmate when developing their recognition template (see Vander Meer & Morel 1998). The simple profile probably also facilitates any transfer of chemicals from *F. occulta* workers through allogrooming.

The acquisition of chemicals from a queen of a host species could facilitate integration of the *P. breviceps* queen among host workers, as well as draw the attention from workers that is often displayed towards reproductive individuals. Indeed, the fantastic change in hydrocarbon profiles of *P. breviceps* queens after having killed a *Formica* queen in conjunction with the dramatic change in worker behavior after the *P. breviceps* queen has ceased her attack indicates critical chemicals are transferred. The significance of the hydrocarbon profile of a newly mated *P. breviceps* queen before she has killed a *Formica* queen also seems worthy of discussion. The cuticular hydrocarbon pattern of newly mated *P. breviceps* queens associated with *F. gnava* was qualitatively almost identical to the hydrocarbon patterns of *F. gnava* and *F. occulta* pupae (Fig. 4.6 b & c), but differed somewhat from hydrocarbon patterns of *P. breviceps* pupae (Fig. 4.6 a). These profiles all contained a relatively abundant, higher molecular weight peak (Peak O). This peak disappeared almost completely from profiles of both the workers (unpublished data) and the *P. breviceps* queens that had killed a *Formica* queen (Fig. 2.1b), even though other new components emerged and increased profile complexity. Workers have been shown to rear different species of pupae in the laboratory. This has lead some to suggest that pupae in general have an odor that is "attractive" to workers (Hölldobler 1977; Zimmerli & Mori 1993), such that the chances for being reared rather than being destroyed by workers is increased (see Chapter 3, *Costs and Benefits of Different Odors*). The discovery of similar or identical patterns of hydrocarbons from pupae of several unrelated species, a stark contrast to the vastly different hydrocarbon patterns of the adults, seem to support this contention (unpublished data; Kaib *et al.* 1993). Thus, that the hydrocarbon components in profiles of newly mated *P. breviceps* queens and pupae of the species *P. breviceps* usurps are also similar in quality and in relative proportion may not be inconsequential. Although highly speculative, this combination of components in particular proportions may be involved in diminishing aggressive reactions of *Formica* workers towards an invading queen. Conceivably, an invading queen that is pupa-like in odor may have better access to the *Formica* queen than an invader with an odor so distinctly different that relentless attacks by workers prevent her movement towards the host queen. Furthermore, a simple profile may promote the acquisition of host species chemicals that are necessary for nestmate recognition, and provide *Polyergus* with an increased chance of becoming integrated among her host workers. Attacks against invading newly mated *P. breviceps* queens, however, do occur, indicating that substances other than hydrocarbons are involved in species-level recognition, an observation made by numerous other authors (Obin 1986; Franks *et al.* 1990; Kaib 1993; Heinze *et al.* 1994; Vander Meer personal communication).

Visual inspection of hydrocarbon patterns of newly mated *P. breviceps* queens has revealed an additional surprising result. Hydrocarbon components of newly mated *P. breviceps* queens from nests with *F. gnava* slaves differed noticeably and consistently in relative proportions from newly mated *P. breviceps* queens collected from nests with *F. occulta* slaves (compare Fig. 2.1a with Fig. 2.2a). Because *P. breviceps* are obligatorily dependent on their host species for survival, rearing *P. breviceps* without some influence of their *Formica* host species is impossible. It is therefore expected that patterns of hydrocarbons will reflect at least some contamination from *Formica*, or from *Polyergus* (to *Formica*). Nonetheless, if hydrocarbons can be used to identify species groups (e.g., Vander Meer 1986; Vander Meer & Lofgren 1990), the presence (or absence) of chemical peaks in profiles of newly mated *P. breviceps* queens that are present (or absent) neither in profiles of their particular host species nor in profiles of *P. breviceps* queens from nests containing the alternate host species, suggests that the two populations of *P. breviceps* may be two host races or, perhaps, two species. It would be unduly worthwhile to clarify the relationship between these two populations of *P. breviceps*.

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CHAPTER 3

Friend or Foe?: Responses of Two Host Species (*Formica gnava* & *Formica occulta*) to Pupae of the Slave-maker Ant, *Polyergus breviceps* (Hymenoptera: Formicidae)

SUMMARY

Queens of the slave-making ant, *Polyergus breviceps*, take over nests of adult *Formica* workers when establishing new colonies. Although naïve to slave-maker brood, the usurped *Formica* rear *Polyergus* offspring and nests comprising both host and parasite species form. Host worker acceptance of parasite brood has been attributed to the similarity of "brood tending signals" between these closely related taxa and/or the presence of an "attractive pheromone" in the slave-maker brood. By presenting single-species groups of *Formica occulta* and *Formica gnava* (two host species of *P. breviceps*) with a choice of *Formica* pupae of both species or with a choice of *P. breviceps* pupae from both types of mixed-species nests, I show that neither close phylogenetic relatedness nor an attractive brood odor alone seems to account for the propensity of host workers to adopt slave-maker pupae. Significantly greater numbers of *P. breviceps* pupae were adopted by enslaved workers than by free-living workers, and within the enslaved groups and the free-living *F. gnava* group, greater numbers of *P. breviceps* pupae were adopted if they were from nests where the host species was conspecific to workers used in tests. When presented with *F. gnava* and *F. occulta* pupae, *Formica* workers adopted conspecific pupae almost exclusively, and ignored or consumed pupae of the other host species. Taken together, these results imply that *P. breviceps* pupae have both a species-specific odor and a general brood-tending pheromone, upon which a host odor may be imposed. The disparate requirements of immatures at different stages of development for cue specificity or generality in maintaining nest exclusivity and maximizing inclusive fitness are discussed.

INTRODUCTION

The surrender of reproductive capabilities by most members of eusocial insect societies to labor on behalf of related, fecund individuals seems to reflect an adaptive compromise between survival and individual fitness (Wilson 1971). This phenomenon would not persist were it not for beneficial behaviors directed towards individuals sharing a proportion of the altruist's genetic material that harbors the propensity for expression of altruistic behavior (Hamilton 1964; Wilson 1975; Hölldobler & Michener 1980), demanding a system in which nestmates (likely to be kin) are recognized (and accepted) and non-nestmates are rejected (Gadagkar 1985; Gamboa *et al.* 1986). Learned species- and colony-specific chemical "labels", which are found on the epicuticle, play an important role in the recognition and acceptance of nestmates in ants (Bonavita-Cougourdan *et al.* 1987, 1988, 1989; Morel *et al.* 1988; Dahbi & Lenoir 1998), wasps (Espelie *et al.* 1990; Bonavita-Cougourdan *et al.* 1991; Espelie *et al.* 1994), and termites (Howard *et al.* 1982; Takahashi & Gassa 1995). Consequently, a relatively exclusive society made up primarily of related individuals is being conserved[2] (Carlin 1988).

Nonetheless, mechanisms to avoid being detected as "non-nestmate" have evolved in social parasites, and they are thereby able to integrate themselves among their host species and surreptitiously receive beneficial actions that are normally reserved for kin. The socially parasitic ant species, *Polyergus breviceps* Emery, is unable to survive without the feeding, foraging, and brood care behaviors of its host species, all belonging to the ant genus *Formica* (Wheeler 1916). During the yearly cycle of the parasite's colony, *Polyergus* acquire their host workers or "slaves" in two ways: through 1) colony usurpation and 2) slave raids. In existing *Polyergus-Formica* mixed-species colonies, *Polyergus* augment their supply of host workers by raiding *Formica* nests for pupae.

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[3] In colonies with several queens or with queens that mate more than once, the coefficient of relatedness among nestmates decreases.

Many of the pupae brought back to the *Polyergus-Formica* nest are consumed. The *Formica* workers, which emerged from the spared pupae, become functioning members of the nest and perform the majority of the colony maintenance tasks (Topoff *et al.* 1985) even though interactions between non-nestmate adult *Formica* and *Polyergus* are normally aggressive. Presumably the newly eclosed *Formica* learn the characteristic odors of the *Polyergus* colony during a "critical period" as callows and, consequently, accept adults and will rear immature *Polyergus* (see Jaisson 1975a, b; Le Moli & Passetti 1977, 1978; Le Moli 1980; Le Moli & Mori 1982, 1987).

The bonding process between *Polyergus* and *Formica* during colony founding is less well understood. Laboratory tests have shown that the probability for successful takeover of a nest of naïve, adult *Formica* by a newly mated *Polyergus* queen is increased if she is able to attack and kill the host queen. Once the parasite queen has ceased attacking the *Formica* queen, the behavior of *Formica* workers towards the *Polyergus* queen changes dramatically from attacking to tending (Topoff & Zimmerli, 1993). These authors (1993) suggested that during the aggressive interactions between the two queens, the *Polyergus* queen acquires chemicals superficially from the host queen facilitating her adoption by host workers.

Cuticular chemical camouflage by the *Polyergus* queen, however, does not inherently suffice as an explanation for the tendency of host workers to care for *Polyergus* offspring since they are unlikely to be disguised in the same manner. Although the issue of worker adoption of heterospecific brood is not yet resolved, studies have shown that the treatment heterospecific brood receives from workers differs from the treatment conspecific brood receives. Heterospecific brood may be rejected or eaten (Jaisson 1975a, b; Jaisson & Fresneau 1978; Alloway 1982; Goodloe & Topoff 1987; Le Moli &

Mori 1982, 1987; Schumann & Buschinger 1991), nursed to a lessor degree (Isingrini *et al.* 1985; Carlin *et al.* 1987; Carlin & Schwartz 1989; Schumann & Buschinger 1991), or reared to adulthood and then destroyed (Haskins & Haskins 1950; Carlin *et al.* 1987). Zimmerli and Mori (1993) demonstrated that *P. breviceps* pupae are adopted by workers of one potential host species but are rejected by a sympatric, non-host species. These authors (1993) therefore suggested that the odors of *P. breviceps* pupae either (1) resemble the pheromones of host pupae because of the close phylogenetic relatedness between host and parasite taxa (see Carlin 1988; Le Masne 1956) or (2) are "naturally attractive" to host *Formica* workers (Hölldobler 1977).

In the experiment reported here, these two hypotheses were tested. If *P. breviceps* pupae are adopted by host workers because host and parasite taxa are closely related and brood odors are therefore similar, then pupae of one host *Formica* species presented to workers of another host *Formica* species should also be adopted. However, if parasite pupae are adopted because they have an attractive pheromone that is relatively independent of phylogenetic relationship, then parasite pupae should be adopted by different species of potential hosts, while pupae of alternative host species may not necessarily be cross-fostered..

METHODS

Ant Collections & Housing

The study was conducted at the Southwestern Research Station of the American Museum of Natural History in the Chiricahua Mountains of southeastern Arizona, 8.5 km west of Portal. Nests of *Formica gnava* Buckley and of *P. breviceps-F. gnava* were collected from research station grounds (el. 1646 m) vegetated with Arizona oak-alligator juniper. Nests of *Formica occulta* Francoeur and of *P. breviceps-F. occulta* were collected from an area populated with ponderosa pine just east of the Barfoot Peak trailhead (el. 2750 m). All colonies were collected at the end of June and during July 1996, brought into the laboratory, and kept in large Tupperware® boxes lined with Fluon® (Northern Products) to prevent escape. Testing took place within a month from collection dates.

Twenty-four hours prior to testing, groups of 35 workers were removed from brood-containing areas in their natal colony (to increase the likelihood that they were nurse workers and not foragers) and placed into individual Tupperware® boxes (20.5 x 45 x 3.5 cm) with Fluon®-coated rims and soil-lined bottoms. An inverted plastic cup (9 x 9 cm with two entrances 180° apart) that served as a nest was placed in the center of each box and covered with a dark cloth. Workers were fed a solution of 1:1 honey to water and local insects; the soil was moistened when necessary.

Pupa Preparation

Pupae were removed from nests approximately 12 hrs prior to being presented to workers. Half of them were marked with a dot of blue Shannon Luminous paint (Bioquip) to distinguish between (a) the species of pupae or, if *P. breviceps*, (b) the species of host in their natal nest, during observations. Because *F. occulta* workers tended to remove the pupal cases, paint markings were placed on the dorsum of the pupal head through a slight incision I made in the pupal case. Pupae in early stages of metamorphosis absorbed the paint therefore pupae at later stages of development with cuticles sufficiently hardened to resist absorption were selected for use. Generally this stage of cuticle hardening could be assessed if the eye pigment was visible through the pupal case. Marking of the pupae was counterbalanced so that if worker responses were affected by the paint, they would be distributed equally across conditions. In 50% of the tests, one species of pupae was painted, and in the other 50%, the other species was painted.

Behavior Tests

Six groups of workers were tested (see Table 3.1). Each group was presented with a total of eight pupae, four representing one species or one host species association and four representing another. The two "types" of pupae were placed alternately around the artificial nest, at a distance of approximately 2 cm. Nests were observed for 10 minutes after pupae were presented to workers and the following behaviors were recorded to provide a measure of initial acceptance (or rejection). To indicate familiarity (or lack thereof), the number of times a pupa was touched by workers with antennae (antennated) was recorded, as unfamiliar "relevant" odors generally elicit sustained, higher frequency antennal action in ants. In addition, the number of pupae that was retrieved into the nest during this time was counted. For 10 days following initial presentation of pupae, the number of pupae of each type found alive within the artificial nest box was recorded to insure retrieval responses were a function of brood tending and not a function of food recovery.

Groups 1 and 2 were used to determine whether free-living *F. gnava* and *F. occulta* would adopt conspecific and heterospecific *Formica* pupae. In Group 1, seven subcolonies of *F. gnava* were each presented with four alien conspecific pupae and four *F. occulta* pupae. In Group 2, seven subcolonies of *F. occulta* were each presented with four alien conspecific pupae and four *F. gnava* pupae. If *Formica* adopt *P. breviceps* pupae because of conserved odors, then *Formica* workers should then retrieve both conspecific and heterospecific *Formica* pupae into their nests. The frequency with which pupae of each species are antennated by workers also should not differ significantly. In addition, pupae of both species should be found, alive, in nests of the

Table 3.1

Composition of subcolonies used to test responses of *Formica* workers to pupae of *Formica* and *Polyergus breviceps*

Groups	Worker Species	State	Choice of Pupae
1	*F. gnava*	Free-living	*F. gnava* *F. occulta*
2	*F. occulta*	Free-living	*F. gnava* *F. occulta*
3	*F. gnava*	Free-living	*P. breviceps* with *F. gnava* *P. breviceps* with *F. occulta*
4	*F. gnava*	Enslaved	*P. breviceps* with *F. gnava* *P. breviceps* with *F. occulta*
5	*F. occulta*	Free-living	*P. breviceps* with *F. gnava* *P. breviceps* with *F. occulta*
6	*F. occulta*	Enslaved	*P. breviceps* with *F. gnava* *P. breviceps* with *F. occulta*

Formica workers for at least 10 days thereafter.

Groups 3 - 6 were used to compare worker responses of free-living and enslaved *Formica* to *P. breviceps* pupae. In Groups 3 and 4, ten subcolonies of free-living *F. gnava* and ten subcolonies of enslaved *F. gnava*, respectively, were each presented with *P. breviceps* pupae from nests containing *F. gnava* slaves and with *P. breviceps* pupae from nests containing *F. occulta* slaves. In Groups 5 and 6, respectively, ten subcolonies of free-living *F. occulta* and ten subcolonies of enslaved *F. occulta* were each presented with *P. breviceps* pupae from nests containing *F. gnava* slaves and with *P. breviceps* pupae from nests containing *F. occulta* slaves. If host worker adoption of *P. breviceps* can be attributed to odor similarities due to the phylogenetic relatedness of host and parasite taxa then, in addition to retrieving heterospecific *Formica* pupae, free-living and enslaved *Formica* workers should retrieve equal numbers of *P. breviceps* pupae. There should also be no difference in the number of *P. breviceps* pupae retrieved relative to the species of slave found in the nests of those pupae. However, if *Formica* workers do not adopt heterospecific *Formica* pupae but do adopt *P. breviceps* pupae then the "natural attractiveness" hypothesis may be supported.

Statistics

T-tests were used to compare the number of conspecific and heterospecific *Formica* pupae that free-living *Formica* retrieved into their nests during 10 min tests, the number of times that workers antennated the pupae, and the mean number of live pupae found inside nests during the following 10 days (Groups 1 & 2).

ANOVAs (2 x 2 factorial) with replication were used to determine whether the state of *Formica* workers (free-living and enslaved) and the species of slave in nests of *P. breviceps* pupae (conspecific or heterospecific to experimental workers) had an effect on the number of pupae that were retrieved into the nest and on the number of times pupae were antennated during the 10 min tests. ANOVAs (2 x 2 factorial) with replication were also used to determine whether the same factors had an effect on mean numbers of live pupae found inside nests during 10 days following presentation of pupae (Groups 3 – 6).

For all groups, Mann Whitney U tests were used to determine whether the order (i.e., rank) in which pupae were retrieved (if they were retrieved) differed depending on species. Latency scores were divided by the total number of pupae of that species retrieved to provide an adjusted score for ranking.

RESULTS

Immediate Responses:

Free-living Formica *with Conspecific and Heterospecific* Formica *Pupae*

Formica gnava

The mean number of *F. occulta* pupae per box ($M = 0.429$, $SE = 0.3$) that free-living *F. gnava* workers retrieved into their nests did not differ significantly from number of non-nestmate *F. gnava* pupae ($M = 1.57$, $SE = 0.65$, $N = 7$) during 10 min observations, $t_{12} = 1.6$, $p = 0.14$ (Fig. 3.1a, left). There was also no significant difference in the frequency with which free-living *F. gnava* workers antennated heterospecific *F. occulta* pupae ($M = 31.43$, $SE = 13.185$, $N = 7$) and non-nestmate conspecific pupae ($M = 9.286$, $SE = 2.72$, $N = 7$), $t_{12} = 1.645$, $p = 0.13$ (Fig. 3.1b, left).

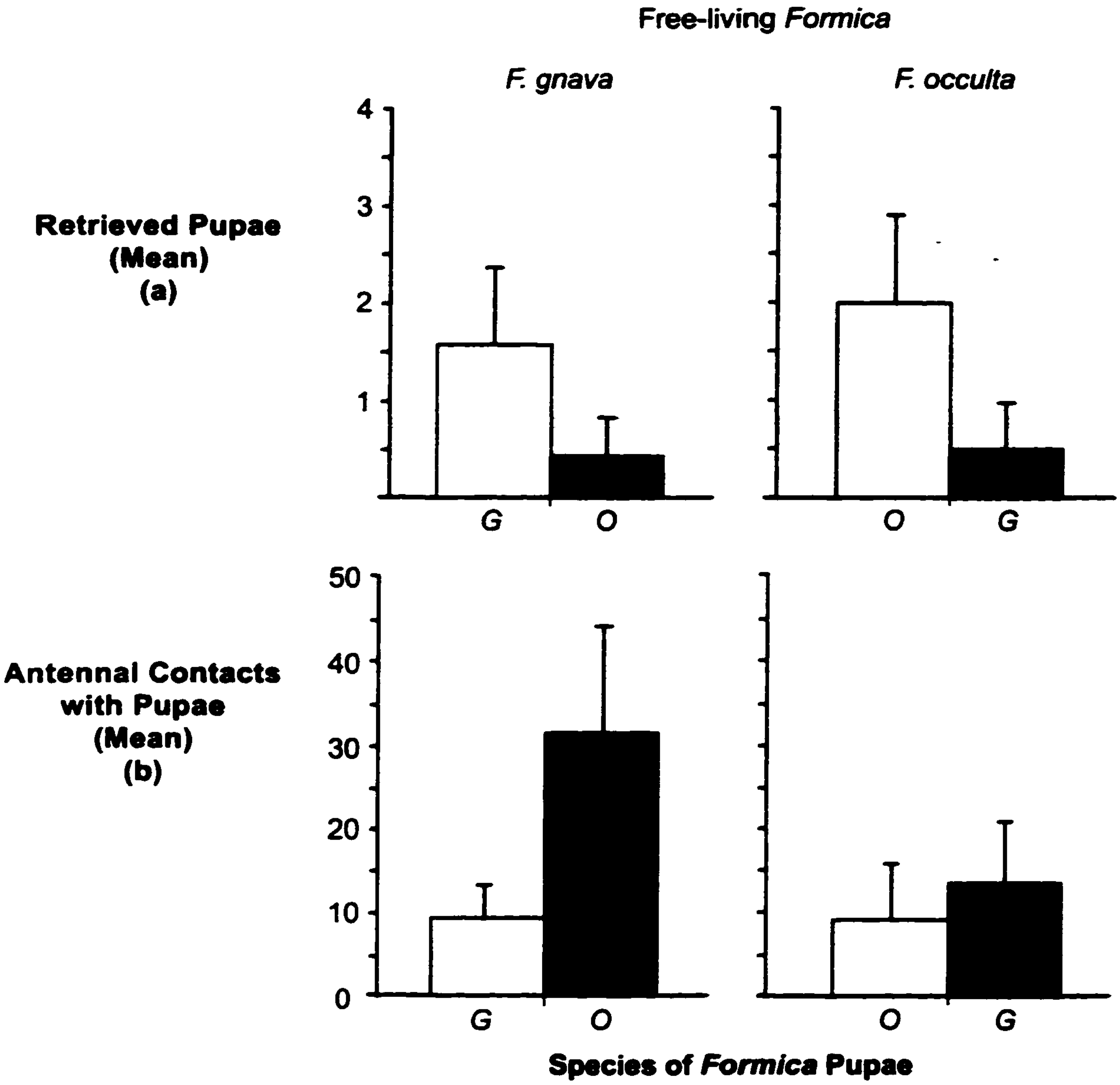

Figure 3.1. Mean numbers of conspecific and allospecific pupae retrieved into nests by free-living *Formica* (a), and mean number of times *Formica* made antennal contacts with pupae of each species (b) during 10 min tests. Although there was no significant difference in the number of pupae retrieved into the nests or in the number of times antennal contacts were made with pupae, the inverse relationship of the two behaviors suggests workers are able to detect differences between the two species of pupae. *G* = *Formica gnava* pupae, *O* = *Formica occulta* pupae.

Formica gnava workers, however, retrieved *F. gnava* pupae significantly sooner than *F. occulta* pupae (Mann-Whitney) $U = 0$, $p = 0.024$. If *F. occulta* pupae were retrieved, *F. gnava* workers tended to bring them into the nest after having retrieved most or all of the conspecific pupae (Fig. 3.2a).

Formica occulta

One subcolony of *F. occulta* was excluded from analysis because all the workers in the experimental chamber died. There was no significant difference between the numbers of non-nestmate *F. occulta* pupae ($M = 2.00$, $SE = 0.78$) and *F. gnava* pupae ($M = 0.5$, $SE = 0.342$) that free-living *F. occulta* workers retrieved into their nests during 10 min tests, $t_{10} = 1.772$, $p = 0.11$ (Fig. 3.1a, right). And the frequency with which free-living *F.* occulta workers antennated *F. gnava* pupae ($M = 13.33$, $SE = 6.9$) did not differ significantly from the frequency with which conspecific pupae ($M = 9.167$, $SE = 5.97$) were antennated, $t_{10} = 0.457$, $p = 0.66$ (Fig. 3.1b, right).

There was no difference in the time it took *F. occulta* to retrieve conspecific pupae into the nests from the time it took *F. occulta* to retrieve *F. gnava* pupae into the nests, $U = 6$, $p = 0.128$ (Fig. 3.2b). This, however, is likely an artifact of the significantly greater number of conspecific pupae that were retrieved as compared to the number of *F. gnava* pupae that were retrieved.

Immediate responses:
Free-living and Enslaved Formica *with* Polyergus breviceps *Pupae*

Formica gnava

The particular slave species in nests of *P. breviceps* pupae had no apparent effect on whether *F. gnava* workers retrieved them into their nests ($F_{1,36} = 0.000$, $p = 1$) during 10

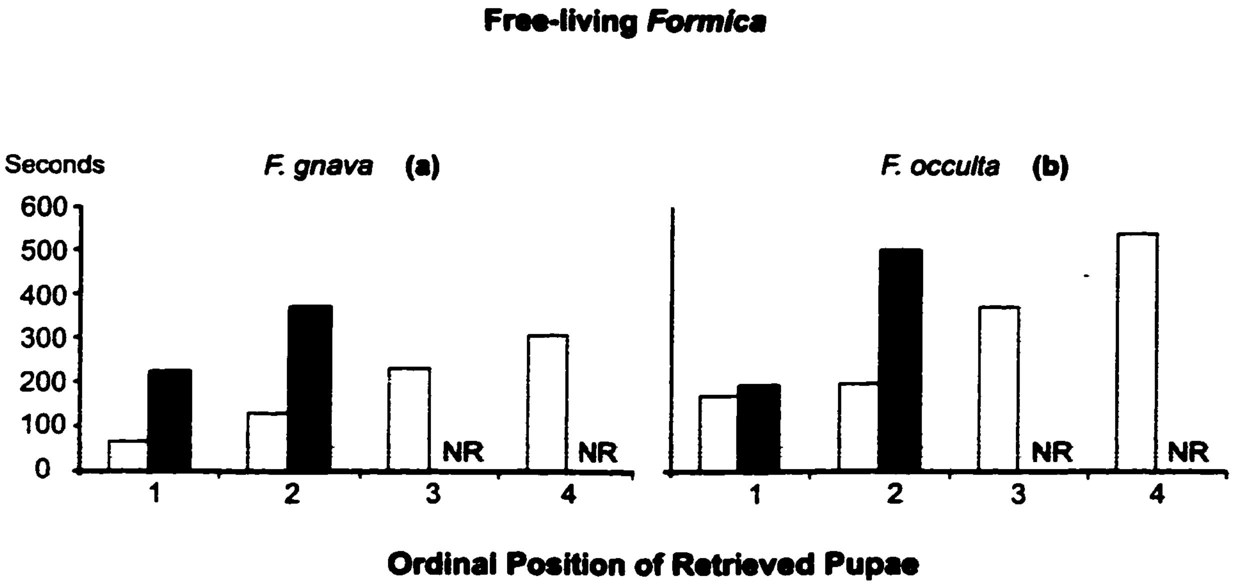

Figure 3.2. Average latencies in seconds to retrieval of each of the 4 available *F. gnava* pupae and each of the 4 available *F. occulta* pupae by free-living *F. gnava* (a) and *F. occulta* (b) workers. **White bars** represent conspecific pupae. **Black bars** represent pupae of the alternative host species. **NR** indicates pupae of that position were not retrieved. Because not all pupae were retrieved, average latencies for each species in each position of the retrieval order were multiplied by the proportion of non-retrieved pupae to adjust for higher average latencies due to greater numbers of pupae retrieved. *Formica* generally retrieved all 4 conspecific pupae before retrieving allospecific *Formica* pupae, if they retrieved allospecific pupae at all.

min tests. There was also no interaction between slave-species association of *P. breviceps* pupae and whether *Formica* workers were free-living or enslaved ($F_{1,36}$ = 0.000, p = 1). There was, however, a significant effect of worker state on the retrieval of pupae; significantly more *P. breviceps* pupae (from nests containing *F. gnava* and from nests containing *F. occulta*) were retrieved by enslaved *F. gnava* workers than by free-living workers ($F_{1,36}$ = 5.29, p = 0.027) (Fig. 3.3a).

Worker state also had a significant effect on antennation frequency (Fig. 3.3b). *Polyergus breviceps* pupae were antennated significantly more frequently by enslaved workers than by free-living *F. gnava* workers ($F_{1,36}$ = 9.56, p = 0.0038). Again, the species of host in nests of *P. breviceps* pupae did not seem to have an effect on antennation frequency ($F_{1,36}$ = 0.835, p = 0.37). There was also no significant interaction between slave-species association of *P. breviceps* pupae and worker state ($F_{1,36}$ = 0.02, p = 0.89).

The order in which *P. breviceps* pupae were retrieved by enslaved *F. gnava* mirrored the retrieval order of free-living *F. gnava* that were presented with conspecific and hetero-specific *Formica* pupae; free-living workers retrieved no *Polyergus* pupae during the 10 min (Fig. 3.4a). Although enslaved *F. gnava* retrieved many of the four *P. breviceps* pupae from nests with *F. gnava* slaves sooner than they retrieved *P. breviceps* pupae from nests with *F. occulta* slaves, this was not statistically significant, U = 37, p = 0.34 (Fig. 3.4b). This insignificant difference, however, is probably a result of the higher numbers of *P. breviceps* pupae from nests with *F. gnava* slaves retrieved in the first position.

Mean numbers of pupae retrieved by both free-living and enslaved *F. gnava* workers (Fig. 3.3a) during 10 min tests were low (0.5 and 0 respectively). General inactivity of

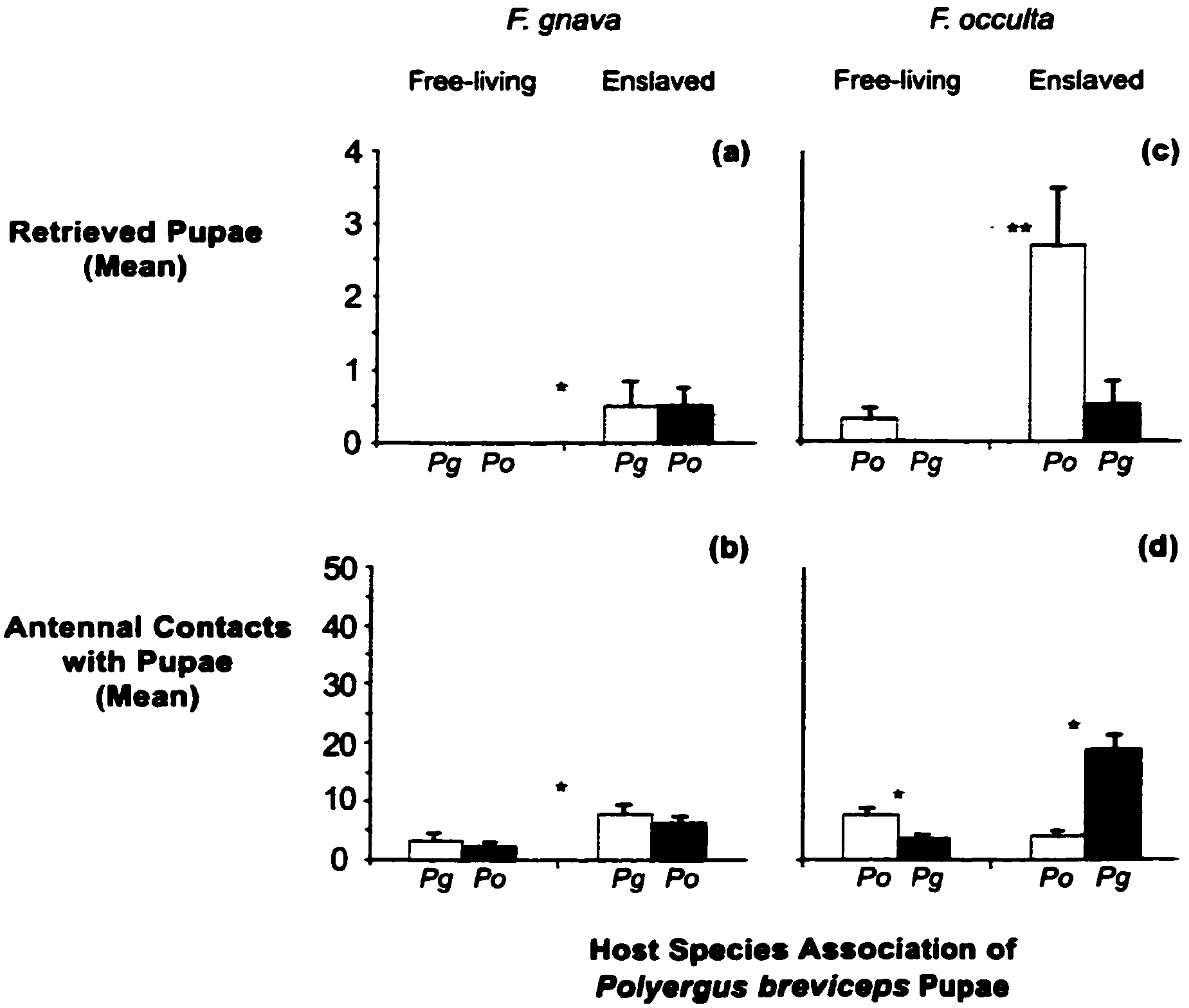

Figure 3.3. Mean numbers of *P. breviceps* pupae associated with *F. gnava* or *F. occulta* host species that were retrieved into nests by free-living or enslaved *F. gnava* **(a)** and free-living or enslaved *F. occulta* **(d)**, and mean number of times *P. breviceps* pupae were antennated by **(b)** *F. gnava* and **(d)** *F. occulta* workers. ***Pg*** = *P. breviceps* pupae from nests containing *F. gnava* slaves, ***Po*** = *P. breviceps* pupae from nests containing *F. occulta* slaves. Single asterisk represents significant main effect at $p < 0.05$, double asterisk represents significant interaction at $p < 0.05$.

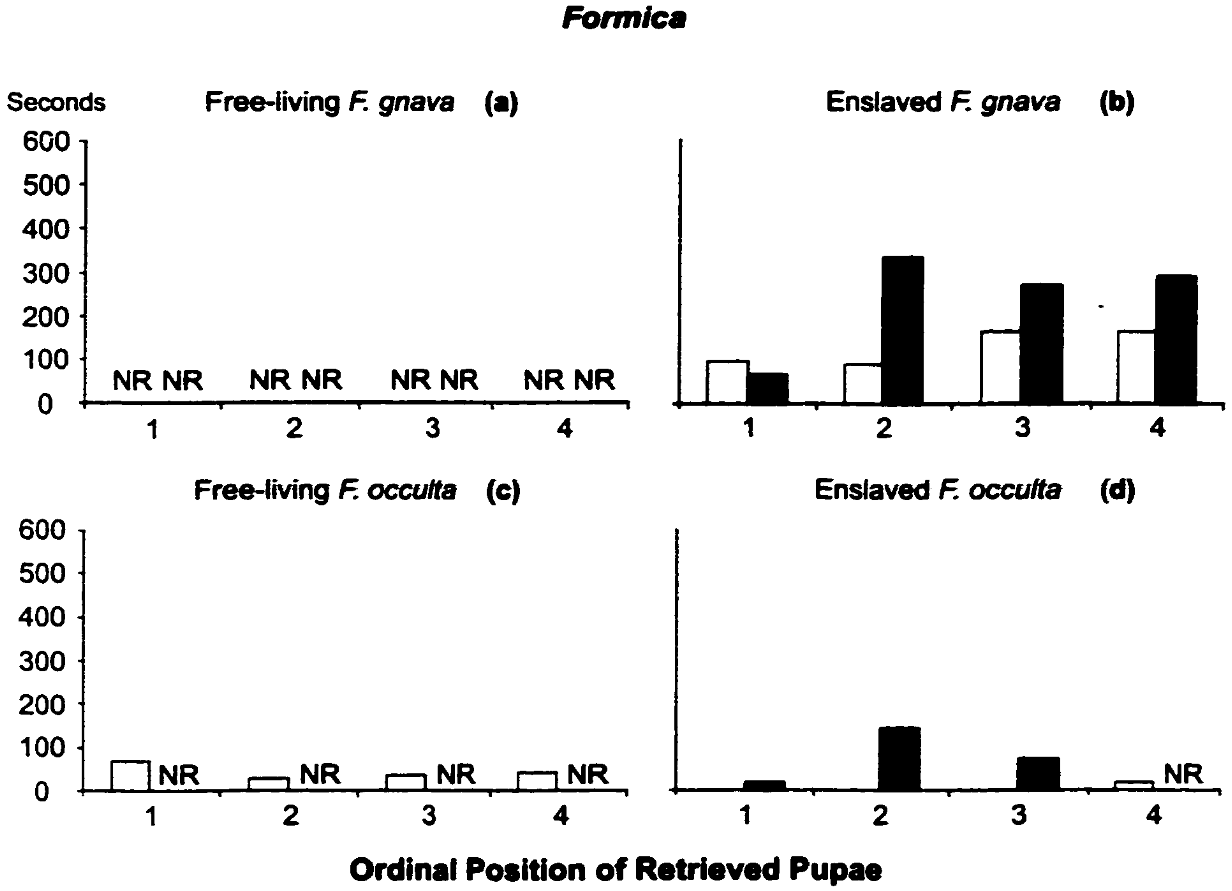

Figure 3.4. Average latencies in seconds to retrieval of each of the 4 available *P. breviceps* pupae from nests with *F. gnava* slaves and each of the 4 available *P. breviceps* pupae from nests with *F. occulta* slaves by free-living **(a)** or enslaved **(b)** *F. gnava* and by free-living **(c)** or enslaved **(d)** *F. occulta* workers. **White bars** represent *P. breviceps* pupae associated with a host species conspecific to workers tested. **Black bars** represent *P. breviceps* pupae associated with a host species heterospecific to workers tested. **NR** indicates pupae of that position were not retrieved. Because not all pupae were retrieved, average latencies for each species in each position of the retrieval order were multiplied by the proportion of non-retrieved pupae to adjust for the higher average latencies due to greater numbers of pupae retrieved. If free-living *Formica* retrieved *P. breviceps* pupae, they generally retrieved the *P. breviceps* pupae that were associated with a host species conspecific to themselves sooner than those that were associated with a host species heterospecific to themselves. Enslaved *Formica* showed a similar pattern.

workers during testing time (almost no workers emerged from the nests) coupled with the dramatic increase of pupae retrieved during the first 24 hours suggests, however, that this is not an indication of *P. breviceps* pupae rejection. In the other conditions, workers were continually emerging from and returning to their nests, and frequently came into contact with the provided pupae. These trials, therefore, appear to reflect active rejection (or acceptance) of pupae.

Formica occulta

During the first 10 min following the presentation of the pupae, there were significant main effects of both worker state (free-living and enslaved) ($F_{1,36}$ = 13.17, p = 0.0009) and the slave-species association of *P. breviceps* pupae ($F_{1,36}$ = 17.73, p = 0.0002) on the retrieval-into-the-nest response by *F. occulta*, as well as a significant interaction ($F_{1,36}$ = 7.6, p = 0.009) (Fig. 3.3b). Therefore, the retrieval responses were analyzed separately by free-living and enslaved groups. *Formica occulta* workers in the enslaved group retrieved into their nests significantly greater numbers of *P. breviceps* pupae from nests with *F. occulta* slaves than *P. breviceps* pupae from nests with *F. gnava* slaves ($F_{1,18}$ = 15.158, p = 0.0011). The host-species association of *P. breviceps* pupae, however, had no significant effect on the retrieval responses of free-living workers ($F_{1,18}$ = 2.647, p = 0.12).

There were also significant main effects of both slave-species association of *P. breviceps* pupae ($F_{1,36}$ = 15.16, p = 0.0004) and worker state ($F_{1,36}$ = 18.56, p = 0.0001), and a significant interaction ($F_{1,36}$ = 49.58, p = 0.0001) on the frequency with which workers antennated the pupae during the first 10 min (Fig. 3.3d). When analyzed separately, again by state, enslaved *F. occulta* workers antennated *P. breviceps* pupae more frequently when the pupae came from nests with *F. gnava* as slaves than when the pupae came from nests with *F. occulta* slaves ($F_{1,18}$ = 40.25, p = 0.0001). Free-living

F. gnava workers, however, antennated *P. breviceps* pupae significantly more when they came from nests with *F. occulta* slaves ($F_{1, 18}$ = 9.627, p = 0.0061).

Free-living *F. occulta* retrieved only *P. breviceps* pupae from nests with *F. occulta* slaves, and no rank comparison could be made. Enslaved *F. occulta*, however, retrieved both types of *P. breviceps* pupae, and retrieved *P. breviceps* pupae from nests with *F. occulta* slaves in all four positions significantly sooner than they retrieved *P. breviceps* pupae associated with *F. gnava*, U = 79, p = 0.0001 (Figs. 3.4c & 3.4d). Taken together, retrieval and latency to retrieval results suggest that the *P. breviceps* pupae from different mixed-species nests are recognizably different, and that the *P. breviceps* pupae are unfamiliar to free-living workers.

Pupa Survivorship as a Measure of Adoption:
Free-living Formica *with* Formica *Pupae*

Formica gnava

Significantly more non-nestmate conspecific *F. gnava* pupae (M = 3.9, SE = 0) than heterospecific *F. occulta* pupae (M = 0.143, SE = 0) were found inside nests of *F. gnava* during the 10 days following the initial presentation of pupae, t_9 = 72.36, p = 0.0001 (Fig. 3.5a).

Formica occulta

Free-living *F. occulta* also maintained significantly more non-nestmate conspecific pupae (M = 2.766, SE = 3.86) than heterospecific *F. gnava* pupae (M = 0.143, SE = 0) inside their nests for 10 days following pupae presentation, t_9 = 21.494, p = 0.0001 (Fig. 3.5d). The temporal decline of live *F. occulta* pupae found in *F. occulta* nests might be evidence

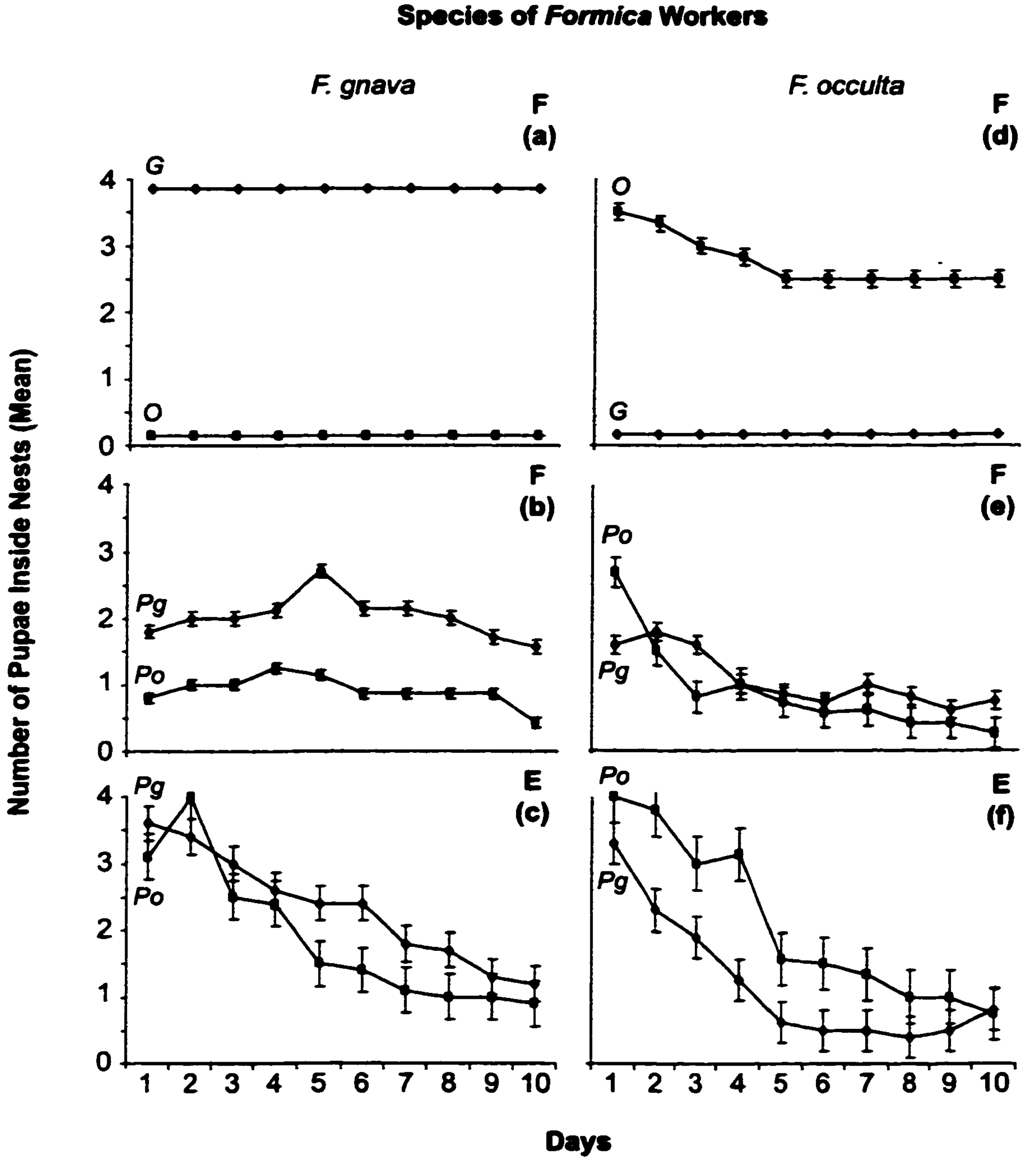

Figure 3.5. Survival curves illustrating the mean number of pupae found alive and inside the nests of two potential *Formica* hosts for the 10 days following the initial presentation of pupae. Bars represent standard error of the mean. *G* = *F. gnava* pupae, *O* = *F. occulta* pupae, *Pg* = *P. breviceps* pupae from nests with *F. gnava* slaves, *Po* = *P. breviceps* pupae from nests with *F. occulta* slaves, **F** = free-living workers, **E** = enslaved workers.

for some rejection of non-nestmate conspecifics.

Free-living and Enslaved Formica *with* Polyergus breviceps *Pupae*

Formica gnava

There were significant main effects of both worker state and slave-species association of *P. breviceps* on the number of pupae that were maintained by *F. gnava* workers inside their nests during the 10 days following the presentation of pupae (Figs. 3.5b and 3.5c). Significantly more *P. breviceps* pupae from nests containing *F. gnava* slaves than *P. breviceps* pupae from nests containing *F. occulta* slaves were found in nests of both free-living and enslaved *F. gnava* ($F_{1,36} = 12.44$, $p = 0.001$). However, enslaved workers maintained significantly more *P. breviceps* pupae overall, having retrieved more of both *P. breviceps* associated with *F. occulta* and *P. breviceps* associated with *F. gnava* either during the initial 10 min or at some point prior to the daily census ($F_{1,36} = 8.62$, $p = 0.006$). The interaction between worker state and slave-species association of *P. breviceps* was not significant ($F_{1,36} = 2.25$, $p = 0.142$).

Formica occulta

The slave species that *P. breviceps* pupae were associated with had no effect on whether *F. occulta* workers maintained them inside their nests during the 10 days following pupae presentation ($F_{1,36} = 1.68$, $p = 0.21$). There was, however, a significant effect of worker state ($F_{1,36} = 5.65$, $p = 0.02$) and a "marginally significant" interaction between worker state and host-association of *P. breviceps* pupae ($F_{1,36} = 3.61$, $p = 0.065$). Significantly more live *P. breviceps* pupae were found inside nests of *F. occulta* for 10 days following pupae presentation when workers were enslaved then when workers were free-living. Enslaved workers also tended to maintain more *P. breviceps* pupae when they were from nests with *F. occulta* slaves than from nests with *F. gnava* slaves (Figs. 3.5e and 3.5f).

DISCUSSION

Social parasites integrate themselves into other societies through chemical and/or behavioral manipulation. Morphological similarities between parasites and their hosts suggest that they evolved from a common free-living ancestral species (Emery 1909; Emery's rule – [Le Masne 1956]) or that the parasite evolved directly from the host species (Buschinger 1986; Bourke & Franks 1991). In either instance, the close relationship gives potential to the currently accepted notion that chemical features of the host or the ancestral unenslaved species are conserved in the parasite and, in the second case, in the host. Chemical conservation in conjunction with derived parasitic behaviors could facilitate parasitization of the host group by enabling the parasite to circumnavigate discrimination (Carlin 1988). Support for the chemical similarity hypothesis as a consequence of close phylogenetic relatedness becomes difficult to support, however, because social parasites are not always restricted to parasitizing one species (ex. *Harpagoxenus sublaevis* (Nylander), *Leptothorax duloticus* Wesson, at least three species of *Polyergus)*. In these instances, assuming that parasite chemistry plays a role in the development of mixed-species nests, the parasite would need to be chemically similar to multiple species simultaneously, a tricky task considering interactions between non-nestmate conspecifics are often aggressive.

Polyergus, which, along with the socially parasitic genus *Rossomyrmex*, forms the sister group to *Formica* (Agosti 1994), enslaves species in the genus *Formica*. Retention of *Formica* olfactory characteristics in *Polyergus* is presumed to be accountable for the successful integration of *Polyergus* offspring. But, when *F. gnava* and *F. occulta* test colonies were presented with a choice of *P. breviceps* pupae from nests with *F. gnava* slaves and from nests with *F. occulta* slaves, workers did not retrieve and maintain *P. breviceps* pupae as they did when presented with non-nestmate conspecific pupae. Furthermore, of the retrieved *P. breviceps* pupae, more tended to be from nests in which the slave species was the same species as the workers retrieving them.

Odor of Polyergus breviceps

The findings of this study are not consistent with the assertion that social parasites are accepted by their host species because they are chemically similar as a consequence of close phylogenetic relationship. This seems particularly true when considering that pupae of heterospecific *Formica* were almost completely rejected and that the pupae that were not retrieved into the nest by workers, during the 10 min tests, were generally antennated more frequently (Figs. 3.1 and 3.3). Instead, the differential retrieval and maintenance responses suggest that the chemical signatures of different species of pupae are variable and, in enslaved groups, that workers had prior exposure to *P. breviceps* odor. Undoubtedly, other factors are involved in the adoption of slave-maker offspring by naïve *Formica* (see Chapter 4).

Variability in signatures of *P. breviceps* pupae, as indicated by the variable reactions of test *Formica* workers, is likely the product of three ingredients that, because of the nature of the parasite/host relationship, act agonistically (Fig. 3.6):

1) ***Species-Specific Odor.*** For 10 days following the presentation of pupae, the average number of *P. breviceps* pupae (from nests with *F. gnava* and nests with *F. occulta* slaves) that were found within nests of enslaved workers was noticeably greater than the average found in nests of unenslaved workers (2.47 and 1.22 respectively). The enslaved workers used in this study had been taken from natural *P. breviceps-Formica* nests that, based on the large number of workers, were likely to

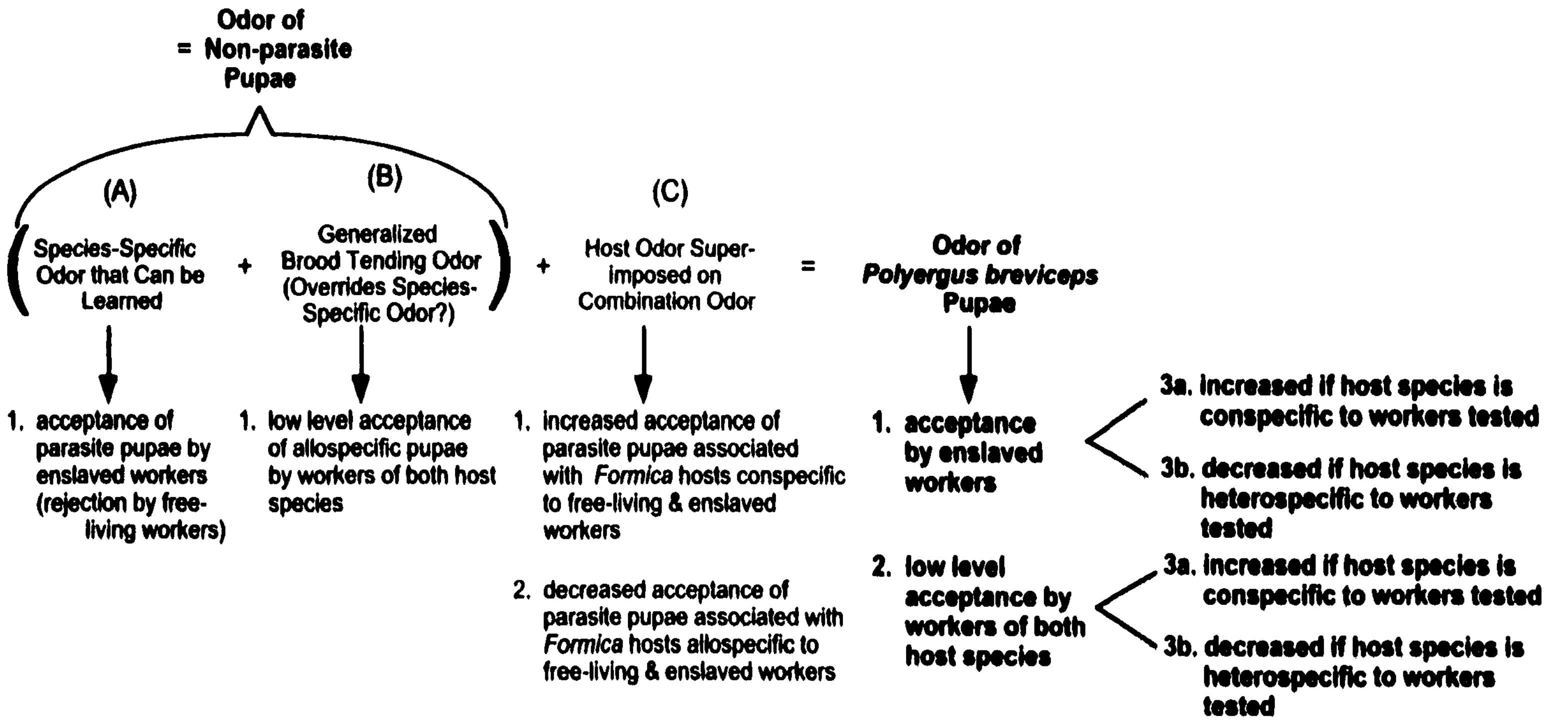

Figure 3.6. Flow chart representing possible contributions to the odor of *P. breviceps* pupae based on the differential brood retrieval and maintenance behaviors of two species of host *Formica*, free-living and enslaved. Letters indicate the odor type; numbers explain the expected behavioral response.

have existed for several years. Most, if not all, *Formica* workers in these nests had probably been acquired as pupae, when their natal nests were raided by *P. breviceps*. As many ant species appear to recognize odors of other ants that they encounter soon after emergence, exposure to *P. breviceps* odors most likely accounts for the greater numbers of *P. breviceps* pupae that these workers adopted (Goodloe & Topoff 1987).

Nonetheless, the disparate numbers of *Polyergus* pupae found within nests of free-living workers and of enslaved workers clearly indicate that *P. breviceps* pupae have olfactory characteristics that are distinct from the odors of their host species. Thus, one contribution to the odor of *P. breviceps* pupae appears to be a heritable, species-specific odor (Fig. 3.6 - A).

2) ***Host Odor.*** Chemical secretions from the post-pharyngeal gland are believed to form, in large part, the chemical signature of a species and, more specifically, the chemical signature of a particular nest (Bagnères & Morgan 1991). Because cuticular lipids may serve as solvents to absorb extrinsic lipid compounds, post-pharyngeal secretions may be incorporated into any one individual's signature through mutual grooming and trophallaxis (Soroker *et al.* 1994, 1995; Vienne *et al.* 1995). Thus, chemicals transferred from queen to workers (Carlin & Hölldobler 1983) and from worker to worker (Stuart 1987; Obin & Vander Meer 1989) create a "gestalt" nest signature (Crozier & Dix 1979; Stuart 1987) and the chemicals are "passed on" from workers to immatures and callows (Morel & Blum 1988). Worker "preference" for *P. breviceps* pupae from nests with a slave species conspecific to test workers (versus from nests with a heterospecific slave species) within both enslaved groups and within the free-living *F. gnava* group indicates that odors of the respective host species are present on *Polyergus* pupae. These "superimposed" odors may mask, to some degree, the *Polyergus* odor, and form the third hypothetical element of the *P. breviceps* pupal signature (Fig. 3.6 - C).

Consequently, *Formica* odors on *P. breviceps* pupae (in addition to *P. breviceps*-specific and generalized odors, see 3 below), which are not recognized across *Formica* species, either are familiar (conspecific) to experimental workers and enhance acceptance of *Polyergus* pupae (Fig. 3.6 - D_{3a}), or fail to provide the appropriate signal (are heterospecific) and result in rejection of *P. breviceps* pupae (Fig. 3.6 - D_{3b}). Hence, adoption of *P. breviceps* pupae by species of *Formica* that are conspecific to the slave species in the *P. breviceps* nest does not necessarily indicate chemical similarity as a consequence of close phylogenetic relationship.

Alternatively, worker preference for *P. breviceps* pupae from nests with a conspecific slave species may reflect the existence of two host races, subspecies, or species. If any one is the case then this may additionally suggest a degree of selected chemical adaptation/convergence of the parasite to its particular host species, though not necessarily negate host odor contributions.

3) ***Generalized Brood Odor.*** The results of this study indicate that pupae of another species, host or parasite, are distinguishable from conspecific pupae. Yet some pupae of heterospecific *Formica* and *P. breviceps* were also adopted, revealing the potential for mixed-species nests to form through interspecific brood raiding alone. Some have suggested that heterospecific pupae are adopted because odors, which are involved in brood tending, are common to diverse taxa (Hölldobler 1977; Kaib *et al.* 1993). The combination of generalized brood tending odors and species specific odors would explain past inconsistencies in the ability to cross-foster heterospecific larval and pupal brood (e.g. Le Moli & Mori 1987; Plateaux 1985; Haskins & Haskins 1950). Consequently, using pupae in adoption tests may not accurately reveal integration mechanisms of slave-makers during colony founding.

Cost and Benefit of Different Immature Odors

Maintaining a closed society (by rejecting allospecific or unrelated offspring) while minimizing loss (incorrectly rejecting conspecific or related offspring) might require offspring to have different odors at different stages of development. In Figure 3.7, three hypothetical scenarios depict the possible odors (species-specific and/or generalized) individuals may have, the stage(s) of development at which they could be present, and the benefit or cost for being present at that stage. In response to these odors, the detector (worker ant) may 1) correctly accept; 2) correctly reject (destroy); 3) incorrectly accept; and 4) incorrectly reject an individual. Arbitrary points indicate a relative gain or loss in inclusive fitness based on a response, beginning with +1/-1 for a correct/incorrect decision at the egg stage, and increasing/decreasing in increments/decrements of 1 for each succeeding stage to reflect invested energy.

Species-specific (used instead of colony-specific for simplification purposes) odors are assumed to involve greater numbers or variations in relative proportions of chemical compounds and thus require greater neural sensitivity within the receiver. The generalized brood tending odor is assumed to be limited to one or a few compounds which either make(s) up the entire chemical profile or override(s) the species-specific signature and require(s) less neural sensitivity. Because the idea of a generalized brood tending odor implies that individuals are "universally" accepted, no points are assigned for correct rejection or incorrect rejection if this odor is present.

Scenario 1. Species-specific odors from the queen are "handed" down to her eggs (by internal [Schal *et al.* 1998] or external transmission [Vander Meer & Morel 1995] of environmental odors; or through genetic transmission) and are maintained by genetic and environmental factors at all stages of development. Maintenance of exclusivity is maximized because heterospecific eggs are distinguishable and, at the earliest stage of

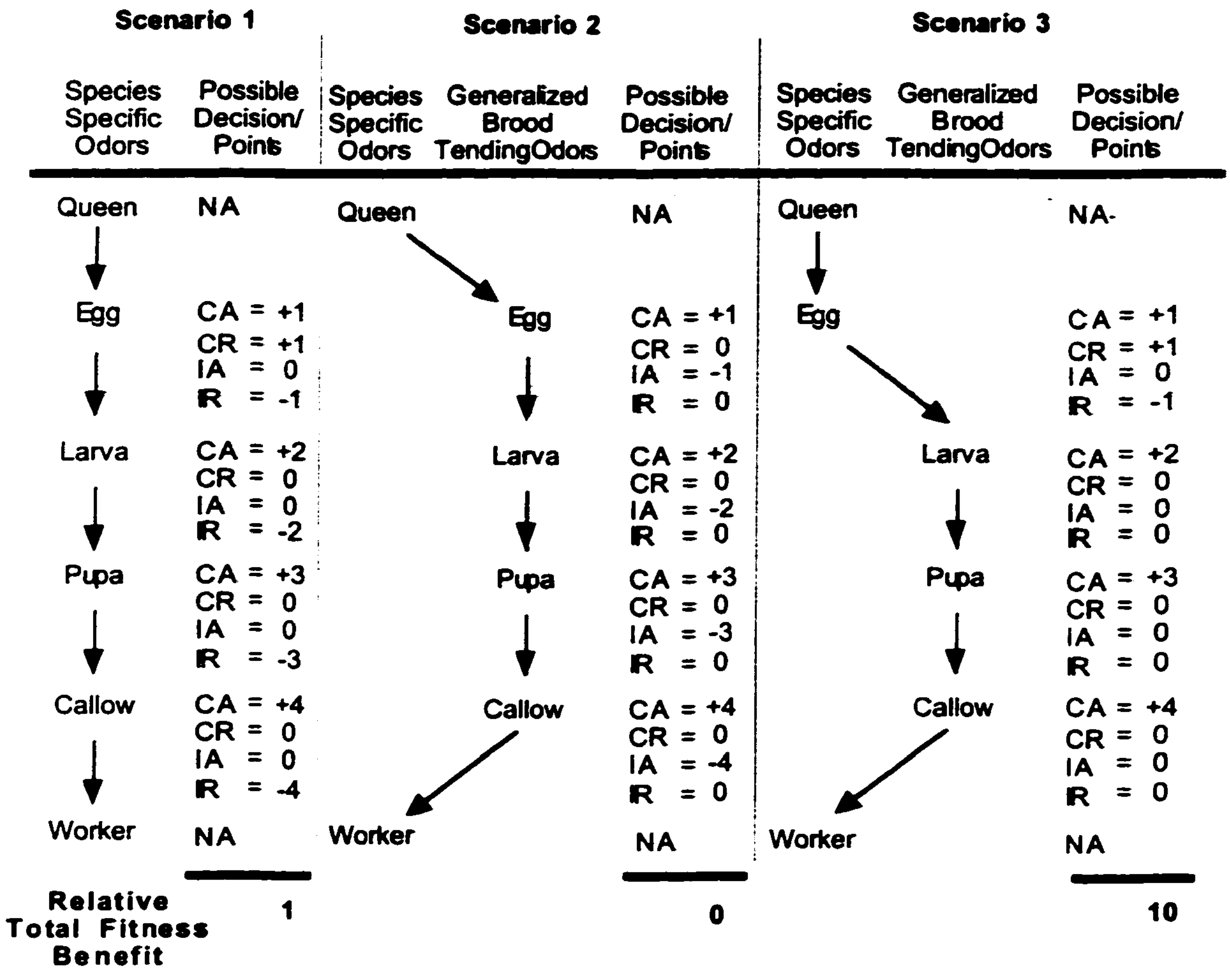

Figure 3.7. Three hypothetical scenarios depicting the costs and benefits associated with having a species-specific odor or generalized brood tending odor at different stages of immature development. At each stage, the detector (worker ant) may make one of four decisions. The decision is assigned +/- points depending on whether the decision would result in an increase or decrease in inclusive fitness. The relative total fitness points, at the bottom of each column, suggest the relative likelihood that the factors in any one scenario would be selected for. See text for point assignment. CA = correctly accept, CR = correctly reject, IA = incorrectly accept, IR = incorrectly reject, NA = not applicable.

development, are destroyed by workers. However, because larvae, pupae, and callows maintain species-specific odors requiring detectors to be acutely sensitive, the chances for incorrect recognition errors (destruction of related nestmates) at later stages are high. And, as development proceeds, the cost of making a recognition error in terms of time and energy expended on an individual swells. Because discrimination has already occurred at the egg stage, any individuals destroyed at succeeding stages are likely to be related and no points are assigned for correct rejection post egg stage. The relative total fitness benefit is 1.

Scenario 2. All immatures possess a generalized brood-tending odor and there is little chance for an incorrect recognition error. This, however, almost guarantees that any alien conspecific and heterospecific immature will be reared. Accepting immatures (alien conspecific or heterospecific) at all stages of development, and then either 1) accepting alien conspecific and heterospecific callows if environmental recognition odors are absorbed or 2) rejecting heterospecific callows/workers if heritable odors become apparent, funnels energy away from rearing related nestmates and reduces inclusive fitness. The relative total fitness benefit here is 0. In addition to being a detriment to fitness, the internal nature of oocyte development suggests that egg odors are unlikely to be generic across species. Recently, the cuticle and the gonads have been shown to be sites for hydrocarbon deposition for several insect taxa (Schal *et al.* 1998). In *Blattella germanica*, the German cockroach, concentrations of hydrocarbons increase significantly during the period of oocyte maturation (Schal *et al.* 1994). Should eggs incorporate hydrocarbons of the queen, they most assuredly would have an odor that is species specific.

Scenario 3. Again the integrity of the nest is maximized as species specific odors are handed down from the queen to her eggs, and minimal energy is invested in rearing alien conspecific or heterospecific immatures. In this scenario, however, a generalized tending odor emerges in larvae and pupae that is maintained until the worker stage. Thus, larvae and pupae are less likely to be incorrectly destroyed by nestmates as they might be in Scenario 1. In this case, the relative total fitness benefit is the highest, 10.

Comparison of hypothetical costs and benefits of having a species specific odor versus an odor accepted across species for every stage of immature development indicates that an egg is more likely to have species-specific odor than a pupa (and their nestmates) which would benefit more by having a generic odor. Evidence for specificity in egg odors comes from observations on the slave-maker *Protomognathus americanus* (Emery) (formerly *Harpagoxenus*) (Buschinger & Pfeifer 1988). During slave raids on nests of *Leptothorax americanus*, *P. americanus* discards eggs and young larvae outside the raided nest and brings back only the older larvae and pupae to the *Protomognathus* nest. Sometimes eggs and small larvae are retrieved during raids, but they tend to be eaten later (Alloway 1979). The slave-makers *Harpagoxenus sublaevis* and *Harpagoxenus canadensis* Smith smear eggs and small larvae from raided colonies with secretions from an abdominal gland to make them "less attractive" to workers of the raided colony, and less likely to be rescued (Buschinger *et al.* 1980). The fact that workers separate eggs from other brood also suggests that eggs (and small larvae) have a chemical signature that is distinct from older immatures. Examination of chemical profiles may reveal differences in relative proportions of cuticular hydrocarbons, if not qualitative differences, among egg signatures of different species and perhaps signature convergence at later stages of development.

The attribution that social parasites are chemically similar to their host species to the extent that interspecific bonds are easily formed on the basis of adoptions of larvae and pupae may be an oversimplification. Immature odors may differ depending on developmental stage and/or older immatures may be "tainted" by chemicals as a result of their relatively lengthy association with adults. Thus, immatures at different stages of development could hypothetically solicit different responses from both conspecifics and allospecifics. Using pupae to investigate mechanisms of integration, whereby the mixed-species colony is initiated through adoption of a parasite queen and her eggs, might not answer properly the question being asked. In the case of *P. breviceps*, pupae undoubtedly bring along numerous confounding variables as discussed above, and cross-fostering eggs might better reflect the processes underlying integration of *P. breviceps* offspring among naïve host workers.

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CHAPTER 4

Do These Eggs Smell Funny to You?: The Discriminating "Nose" of *Formica* Species That Are Hosts to the Slave-maker, *Polyergus breviceps*

SUMMARY

Offspring of the slave-maker ant, *Polyergus*, are believed to be adopted and reared by their *Formica* host species because, as close phylogenetic relatives, brood tending odors have been retained in the derived species. Pupae are commonly used in laboratory tests to examine this problem, but because they usually have a lengthy association with tending host workers (from eggs on), there is the risk that pupae are contaminated with host odors. The preference of host species to tend *Polyergus* pupae over other species that have not had contact with host workers may merely reflect a response to conspecific signals acquired by pupae through allogrooming. This study reports the findings of tests in which free-living and newly enslaved *Formica* had been offered conspecific eggs, heterospecific *Formica* eggs, and *Polyergus breviceps* eggs. Both *Formica gnava* and *Formica occulta* (free-living and newly enslaved) adopted and reared many of the conspecific eggs presented to them, and rejected almost all heterospecific *Formica* and *P. breviceps* eggs. After approximately five months, an increase in the number of *P. breviceps* eggs that were reared was observed. Examination of hydrocarbon patterns of eggs and pupae reveals minimal correlation with rearing tendencies, and suggests that some other mechanism accounts for the eventual rearing of *Polyergus breviceps* offspring during colony founding, when queens take over nests of adult *Formica*.

INTRODUCTION

The slave-maker, *Polyergus breviceps* Emery, raids nests of *Formica* for pupae. The pupae are brought back to the slave-maker nest, where they mature and become active members of the slave-maker colony, caring for the slave-makers as well as for the *Formica* retrieved on other raids. Generally, ant societies maintain exclusivity by rejecting conspecific non-nestmates or heterospecifics on the basis of their epicuticular chemicals, which differ from their recognition template (Vander Meer & Morel 1998). In many ant species, the recognition template is believed to form largely through exposure to odors of other individuals during early periods of development, although templates are probably modified throughout life (Vander Meer & Morel 1998). The recognition template of "kidnapped" *Formica* in *Polyergus* nests will include the *Polyergus* odor, as well as the odor of other *Formica*, and most likely accounts for their adopting *Polyergus* (Goodloe & Topoff 1987). During colony establishment, however, newly mated *P. breviceps* queens take over nests of naïve, adult *Formica*. Where we might expect offspring of the alien invader to be rejected, the existence of incipient mixed species nests with callow *P. breviceps* although a *P. breviceps* queen is unable to rear her own first brood suggests that the "child-care" is provided by the newly enslaved *Formica*.

Attempts to ascertain why offspring of *Polyergus* and other slave-makers are adopted by host colonies have primarily involved observations on the behavioral responses of workers to pupae of their own species, the parasite species, and a non-parasite species (Alloway 1982; Zimmerli & Mori 1993; see Chapter 3). Certain responses, such as bringing a pupa into the nest, have been, in some cases, useful taxonomic tools (Rosengren & Cherix 1981). For example, if pupae of another species are placed outside an artificial nest, they tend not to be carried into the nest, whereas conspecific pupae are retrieved and reared (Zimmerli & Mori 1993; see Chapter 3). Other similar tests have shown that, when two species are intermingled, heterospecific pupae are groomed to a lesser degree than conspecific pupae (Carlin *et al.* 1987; Carlin & Schwartz 1989), or ignored altogether (Jaisson 1975; Jaisson & Fresneau 1978; Alloway 1982; Le Moli & Mori 1982, 1987; Schumann & Buschinger 1991).

Immatures of other species, however, seem to be cross-fostered easily (Fielde 1903; Plateaux 1960; Jaisson 1971). On the basis of these findings, it has been suggested that the chemical substance of immatures elicits tending behaviors across generic taxa (Hölldobler 1977). A generalized odor as such might potentially ensure that immatures are cared for. The finding that cuticular hydrocarbon profiles of pupae from different species (see Fig. 4.6) and even different genera are similar is compelling (Yamaoka 1990; Kaib *et al.* 1993).

Laboratory adoption tests using *Polyergus* pupae have also relied on worker ability to recognize chemical distinctions between different species of pupae. Findings show that not all pupae are treated equally. Whereas heterospecific *Formica* pupae were rejected, *Polyergus* pupae were reared by small subcolonies of free-living and enslaved *Formica*, leading to the hypothesis that *Polyergus*, as a derived, phylogenetic relative, has retained certain chemical features of their *Formica* hosts[3] (Zimmerli & Mori 1993).

On the other hand, there was a diminished tendency for *Formica* workers to adopt *P. breviceps* pupae when the pupae came from nests where the slaves were of a different species of *Formica* (see Chapter 3). This suggests that the lengthy association with host workers may effect pupae odors soliciting worker responses. Because the relationship that *Polyergus* entertains with its host species is an obligatory one, *Polyergus* are never without their host species. Any *Formica* chemicals that are involved

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[3] Or, if host and parasite evolved through allopatric speciation, then *Polyergus* pupae are adopted because host and parasite have retained chemical features of their common ancestor.

in producing a recognition signature and are transferable from worker to pupae could feasibly taint *Polyergus* pupae. In choice tests, preference for *Polyergus* pupae over other species, which have not had any contact with the particular host species involved, might merely reflect a response to conspecific signals that have been acquired by *Polyergus* pupae through allogrooming. If this is true, then such tests involve a critical confounding variable and might not accurately reflect the mechanisms underlying the adoption of parasite host workers during colony founding.

Eggs receive a great deal of care, at first by their mothers and then by their sisters who take over the brood-care responsibilities. This alone is indicative of a chemical signal that directs the attention of both queen and sisters towards eggs[4]. Because newly laid eggs have had little contact with the outside world, immatures at the egg stage are likely to be the most "pure" in chemical form as compared to immatures at the larval or pupal stages. Most likely, the chemical profile of eggs is made up of chemicals that come from the queen herself, as ovarian tissue, as well as the cuticle, have been shown to be targets for hydrocarbon deposits in several insect orders (Schal *et al.* 1998). Therefore, using newly laid eggs in adoption tests would better clarify the relationship naïve *Formica* workers have with *P. breviceps* eggs than using larvae or pupae. Moreover, contamination with host odors can be minimized and, if there are changes in odor during immature development (see Chapter 3, *Cost and Benefits of Immature Odor* and Fig. 3.7), testing responses to a developmentally modified signature can be avoided.

In this study, I examined whether eggs of *Polyergus breviceps* and eggs of *Formica gnava* Buckley and *Formica occulta* Francoeur would be reared by *F. gnava* and *F. occulta* workers. Workers used in tests either were free-living, residing in queenright

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[4] Some authors suggest that cues other than pheromones account for rearing (Vander Meer & Morel 1988; Vander Meer & Alonso 1998).

natal nests until testing time, or were newly enslaved, residing with the *Polyergus* queen that had recently taken over their nest. Test colonies were checked daily for the presence or absence of egg clumps, and then the number of larvae, pupae, and callows in each nest were counted until callows emerged. Cuticular hydrocarbons from immatures at the egg and pupa stages were analyzed to ascertain whether rearing tendencies could be correlated with the respective patterns of hydrocarbons. *Formica* generally rejected allospecific eggs, and newly enslaved *Formica* only began rearing *P. breviceps* eggs after they (the *Formica* workers) had been living with the *P. breviceps* queen in the laboratory for approximately 5 months. Hydrocarbon patterns from egg extracts of the three species were, for the most part, species specific. Although hydrocarbon profiles of pupae appeared similar across species, they, too, were different according to principal component analysis. Hydrocarbon profiles of eggs and pupae also differed.

METHODS

Ant Collections, Housing, & Food

Ant colonies were collected during June, July, and August of 1997 in the Chiricahua Mountains of southeastern Arizona. Fourteen queenright colonies of *Formica gnava* were collected from the Arizona oak - alligator juniper woodlands of the Southwestern Research Station (SWRS) of the American Museum of Natural History (el. 1646 m). Eleven queenright colonies of *Formica occulta* were collected from an area just east of the Barfoot Peak trailhead (el. 2750 m) in Coronado National Forest populated with ponderosa pine. Colonies were brought into the laboratory at SWRS and placed in large Tupperware® containers lined with Fluon® (Northern Products) to prevent the escape of workers. Colonies were provided with local insects when in Arizona or with crickets

(Fluker Farms) when in New York, the Bhatkar & Whitcomb (1970) diet, 1:1 honey/water solution, and water when needed.

Dealate *P. breviceps* queens from nests with *F. gnava* slaves (n = 6) and from nests with *F. occulta* slaves (n = 10) were individually collected in 4 dram vials containing a moistened ball of cotton as they approached the *Formica* nest being raided by their non-reproductive nestmates during July 1997.

Incipient Polyergus breviceps *Colonies*

Incipient *P. breviceps* nests are difficult to find; therefore incipient *P. breviceps* nests were created in the laboratory to supply the newly enslaved workers and the *P. breviceps* eggs for testing. Six *F. gnava* colonies and ten *F. occulta* colonies were selected for takeover by newly mated *P. breviceps* queens. *Formica* queens were removed from these nests and placed in individual Tupperware boxes (20.5 x 30 x 9 cm) lined with a thin layer of soil. A single newly mated *P. breviceps* queen was introduced into the box and allowed to attack the *Formica* queen. Once the *P. breviceps* queen ceased attacking the *Formica* queen, she was placed with the workers of the queen she had killed. The remaining *F. gnava* and *F. occulta* queenright colonies provided the free-living *F. gnava* and *F. occulta* workers, and the *F. gnava* and *F. occulta* eggs.

Experimental Test Colonies

Four groups of workers were tested with four egg types (see Table 4.1 for mean and total numbers of eggs presented, and the number of trials in each condition). Free-living or enslaved *F. gnava* or *F. occulta* were presented with a clump of 5 – 8 *F. gnava*, *F. occulta*, *P. breviceps* associated with *F. gnava*, or *P. breviceps* associated with *F. occulta* eggs.

Table 4.1

Numbers (mean & total) of eggs presented to *Formica* workers, and numbers of those eggs reared to larval, pupal, and callow stages

			EGGS PRESENTED				LARVAE REARED				PUPAE REARED				CALLOWS REARED			
			Gnava	*Occulta*	*Breviceps w/ gnava*	*Breviceps w/ occulta*	*Gnava*	*Occulta*	*Breviceps w/ gnava*	*Breviceps w/ occulta*	*Gnava*	*Occulta*	*Breviceps w/ gnava*	*Breviceps w/ occulta*	*Gnava*	*Occulta*	*Breviceps w/ gnava*	*Breviceps w/ occulta*
Formica gnava	Free-living	**Mean**	**6.09**	**5.62**	**5.84**	**6.43**	**4.00**	**0.46**	**0.35**	**0.00**	**3.18**	**0.46**	**0.24**	**0.00**	**3.18**	**0.46**	**0.16**	**0.00**
		SE	0.56	0.33	0.21	0.57	0.99	0.46	0.23	0.00	1.08	0.46	0.15	0.00	0.93	0.46	0.10	0.00
		Sum	**67**	**73**	**216**	**45**	**44**	**6**	**13**	**0**	**35**	**6**	**9**	**0**	**35**	**6**	**6**	**0**
		N	11	13	37	7	11	13	37	7	11	13	37	7	11	13	37	7
	Enslaved	**Mean**	**6.00**	**5.00**	**6.86**	**6.00**	**2.43**	**0.00**	**1.27**	**0.17**	**1.86**	**0.00**	**1.27**	**0.17**	**1.86**	**0.00**	**0.64**	**0.00**
		SE	0.38	0.00	0.43	0.58	0.84	0.00	0.46	0.17	0.99	0.00	0.46	0.17	0.99	0.00	0.26	0.00
		Sum	**42**	**20**	**151**	**36**	**17**	**0**	**28**	**1**	**13**	**0**	**28**	**1**	**13**	**0**	**14**	**0**
		N	7	4	22	6	7	4	22	6	7	4	22	6	7	4	22	6
Formica occulta	Free-living	**Mean**	**5.67**	**6.19**	**5.63**	**6.57**	**0.11**	**4.44**	**0.00**	**0.00**	**0.00**	**2.94**	**0.00**	**0.00**	**0.00**	**2.44**	**0.00**	**0.00**
		SE	0.33	0.36	0.24	0.57	0.10	0.58	0.00	0.00	0.00	0.77	0.00	0.00	0.00	0.63	0.00	0.00
		Sum	**51**	**99**	**135**	**46**	**1**	**71**	**0**	**0**	**0**	**47**	**0**	**0**	**0**	**39**	**0**	**0**
		N	9	16	24	7	9	16	24	7	9	16	24	7	9	16	24	7
	Enslaved	**Mean**	**5.80**	**5.83**	**6.58**	**6.41**	**0.00**	**3.00**	**0.00**	**0.41**	**0.00**	**2.83**	**0.00**	**0.12**	**0.00**	**2.17**	**0.00**	**0.06**
		SE	0.80	0.48	0.54	0.32	0.00	0.89	0.00	0.21	0.00	0.98	0.00	0.12	0.00	0.79	0.00	0.06
		Sum	**29**	**35**	**79**	**109**	**0**	**18**	**0**	**7**	**0**	**17**	**0**	**2**	**0**	**13**	**0**	**1**
		N	5	6	12	17	5	6	12	17	5	6	9	17	5	6	9	17

SE = standard error of the mean, **SUM** = total number of eggs presented, or larva, pupae, and callows reared, **N** = number of test colonies. **Free-living** = workers in test colonies were taken from queenright *Formica* colonies, **Enslaved** = workers in test colonies had been acquired by *P. breviceps* as adults and were living with a *P. breviceps* queen.

Supply nests were checked for eggs every four hours during the first week after *P. breviceps* nests were created. Thereafter, nests were checked once daily. While *F. gnava* and *F. occulta* queens produced eggs regularly, only a few eggs (between 3 and 7) were found in a few nests of *P. breviceps* queens. In other *P. breviceps* nests there were no eggs at all. At first, because there were rarely enough *P. breviceps* eggs to conduct a single trial, eggs were not immediately harvested but left until five eggs could be removed. However, when nests were subsequently checked, all the *P. breviceps* eggs were gone. A single nest produced enough eggs to conduct one trial every 1 – 2 weeks. For the most part, consistent and sufficient egg laying by most of the *P. breviceps* queens did not begin until early December.

Once *P. breviceps* eggs were available, they were always immediately harvested and used in trials. If eggs were available for use, 35 – 40 workers were removed from brood-containing areas in their colony, placed in a Tupperware® container (18 x 30 x 9 cm) with Fluon®-coated rims and soil-lined bottoms, and allowed to acclimate for one hour before eggs were introduced. An inverted plastic petri dish (5.5 cm diameter) with two entrances 180° apart served as an artificial nest and was placed in the center of each box and covered with a dark cloth. With the aid of a dissecting scope, eggs were carefully removed from their respective colonies with fine forceps, which had been cleaned with hexane and air dried, and placed 3 cm from one of the nest entrances.

Nests were checked daily for the presence or absence of egg clumps. Once eggs matured into larvae, the number of larvae, pupae, and callows were counted. Fisher's Exact Probability levels were calculated to compare the presence or absence of immatures within a nest for each stage of immature development for the following:

1) **free-living *Formica* with conspecific eggs** (free-living *F. gnava* with *F. gnava* eggs vs free-living *F. occulta* with *F. occulta* eggs);
1) **free-living *Formica* with heterospecific *Formica* eggs** (free-living *F. gnava* with *F. occulta* eggs vs free-living *F. occulta* with *F. gnava* eggs);
1) **free-living *Formica* with *P. breviceps* eggs from nests containing slave species conspecific to test workers** (free-living *F. gnava* with *P. breviceps* eggs from *P. breviceps-F. gnava* nests vs free-living *F. occulta* with *P. breviceps* eggs from *P. breviceps-F. occulta* nests);
1) **free-living *Formica* with *P. breviceps* eggs from nests containing slave species heterospecific to test workers** (free-living *F. gnava* with *P. breviceps* eggs from *P. breviceps-F. occulta* nests vs free-living *F. occulta* with *P. breviceps* eggs from *P. breviceps-F. gnava* nests).

Groups 5 – 8 were the same except *Formica* workers were newly enslaved. If like groups were not significantly different, the data were pooled and analyzed as:

a) Pooled Group 1 vs pooled Group 2;
b) Pooled Group 3 vs pooled Group 4;
c) Pooled Group 5 vs pooled Group 6;
d) Pooled Group 7 vs pooled Group 8.

Repeated measures ANOVAs were used to determine whether there was a difference in the number of larvae that were reared to pupae and the number of pupae that were reared to callows between paired, pooled groups. Factorial ANOVAs were used to determine whether the number of days it took reared eggs to emerge as larvae, pupae, and callows differed within the following groups of *Formica* that had reared them:

1) ***Formica gnava*** (free-living, newly enslaved without *P. breviceps* queen and newly enslaved with *P. breviceps* queen) presented **with conspecific eggs**;
1) ***Formica occulta*** (free-living, newly enslaved without *P. breviceps* queen and newly enslaved with *P. breviceps* queen) presented **with conspecific eggs**;
1) ***Formica gnava*** (free-living, newly enslaved without *P. breviceps* queen and newly enslaved with *P. breviceps* queen) presented **with *P. breviceps* eggs** from *P. breviceps-F. gnava* nests.

Scheffé's post-hoc tests were used to indicate which condition(s) differed significantly within each group.

Chemical Analysis

Cuticular components were extracted from clumps of 10 – 20 eggs or from 5 pupae by immersing them respectively in 1 or 2 ml of high purity hexane (B & J, GC^2 Grade) for 10 min in 7 ml scintillation vials. Solvent extracts were transferred from the sample with a fresh Pasteur pipette to a 2 ml scintillation vial, allowed to evaporate, and transported to United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology in Gainesville, Florida where chemical analyses were performed. Ethyl alcohol (70%) was added to 7 ml vials to preserve specimens for voucher. Evaporated solvent extracts were reconstituted with 0.2 ml hexane, vortexed for 1-2 sec, and then applied to a small silicic acid (70-230 mesh, 60Å, Aldrich Chemical Co., Inc.) Pasteur pipette column. Hydrocarbons were isolated from other lipids by eluting the column with hexane. The elutant, containing purified hydrocarbons, was evaporated to ca. 10 μl under a stream of nitrogen. Samples were analyzed by gas chromatography (Varian 3700 equipped with a split-splitless injector, a capillary column [J & W DB – 1 30 m] and flame ionization detector). The injector and detector were set at 300° C; the oven was programmed from 190° to 290° C at 5° C/min, and then held at 290° C for 5 min. Hydrogen was the carrier gas and nitrogen was the make-up gas. Standards were injected at regular intervals during sample analysis and used to calculate KI values (C16, C18, C20, C22, N–Parafin mix, 500 mg/5 ml in hexane stock, #NP-MIX-H, Alltech Associates, Inc.). Retention times were converted to KI values using the linear regression formula:

$$Y = 1.340x - 23.131$$

Each peak was labeled with a KI value and was considered an individual variable (although correlated) before transforming them into new unrelated variables and subjecting them to principal component analysis. The data were analyzed using PE Nelson Turbochrom Navigator 6.1.0.1FO4 (Perkin Elmer Corp., 1998).

Hydrocarbon constituents from eggs and from pupae were analyzed first separately to determine whether profiles differed with respect to species, and then together to determine whether hydrocarbon profiles of the same species differ at egg and pupa stages of immature development. Analysis of principal components was conducted on 42 normalized variables for eggs (N = 30), 43 normalized variables for pupae (N = 18), and 43 normalized variables for eggs and pupae (N = 48). The relative proportions of cuticular hydrocarbons were calculated by dividing the percent area given for each cuticular hydrocarbon by the total percent area of that profile. Singular value decomposition was used to implement principal component analysis. Multivariate analyses were conducted using Pirouette software (Infometrix, Woodinvill, WA).

RESULTS

Rearing

Means and total numbers of the eggs presented to workers in each condition and of the larvae, pupae, and callows that were reared from those eggs are presented in Table 4.1. All Fisher's Exact Probabilities (FEP) are presented in Table 4.2.

Free-living Formica *Workers:*

With Formica *Eggs*

The number of free-living *F. gnava* test colonies that retrieved *F. gnava* eggs, or reared *F. gnava* eggs to larvae, pupae, and callows did not differ from the number of free-living *F. occulta* test colonies that retrieved *F. occulta* eggs, or reared *F. occulta* eggs to larvae, pupae, and callows. The number of free-living *F. gnava* test colonies that retrieved *F. occulta* eggs, or reared *F. occulta* eggs to larvae, pupae, and callows did not differ from the number of free-living *F. occulta* test colonies that retrieved *F. gnava* eggs, or reared *F. gnava* eggs to larvae, pupae, and callows. Thus, conspecific worker/egg pairings were pooled and heterospecific worker/egg pairings were pooled, and the numbers of test colonies that retrieved eggs, or reared larvae, pupae, and callows were compared. There was a significant difference between conspecific and heterospecific pooled groups for each stage compared (Tables 4.1 & 4.2).

Free-living *Formica* reared 51% of the conspecific eggs presented to them to eclosion, whereas only 4.8% of heterospecific *Formica* eggs presented to them were reared to eclosion (Fig. 4.1).

Table 4.2

Fisher's Exact Probabilities for the presence or absence of conspecific and heterospecific offspring in test nests of *Formica*

FORMICA **WORKERS WITH** ***FORMICA*** **EGGS**

	Worker Species	Egg Species	Eggs	Larvae	Pupae	Callows
Unenslaved	*Gnava* *Occulta*	*Gnava* *Occulta*	0.4074	0.9999	0.4267	0.4267
	Gnava *Occulta*	*Occulta* *Gnava*	0.9999	0.9999	0.9999	0.9999
	POOLED DATA: CONSPECIFIC VS HETEROSPECIFIC PAIRINGS		**0.0001***	**0.0001***	**0.0001***	**0.0001***
Enslaved	*Gnava* *Occulta*	*Gnava* *Occulta*	0.9999	0.9999	0.5921	0.5921
	Gnava *Occulta*	*Occulta* *Gnava*	0.4444	>0.9999	>0.9999	>0.9999
	POOLED DATA: CONSPECIFIC VS HETEROSPECIFIC PAIRINGS		**0.0001***	**0.0002***	**0.0167***	**0.0167***

FORMICA **WORKERS WITH** ***BREVICEPS*** **EGGS**

	Worker Species	Egg Species	Eggs	Larvae	Pupae	Callows
Unenslaved	*Gnava* *Occulta*	*Breviceps* w *gnava* *Breviceps* w *occulta*	0.6395	0.9999	0.9999	0.9999
	Gnava *Occulta*	*Breviceps* w *occulta* *Breviceps* w *gnava*	0.2258	>0.9999	>0.9999	>0.9999
	POOLED DATA: CONSPECIFIC VS HETEROSPECIFIC PAIRINGS (of species of *breviceps* host)		**0.0396**	**0.2627**	**0.2627**	**0.2627**
Enslaved	*Gnava* *Occulta*	*Breviceps* w *gnava* *Breviceps* w *occulta*	0.082	0.494	0.1061	0.3634
	Gnava *Occulta*	*Breviceps* w *occulta* *Breviceps* w *gnava*	0.043*	0.3333	>0.9999	>0.9999
	POOLED DATA: CONSPECIFIC VS HETEROSPECIFIC PAIRINGS (of species of *breviceps* host)		**UAP**	**0.0442***	**.0463***	**0.1681**

* = statistically significant difference, **UAP** = unable to pool data

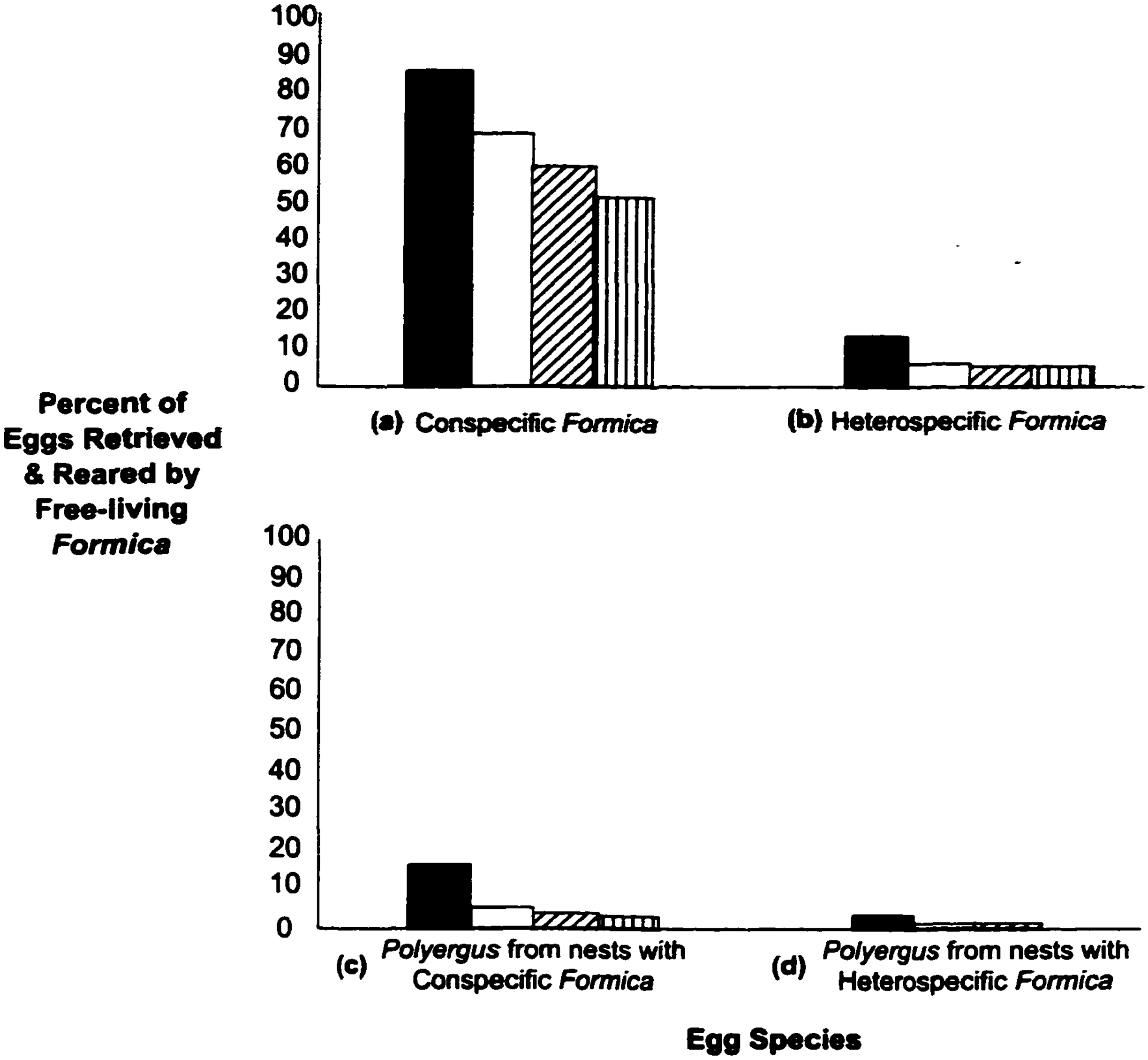

Figure 4.1. Percent of eggs presented to free-living *Formica* that were accepted as eggs and reared to larval, pupal or callow stage. (a) Free-living *Formica* workers reared 51% of conspecific eggs to callowhood but (b) only 4.8% of heterospecific *Formica* eggs. Free-living *Formica* workers generally rejected *P. breviceps* eggs regardless if they were from nests in which the host species was conspecific (c) or heterospecific (d) to test workers. ■ = accepted as eggs, □ = reared to larva, ▨ = reared to pupa, ▥ = reared to callow.

With Polyergus *Eggs*

Similarly, the number of free-living *F. gnava* test colonies that retrieved *P. breviceps* eggs that came from *P. breviceps-F. gnava* nests, or reared these eggs to larvae, pupae, and callows did not differ from the number of free-living *F. occulta* test colonies that retrieved *P. breviceps* eggs that came from *P. breviceps-F. occulta* nests, or reared these eggs to larvae, pupae, and callows. The number of free-living *F. gnava* test colonies that retrieved *P. breviceps* eggs that came from *P. breviceps-F. occulta* nests, or reared these eggs to larvae, pupae, and callows did not differ from the number of free-living *F. occulta* test colonies that retrieved *P. breviceps* eggs that came from *P. breviceps-F. gnava* nests, or reared these eggs to larvae, pupae, and callows. Thus, conspecific worker/egg pairings were pooled and heterospecific worker/egg pairings were pooled, and the numbers of test colonies that retrieved eggs, or reared larvae, pupae, and callows were compared. The number of test colonies that retrieved *P. breviceps* eggs that came from nests in which the host species was conspecific to test workers, differed significantly from the number of test colonies that retrieved *P. breviceps* eggs that came from nests in which the host species was heterospecific to test workers. These test colony groups, however, did not differ with respect to all other developmental stages reared (Tables 4.1 & 4.2). In other words, workers initially responded differently towards eggs relative to their host species association, but eventually this difference disappeared sometime before or at the larval stage.

Hence, although some *P. breviceps* eggs were accepted (21%), they were eventually rejected regardless of whether they were from nests with a conspecific slave species (2.3% reared) or heterospecific slave species (0% reared) (Fig. 4.1 c & d).

Number of Immatures Reared to the Next Stage

Of the eggs (of all species) that free-living *Formica* reared to the larval stage, the number reared to the pupa stage did not differ from the number reared to the callow stage ($F_{2, 34} = 0.407$, $p = 0.67$) for any of the species that were reared ($F_{3, 17} = 0.95$, $p = 0.44$). There was also no significant interaction ($F_{6, 34} = 0.55$, $p = 0.77$). In other words, once eggs were reared to larvae, they were likely to be reared to pupae and then to callows, regardless of whether they were conspecific or heterospecific or, in the case of *P. breviceps*, associated with conspecific or heterospecific host species (Fig. 4.1).

Newly Enslaved Formica *Workers:*

With Formica *Eggs*

The number of enslaved *F. gnava* colonies that retrieved *F. gnava* eggs into the nest did not differ significantly from the number of enslaved *F. occulta* colonies that retrieved *F. occulta* eggs into the nest. Nor were there statistically significant differences in the number of enslaved test colonies that had reared larvae, pupae, or callows from these eggs. The same is true for enslaved *Formica* presented with heterospecific *Formica* eggs (see Table 4.2). Thus, conspecific worker/egg pairings were pooled and heterospecific worker/egg pairings were pooled, and the numbers of test colonies that retrieved eggs, or reared larvae, pupae, and callows were compared. The number of test nests that retrieved conspecific eggs differed significantly from the number of test nests that retrieved heterospecific *Formica* eggs. The groups also differed significantly in the number of test colonies that reared larvae, pupae, and callows from the eggs that had been retrieved.

Twenty-eight percent of the conspecific eggs presented to newly enslaved *Formica* workers were reared to callowhood (Fig. 4.2a). Heterospecific *Formica* eggs were completely rejected (Fig. 4.2 b).

With Polyergus *Eggs*

The number of newly enslaved *F. gnava* colonies did not differ significantly from the number of newly enslaved *F. occulta* colonies in retrieving *P. breviceps* eggs from nests containing a slaves species the same as the species of test workers, nor did the numbers differ in rearing these eggs to larvae, pupae, or callows. However, because the two groups differed in the number of test colonies that retrieved *P. breviceps* eggs from nests with a slave species different from test workers, results were not pooled. At the other developmental stages, the groups did not differ and results were pooled. The number of test colonies that had reared *P. breviceps* eggs associated with a conspecific slave species to larvae and pupae and that had reared *P. breviceps* eggs associated with a heterospecific slave species to larvae and pupae differed significantly. No difference between these two groups was found, however, in the rearing of eggs to callows (Table 4.2).

Only 2% of *P. breviceps* eggs were reared to callowhood and they were from nests in which the slave species was conspecific to test workers (Fig. 4.2 c & d).

Number of Immatures Reared to the Next Stage

Of the eggs (of all species reared) that were reared to the larval stage by enslaved *Formica*, there was no statistically significant difference between the numbers reared to the pupa or callow stage ($F_{2,14} = 0.52$, $p = 0.6$). There was a difference, although just shy of being statistically significant, between the species of eggs that were reared

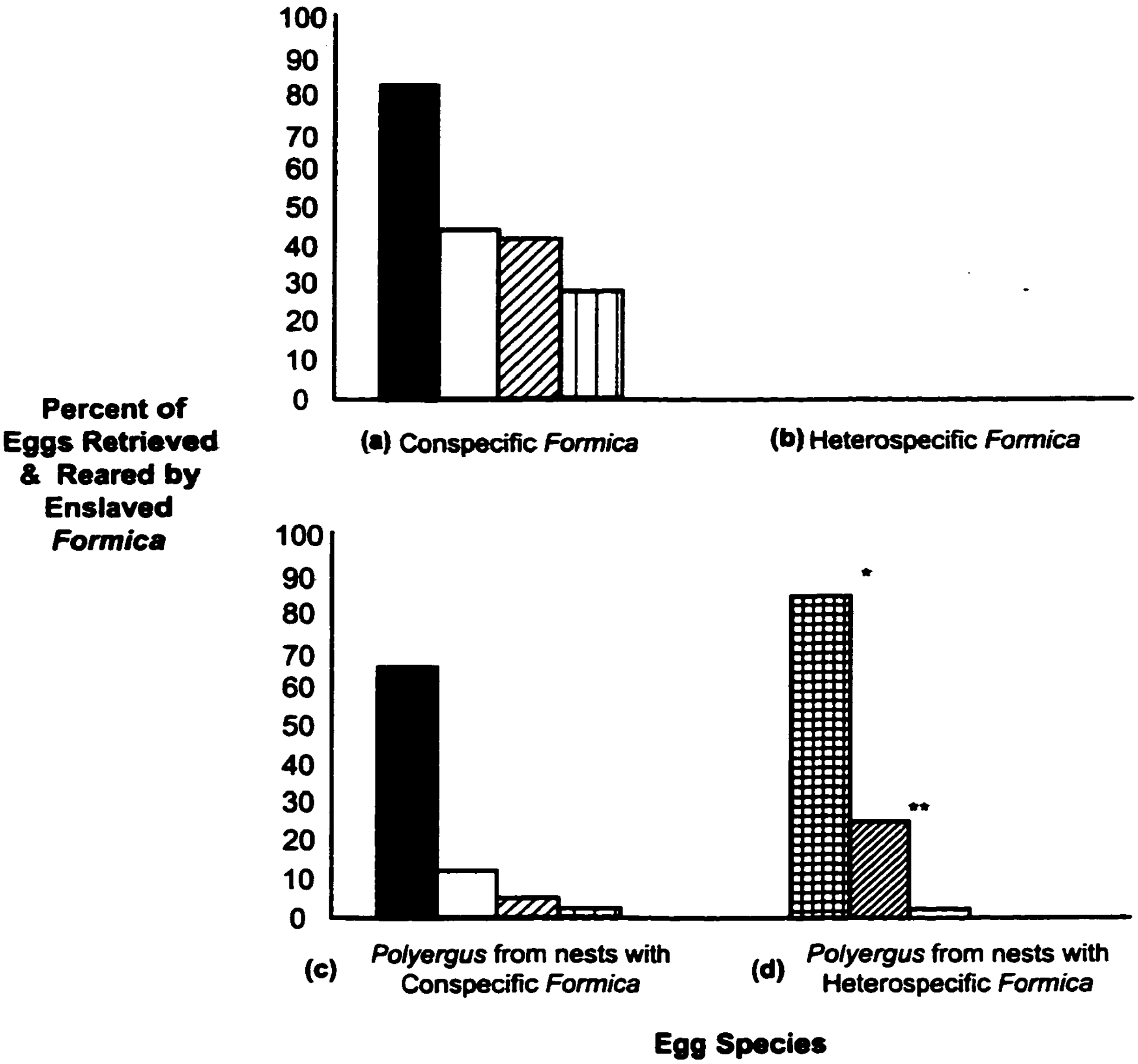

Figure 4.2. Percent of eggs presented to enslaved *Formica* that were accepted as eggs and reared to larval, pupal or callow stage. **(a)** Enslaved *Formica* workers reared 28.6% of conspecific eggs to callowhood but **(b)** 0% of heterospecific *Formica* eggs. **(c)** Enslaved *Formica* workers retrieved *P. breviceps* eggs if they were from nests in which the host species was conspecific to test workers, but generally did not rear them. **(d)** Significantly more *P. breviceps* eggs from *F. gnava* nests were retrieved by enslaved *F. occulta* than vice versa (see Table 4.2), however, enslaved workers did not rear any *P. breviceps* eggs from nests in which the slave species was a different species than themselves. * ▦ = accepted as eggs (*P. breviceps* with *F. gnava*), ** ▨ = accepted as eggs (*P. breviceps* with *F. occulta*), □ = reared to larva, ▨ = reared to pupa, ▥ = reared to callow.

($F_{2,7} = 4.52$, $p = 0.055$). The interaction was not significant ($F_{4,14} = 2.74$, $p = 0.07$), but indicates that the stage of immaturity and species of immature had a slight effect on whether *Formica* would rear them to the next stage. By examining the percent of eggs reared by enslaved *Formica* (Fig. 4.2a), it appears that conspecific larvae were more likely to be reared to pupae than any other species or any other stage to the next stage, i.e., pupae to callow stage.

"Growth Rates":

In Figure 4.3, the average number of days it took the first larva and pupa, and each of the callows to emerge from reared eggs are plotted. Results are based on only the individuals that emerged at each developmental stage. In the free-living *F. gnava* graph, emergence curves for *F. gnava* in queenright *F. gnava* nests and for *P. breviceps* in queenright *P. breviceps-F. gnava* nests are included to provide a rough comparison of "growth rates" between queenright laboratory nests and queenless test colonies. Growth rates, which here refer to the number of days an immature spends at each stage of development (excluding larval instars), for the *F. occulta* that were reared in *F. gnava* nests were compared with growth rates for *F. occulta* in free-living and newly enslaved *F. occulta* nests.

Formica gnava

The growth rates of *F. gnava* larvae ($F_{2,18} = 0.18$, $p = 0.84$) or pupae ($F_{2,15} = 1.31$, $p = 0.3$) were not significantly different in test colonies of free-living *F. gnava*, in *P. breviceps* test colonies of enslaved *F. gnava*, and in queenright laboratory colonies of *F. gnava*. There was, however, a significant difference in the growth rate of *F. gnava* pupae relative to the different nest types ($F_{2,12} = 35.76$, $p = 0.0001$). *Formica gnava* pupae developed significantly faster in queenright *F. gnava* nests than in free-living test nests (Scheffé test, $p < 0.0001$) or newly enslaved test nests (Scheffé test, $p = <$

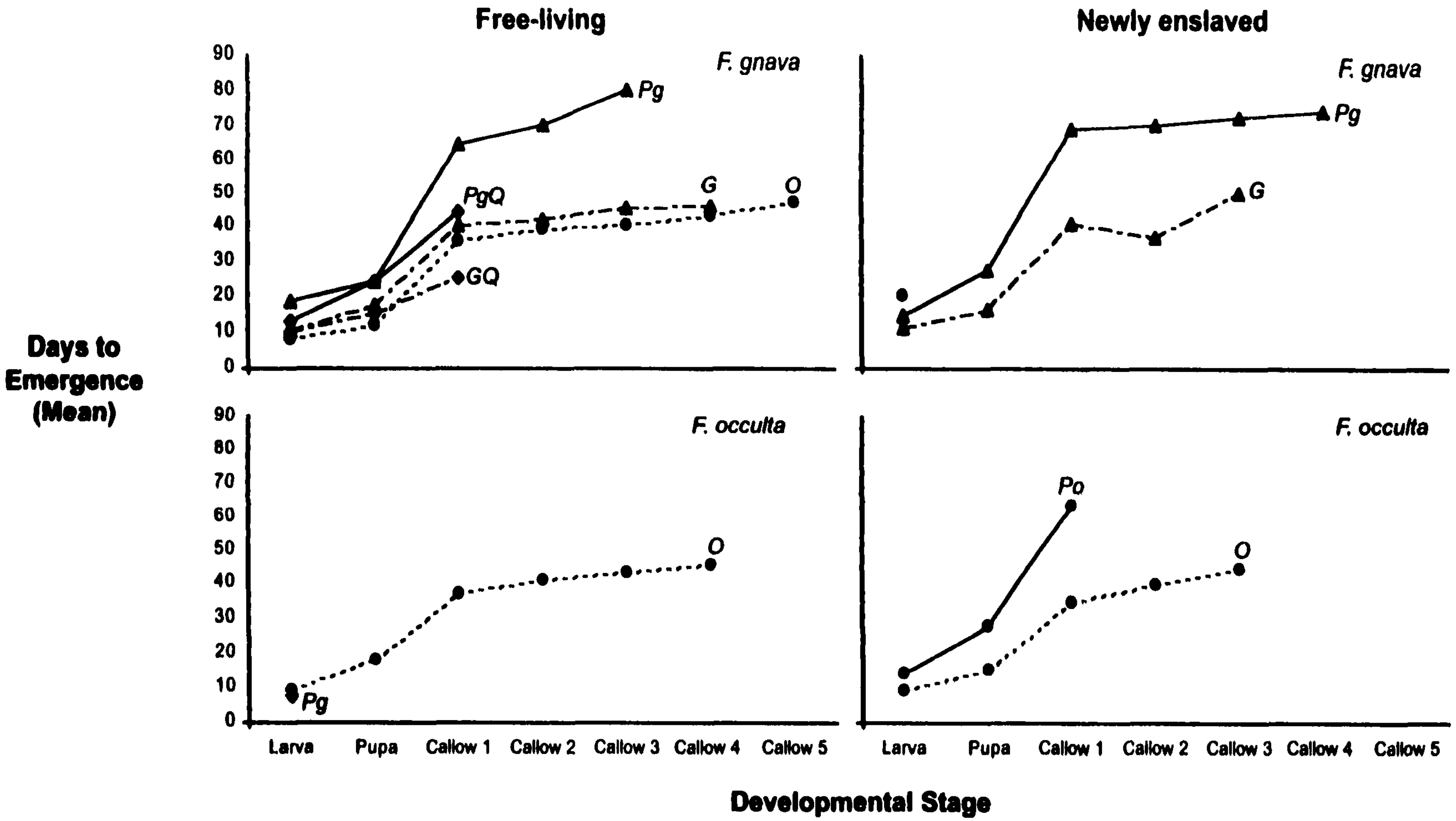

Figure 4.3. Mean number of days until emergence of the first larva and pupa, or callows, in their respective order, in experimental nests of *Formica*. For comparison of development times in queenright nests, emergences of *F. gnava* offspring in queenright *F. gnava* nests and of *P. breviceps* offspring in queenright *P. breviceps-F. gnava* nests are provided in the free-living *F. gnava* graph. *G* = *F. gnava*, *GQ* = *F. gnava* in queenright *F. gnava* nests, *O* = *F. occulta*, *Pg* = *P. breviceps* from *P. breviceps-F. gnava* nests, *PgQ* =*P. breviceps* in queenright *P. breviceps-F. gnava* nests, *Po* = *P. breviceps* from *P. breviceps-F. occulta* nests.

0.0001). There was no difference between growth rates of *F. gnava* in free-living or enslaved *F. gnava* test nests (Scheffé test, $p = 0.99$).

Formica occulta

The growth rates of *F. occulta* larvae ($F_{2,16} = 0.07$, $p = 0.93$), pupae ($F_{2,13} = 2.92$, $p = 0.09$), or callows ($F_{2,11} = 0.11$, $p = 0.9$) did not differ significantly among free-living *F. occulta* test nests, newly enslaved *F. occulta* test nests, and free-living *F. gnava* test nests.

Polyergus breviceps

Although not statistically significant, there was a tendency for *P. breviceps* eggs to develop in larvae at different rates in the different types of nests ($F_{2,13} = 3.42$, $p = 0.06$). Eggs matured into larvae sooner in queenright *P. breviceps-F. gnava* nests than in test nests of free-living *F. gnava* (Scheffé test, $p = 0.06$), but not sooner than in test nests of newly enslaved *F. gnava* (Scheffé test, $p = 0.55$). And there was no difference between free-living test nests and newly enslaved test nests in the growth rate of *P. breviceps* larvae (Scheffé test, $p = 0.21$). The rate of development from larva to pupa also was not significantly different ($F_{2,12} = 3.4$, $p = 0.07$). The growth rate of *P. breviceps* pupae tended to be slightly lower in free-living *F. gnava* test colonies (Scheffé test, $p = 0.07$) than in enslaved *F. gnava* test colonies, but did not differ from the growth rate of *P. breviceps* pupae in queenright *P. breviceps-F. gnava* nests (Scheffé test, $p = 0.33$). There were no differences between enslaved *F. gnava* test nests and queenright *P. breviceps-F. gnava* nests (Scheffé test, $p = 0.55$). There was a significant difference among the different nest types in the time it took callows to emerge from pupae ($F_{2,11} = 34.08$, $p = 0.0001$). *Polyergus breviceps* pupae developed into callows significantly faster in queenright *P. breviceps-F. gnava* colonies than in either enslaved *F. gnava* test nests (Scheffé test, $p = 0.0006$) or in free-living *F. gnava* test nests (Scheffé test, $p =$

0.0004). The development rate did not differ between free-living and enslaved *F. gnava* test nests (Scheffé test, $p = 0.91$).

Hydrocarbon Profiles:

Eggs

Cuticular hydrocarbon profiles of eggs from *F. gnava*, *F. occulta*, *P. breviceps* associated with *F. gnava* and *P. breviceps* associated with *F. occulta* were distinctive (Fig. 4.4). While there were qualitative similarities among all three species, they differed with respect to their relative proportions.

The first and second principal components of hydrocarbon extracts from eggs accounted for 63% of the total cumulative variance. On the basis of the first and second principal components, *F. gnava* eggs and *P. breviceps* eggs from *P. breviceps-F. gnava* nests were separated from each other and from *F. occulta* eggs and *P. breviceps* eggs from *P. breviceps-F. occulta* nests, which clustered together (Fig. 4.5).

Pupae

No *P. breviceps* pupae from nests with *F. occulta* slaves were extracted. Hydrocarbon profiles of *F. gnava* and *F. occulta* pupae were similar both in simplicity and quality. The pupa profile of *P. breviceps* associated with *F. gnava* was qualitatively different from the profiles of both *F. gnava* and *F. occulta*. The profiles of all three species, however, were dominated by two peaks (Fig. 4.6, Peaks K, O) and appeared to differ from the others in relative proportions of shared components.

Nonetheless, on the basis of the first principal component, *F. occulta* pupae separated from *F. gnava* pupae. And, *P. breviceps* pupae separated from *F. gnava* pupae on

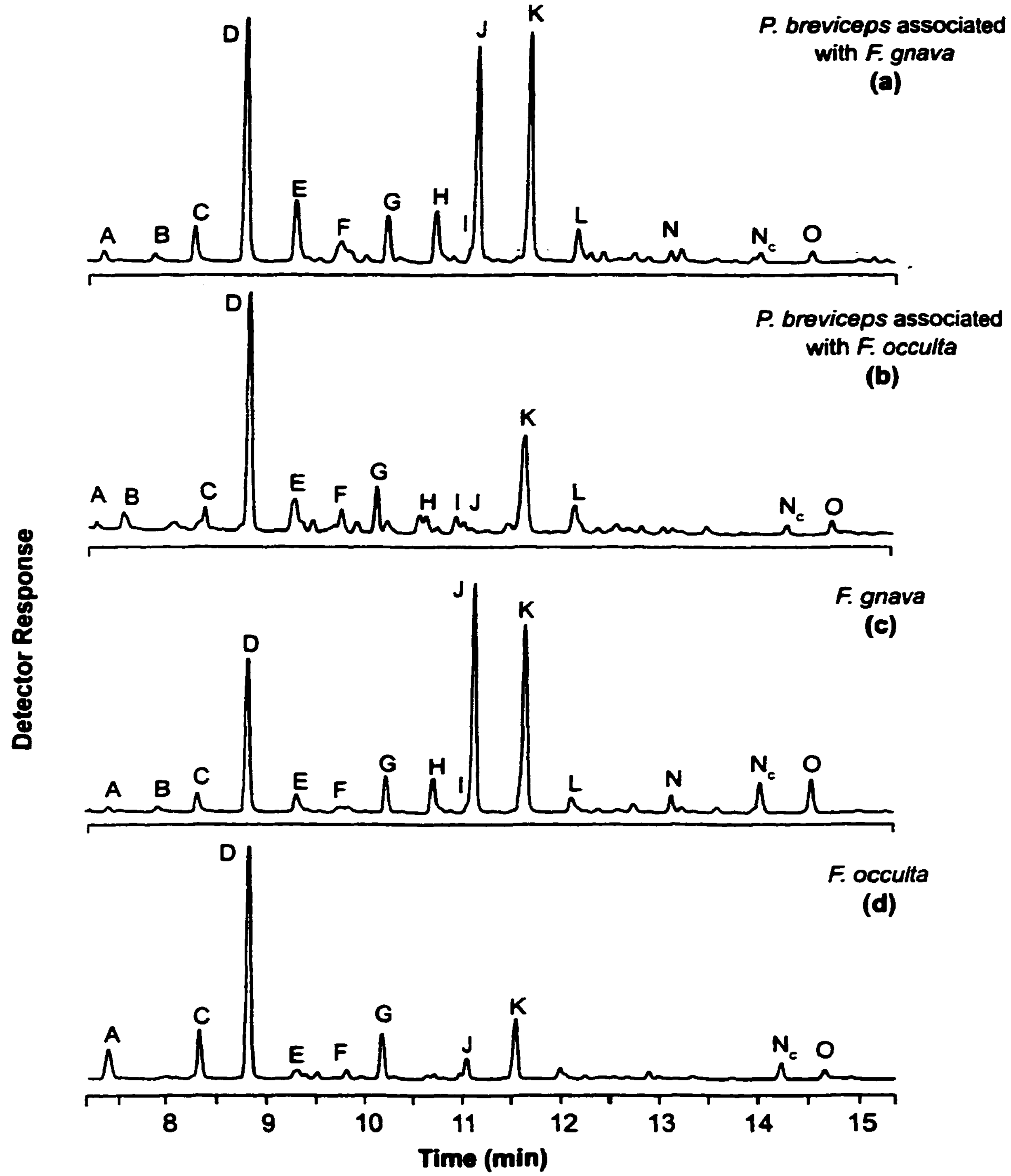

Figure 4.4. Cuticular hydrocarbon profiles of eggs from **(a)** *P. breviceps* associated *F. gnava*, **(b)** *P. breviceps* associated with *F. occulta*, **(c)** *F. gnava*, and **(d)** *F. occulta*. Profiles are qualitatively similar but are distinctive in the relative proportions of the shared components.

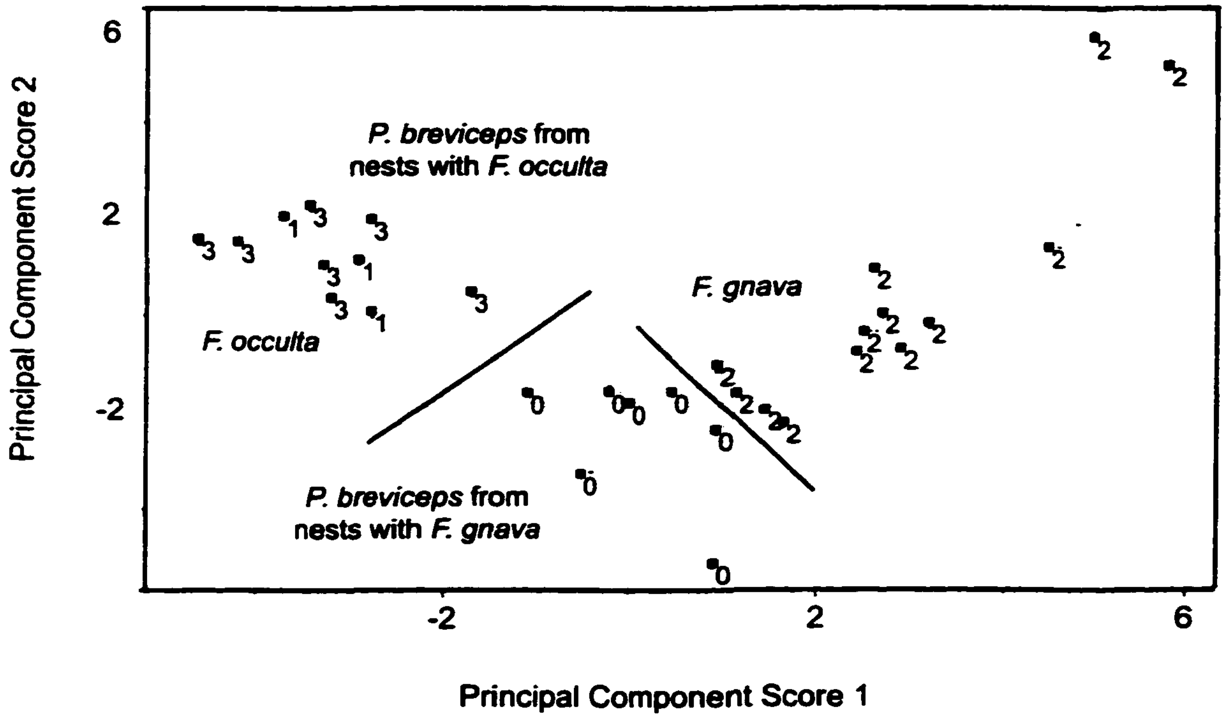

Figure 4.5. Principal component map of cuticular hydrocarbons from eggs of *P. breviceps*, *F. gnava*, and *F. occulta*. On the basis of the first and second principal components, hydrocarbon extracts of *F. gnava* eggs and of *P. breviceps* eggs from nest with *F. gnava* slaves are separated from each other and from hydrocarbon extracts of *F. occulta* eggs and *P. breviceps* eggs from nests with *F. occulta* slaves, which clustered together. First and second principal components account for 63% of the total cummulative variance. 0 = *P. breviceps* from *P. breviceps-F. gnava* nests, 1 = *P. breviceps* from *P. breviceps-F. occulta* nests, 2 = *F. gnava*, 3 = *F. occulta*.

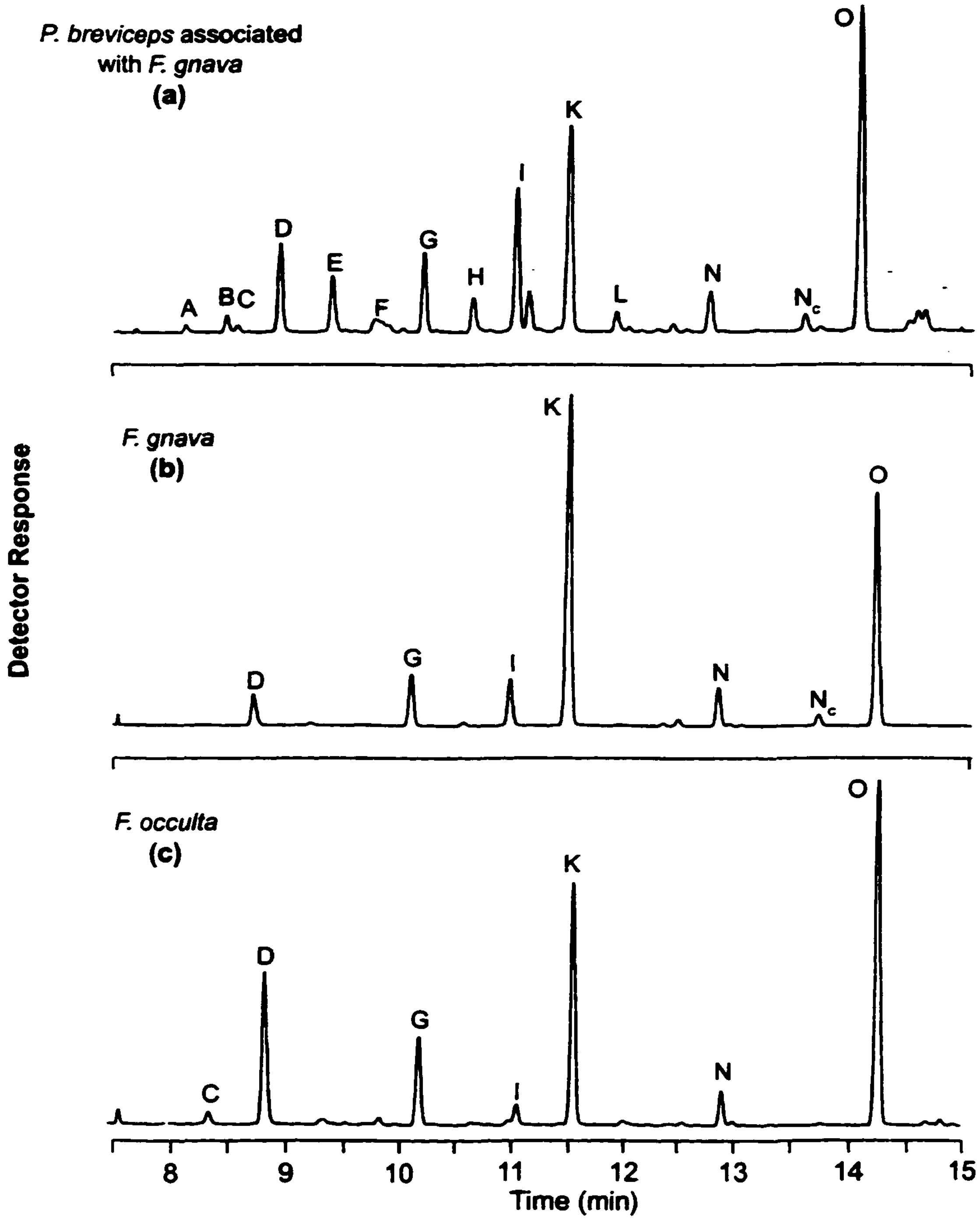

Figure 4.6. Cuticular hydrocarbon profiles of pupae from **(a)** *P. breviceps* associated with *F. gnava*, **(b)** *F. gnava*, and **(c)** *F. occulta*. Profiles are similarly simple but differ somewhat in quality and in the relative proportion of shared components.

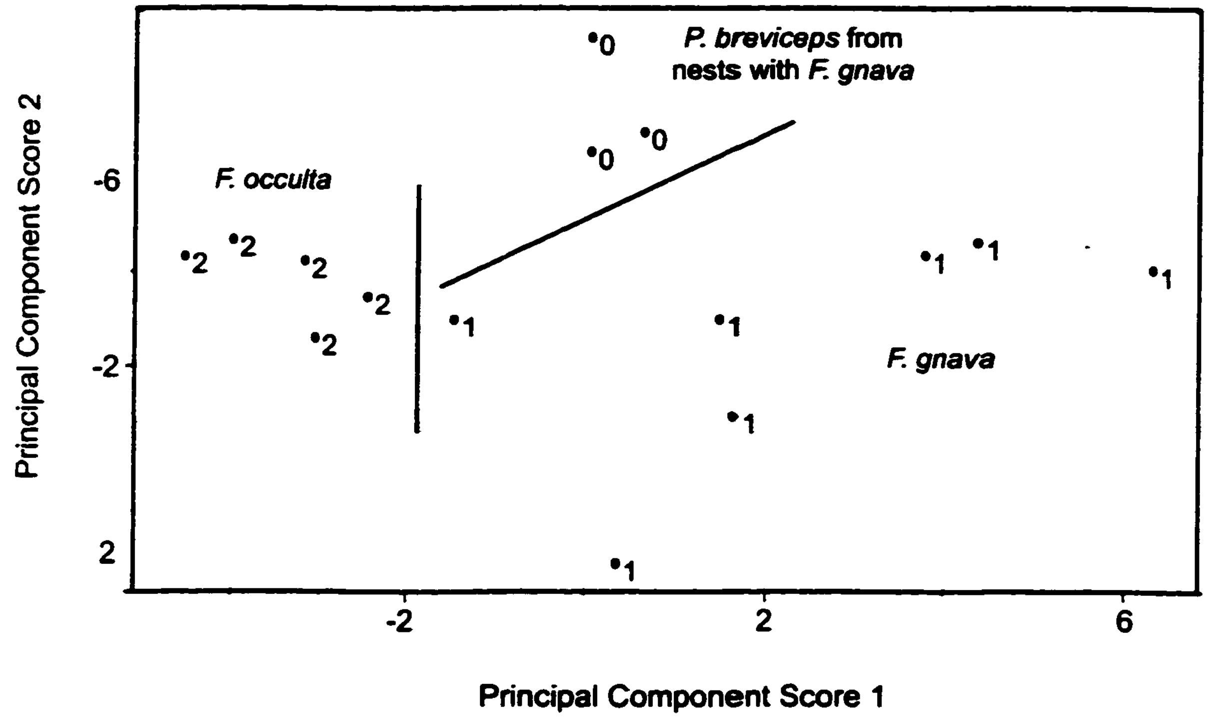

Figure 4.7. Principal component map of cuticular hydrocarbons from pupae of *P. breivceps*, *F. gnava*, and *F. occulta*. On the basis of the first principal component, *F. occulta* pupae separated fromboth *F. gnava* pupae and *P. breviceps* pupae from nests containing *F. gnava* slaves. *Polyergus breviceps* pupae from nests containing *F. gnava* slaves separated from *F. gnava* pupae on the basis of the first and second principal components. 0 = *P. breviceps* from nests containing *F. gnava* slaves, 1 = *F. gnava*, 2 = *F. occulta*.

the second principal component and from *F. occulta* pupae on the first and second principal components (Fig. 4.7).

Eggs and Pupae

Analysis of principal components from egg and pupae hydrocarbon extracts revealed that eggs and pupae separated on the basis of the first and second principal components (Fig. 4.8).

DISCUSSION

The results of this study demonstrate first, that eggs of *F. gnava* and *F. occulta* are not easily adopted and reared by the other species, even though these species are *Formica* congeners. Second, these results demonstrate that at least two species of *Formica* that are host to slave-maker, *Polyergus breviceps*, do not spontaneously adopt and rear the slave-maker's eggs. Only 2.6% of 817 *P. breviceps* eggs as compared to 41% of 243 conspecific eggs that were presented to *Formica* (of both species and of both states) were reared to callows (Figs. 4.1 & 4.2). These findings suggest that *P. breviceps* eggs are neither "naturally attractive" nor chemically similar to their host species such that they elicit tending behavior. Furthermore, these results support the contention that rules for membership in an exclusive society are stringent, even for eggs.

Rearing of *P. breviceps* eggs by newly enslaved *Formica* did not begin until 5 months after workers had been enslaved and were living with the *P. breviceps* queen, in both test colonies and laboratory supply colonies. During this time, however, newly enslaved workers continued to rear conspecific eggs. At first, I questioned whether the Tupperware containers influenced worker sensations or egg odors. But when eggs still

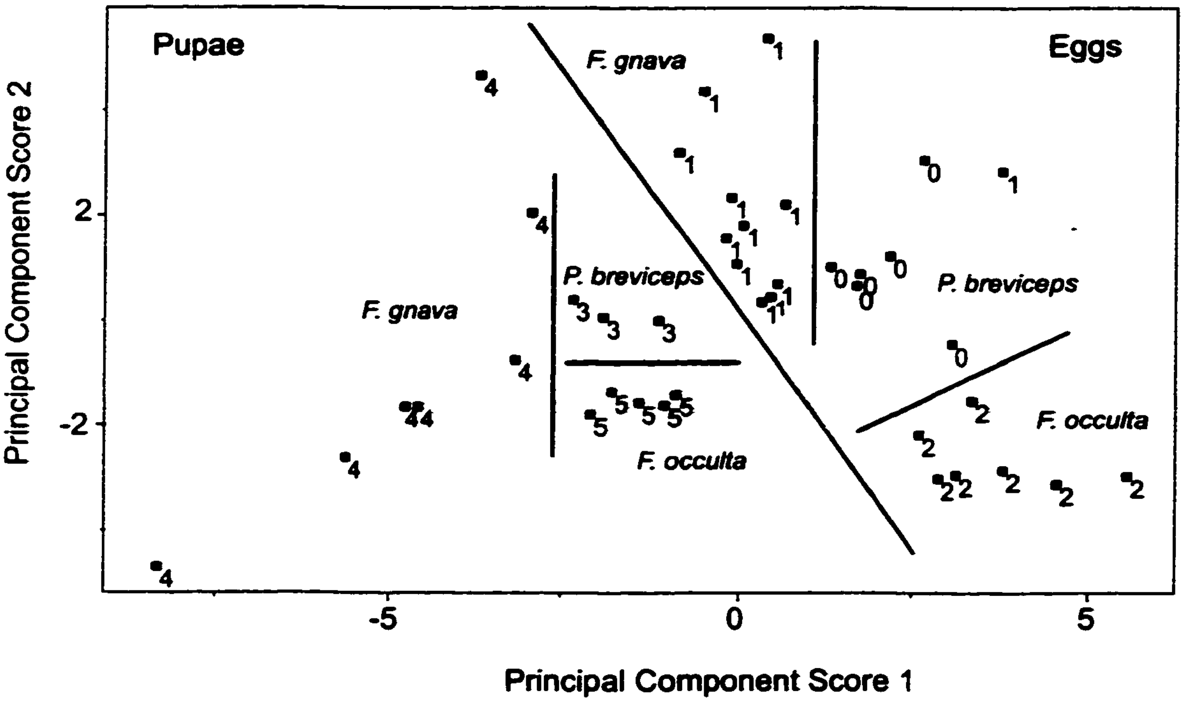

Figure 4.8. Principal components map of cuticular hydrocarbons from eggs and pupae of *P. breviceps*, *F. gnava*, and *F. occulta*. Eggs are separated from pupae on the basis of the first and second principal components. **EGGS**: 0 = *P. breviceps* from *P. breviceps-F. gnava* nests, 1 = *F. gnava*, 2 = *F. occulta*; **PUPAE**: 3 = *P. breviceps* from *P. breviceps-F. occulta* nests, 4 = *F. gnava*, 5 = *F. occulta*.

did not appear after some colonies were transferred to glass aquariums and another colony was transferred to a fine-mesh wire cage, which I had constructed and transplanted into the ground at the Barfoot field site, I concluded that workers were destroying the *P. breviceps* eggs. Although some of the *P. breviceps* queens may not have produced eggs during this time, this was excluded as a general explanation for the lack of eggs because, periodically, *P. breviceps* eggs were found (and harvested). Furthermore, *P. breviceps* eggs that had been presented to *Formica* in tests either were retrieved into the nests and disappeared subsequently, or were left outside the nest and eventually molded.

Others have also noted a delay in egg production by newly mated *Polyergus* queens. Zimmerli (1992) observed that a *P. breviceps* queen, which had effectively taken over a *F. gnava* nest on July 20, 1992, still had not produced eggs by October 4, 1992. Eggs of *Polyergus rufescens* also did not appear in *Formica cunicularia* nests until spring (D'Ettorre *et al.* 1997). In both cases, the destruction of *Polyergus* eggs by newly enslaved workers is a reasonable alternative to the delayed production hypothesis, particularly since those colonies had not been checked regularly for eggs.

The rejection of *P. breviceps* eggs in this study was surprising, even with the abundant evidence that eggs are discernible (Franks & Sendova-Franks 1992; Bourke 1994; Monnin & Peeters 1997) and with the conjecture that egg signatures may be made up of species specific queen chemicals (see Chapter 3, *Cost & Benefits of Different Odors*). Worker discrimination against parasite eggs indicates that eventual adoption is a function of some mechanism other than natural chemical similarity or an "attractive" pheromone. What might these mechanisms be? I consider several, not mutually exclusive, hypotheses. The first hypothesis is that with time newly enslaved *Formica* workers habituate to the odors of *P. breviceps* eggs, and this eventually leads to adoption.

Recognition signals can be derived from heritable and/or environmental sources. Both types of cues tend not to remain constant but vary depending on circumstance. For example, in *Polistes dominulus* Christ wasps, the size of the oocytes, which is indicative of a particular hormonal state, is highly correlated with specific proportions of certain cuticular hydrocarbons (Bonavita-Cougourdan *et al.* 1991). Repeated sampling from colonies of *Solenopsis invicta* Buren revealed that over time, patterns of hydrocarbons vary (Vander Meer *et al.* 1989). The composition of cuticular hydrocarbons from *Camponotus vagus* Scopoli workers differ consistently, relative to whether they conduct most of their activities inside or outside the nest (Bonavita-Cougourdan 1993). Similarly, *Pogonomyrmex barbatus* Smith workers belonging to different task groups have specific cuticular hydrocarbon profiles, and the composition of the profile may be influenced by age-related changes (heritable) or by a change in abiotic conditions, due to switching to a new age-related task (Wagner *et al.* 1998). Thus, it would seem counterproductive for other members in a nest not to be able to update their recognition template with the new cues of their nestmates. Most likely, individuals habituate regularly to slightly modified odors (Vander Meer & Morel 1998). If this is the case, we might expect workers eventually to adopt eggs of the alternative host species of *Formica*. Although the results of this study suggest that the probability for adoption of heterospecific eggs may be low - only one test colony of both *Formica* species given heterospecific *Formica* eggs reared them to callows – it is not impossible.

Second, workers may adopt *P. breviceps* eggs once temperatures become steadily cooler in late summer-early fall. It has been hypothesized that decreased temperatures may dull or slow the chemosensory system making individuals less responsive to various chemical stimuli (Hölldobler & Wilson 1990). Most species of *Polyergus* occur in mountainous regions where in early to mid-September, and sometimes sooner, temperatures may drop to 4°C in the evenings. But because laboratory temperatures were relatively constant during this study, never dropping to 4°C, earlier adoption of *P. breviceps* eggs may have been prevented. Experimental manipulation of ambient temperatures could demonstrate whether periods of chemosensory dulling might result in increased adoptions. Adjusting the duration of the chemosensory dulling period, in combination with surface chemical analysis, could reveal whether adoption is a function of dulling alone or whether this period allows eggs to absorb sufficient environmental odors, preventing them from being destroyed.

Third, in these experiments, *P. breviceps* queens took over colonies that consisted only of workers, and not immatures. Under natural circumstances during colony founding, *Polyergus* queens enter and take over nests that are being raided by their non-reproductive nestmates. Raiders, for the most part, pillage only the pupae; presumably, eggs and larvae remain within the *Formica* nest. In laboratory tests, once *P. breviceps* queens have killed the host queen, they almost always place themselves on the brood pile (Topoff *et al.* 1988). If the *Polyergus* queen lays or mixes her eggs amongst host eggs, her eggs might become camouflaged by absorbing odors of the *Formica* eggs or might be hidden by them. Similar behaviors have been reported in queens of the polygynous ant, *Pachycondyla obscuricornis* Emery, which eat each other's eggs. To minimize agonistic consumption, queens shuffle their eggs with the eggs of other queens, which probably mixes borne chemicals (Oliveira & Hölldobler 1991).

The role that hydrocarbons play in stimulating rearing behaviors in *Formica* is a bit difficult to evaluate based on the findings of this study. This is, for the most part, because *P. breviceps* eggs from nests with *F. occulta* slaves had hydrocarbon profiles that were almost identical to hydrocarbon profiles of *F. occulta* eggs, yet only one of these individuals was reared to adulthood. Thus, hydrocarbons may have little involvement in stimulating rearing behaviors in *Formica* workers. Nonetheless, the behavioral results clearly indicate that odors of *P. breviceps* eggs that arbitrate rearing responses have undergone substantial modification from odors of their sister-taxa, otherwise more *Polyergus* would have been adopted overall and adoption would have occurred much earlier than five months after enslavement. Thus, it seems unlikely that *Polyergus* evolved directly from their host species as intraspecific social parasites, as has been suggested for other slave-making and socially parasitic species (Buschinger 1970, 1986, 1990; Pearson & Child 1980; Pearson 1981; see Chapter 5, Transport/Sympatric Speciation Hypothesis). Instead the lack of conservation in odors involved in brood rearing of *Polyergus* eggs (as implied by rejection of eggs and, possibly, differences in hydrocarbon profiles) suggests that *Polyergus* was at one point a free-living species and that, at least, dependent colony founding evolved later on.

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CHAPTER 5

Dependent Colony Founding in Slave-maker Ants: Precursor to Slave Raiding or Chance Encounter with Raiding Nestmates?

The prominent hypotheses regarding the evolution of slave-making are reviewed and discussed. An alternative conjecture, the Chance Encounter Hypothesis, is presented, which suggests that dependent colony founding and slave raiding arose from exposure of newly mated queens to signals recruiting workers to a territory invader. Queens exposed to recruitment signals follow raiding workers to the targeted nest. However, instead of pillaging brood and fighting with workers of the territory invader, queens enter the nest as they would during immigration. As the residents have fled the nest temporarily, the slave-maker queen easily locates and places herself on the remaining brood pile. When the resident queen returns, aggressive interactions ensue and a transfer of chemicals from resident queen to slave-maker queen takes place that result in her adoption by workers. The slave-maker offspring are reared, and they learn the odors of both slave-maker and host species. When they encounter nests of either species during foraging, they incite a territorial raid, as both species are now perceived as conspecific. However, because odors of both species have been learned, not all pillaged brood is destroyed and raiding for slaves evolves.

INTRODUCTION

Ants are most impressive in their ability to maintain exclusive societies. Most members of the society (nestmates) relinquish their reproductive capabilities and labor on behalf of the fecund individuals (Wilson 1971). Frequently, nestmates are to some degree genetically related, and the acceptance of nestmates (or the rejection of non-nestmates) appears to involve the recognition of heritable conspecific and acquired extrinsic chemical signals. Individuals learn these signals by being exposed to them during critical periods of their immature (Isingrini *et al.* 1985) or adult life (Morel 1983; Errard 1984; Morel *et al.* 1988). This context makes the relationship between socially parasitic ants and their allospecific host species an enigma, particularly among the specialized slave-makers. Slave-making (dulotic) ant species do not merely reside with other species of ants but rely on them (facultatively or obligatorily depending on the species) to provide the services needed for a colony to function, such as foraging, feeding and brood care. Slave-makers replenish their host worker supply by raiding nests of other species for their brood. Individual slave-makers (scouts) search for nests of their host species to raid, return to their nest, and recruit nestmates to the site. Brood is pillaged from these nests and is transported back to the home nest where some of the captured are eaten. The uneaten brood matures and the emerged workers become an integral part of the colony (Buschinger *et al.* 1980; Topoff *et al.* 1984, 1985; Buschinger 1986; Hölldobler & Wilson 1990). Queens of dulotic species are typically incapable of founding new colonies alone because they lack the fat stores found in most non-parasitic ants that nourishes them during the period of isolation before the first brood matures (Keller & Passera 1989). Occasionally dulotic queens form new colonies by departing from their natal nests with a supply of host workers (Marlin 1968, 1969; Wilson 1971; Buschinger *et al.* 1980; Kwait & Topoff 1983; pers obs 1997). More commonly, dulotic queens invade nests of their host species and either eliminate the resident queen(s), thereby gaining attention from the expunged's assembly of workers, or expel all adults and appropriate immatures.

Since Darwin (1859) first proposed predation as an evolutionary precursor to slave-raiding, numerous hypotheses bearing alternative explanations have emerged. Current research, while buttressing various speculations, continues to contribute to the emergence of new, significant questions. Research to date has focused primarily on raiding tendencies and colony founding methods of slave-makers and their host species to reveal the behavioral sequence of events that accurately reflects the evolution of slave-making. Problems in forming a unified evolutionary theory, however, seem to stem first from attempts to account for both slave-raiding and non-independent colony founding, and second by assuming this theory is plausible for both distantly-related taxa in which slave-making occurs (Formicoxenini and Formicini). Is non-independent colony founding or slave-raiding a prerequisite for the other to occur? Do the behaviors that are involved in forming polygynous and polydomous nests facilitate nest invasion and brood raiding by the parasite? Is olfactory imprinting responsible for territorial reactions against allospecifics?

Clearly the unresolved status of these questions necessitates further analysis, if not to iron out the wrinkles, then at least to trigger new questions that might lead to experimentation on a new trajectory. In this chapter, I will examine the behaviors that are typical of queens establishing a colony in slave-maker formicine and myrmecine species, with a particular emphasis on *Polyergus*. Where and when do they mate? How do they find a host nest and take it over? Why do naïve host workers adopt these queens and their offspring? By pursuing these questions further, how non-independent colony founding evolved in these groups, albeit differently, and the relationship between non-independent founding and slave-raiding might be better understood. As colony founding in slave-makers appears to be integrally linked to slave-raiding (or vice versa), I will first review the four major hypotheses and the relevant literature on the evolution of slavery. I will then provide an alternative conjecture and present experimental data on *Polyergus breviceps*.

The Phylogeny of Slave-makers

Raiding for "slaves" is a rare behavior among formicids and is conspicuously convergent. The majority of socially parasitic species are found in two distantly related subfamilies, Formicinae and Myrmecinae. Slave-makers are further concentrated in two tribes, Formicini (Formicinae) and Formicoxenini (Myrmicinae) (Bolton 1997; Hölldobler & Wilson 1990). In Formicini, slavery evolved independently at least three times as the facultative *Formica* slave-makers and the obligate slave-makers *Polyergus* and *Rossomyrmex*. Among Formicoxenini, slavery probably evolved 5 times, producing *Chalepoxenus*, *Epimyrma*, *Harpagoxenus*, *Leptothorax*, and *Protomognathus*[5] (Buschinger *et al.* 1980; Stuart & Alloway 1983; Buschinger 1990). In both subfamilies, the parasitic group usually forms the sister group to the host group at the generic level (Wesson 1937, 1939; Wilson 1971, 1975; Agosti 1994; Baur *et al.* 1995). On the basis of morphological, behavioral, and allozyme data, some have suggested that slave-makers could have evolved directly from the host species (see Transport/Sympatric Speciation Hypothesis; Wheeler 1919; Creighton 1927; Buschinger 1970, 1990; Elmes 1978; Pearson & Child 1980; Bourke & Franks 1991). However, this scenario seems unlikely (see Transport/Sympatric Speciation Hypothesis - *Some Problems*) and is not generally accepted.

The impact of sibling-group relationship between host and slave-maker may be great. Although the parasitic species has presumably diverged from the host species (or

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[5] Previously, *Protomognathus americanus* was transferred to *Harpagoxenus* from *Tomognathus*. However, *Harpagoxenus* was recognized as polyphyletic (Buschinger *et al.* 1980) and differences in sexual behaviors, host specificity, and ecological requirements strongly suggested that *P. americanus* did not belong in *Harpagoxenus* with *H. canadensis* and *H. sublaevis* (Buschinger & Alloway 1979; Buschinger 1981). The transfer of *H. americanus* to *Protomognathus* by Hölldobler and Wilson (1990) and Buschinger (1990), although not formal, has been generally accepted.

parasite and host species from a common ancestor), ecological requirements and even chemical makeup are likely to be similar as a result of their close phylogenetic relatedness, which surely contributes to general cohabitation compatibility. As with any trait, the convergent evolution of slavery in Formicini and Formicoxenini also suggests that these groups have similar behavioral and ontogenetic tendencies that make them susceptible to forming socially parasitic relationships. Environmental and/or ecological conditions have undoubtedly contributed to the evolution of these tendencies as well as further increased general susceptibility (Wilson 1990). Shared behavioral and ecological traits in both parasite and host species of these two tribes have been thoroughly examined. The behavioral traits relevant to colony foundation and certain colony characteristics for several species of slave-makers and their hosts in both tribes are summarized in Table 5.1. This list is by no means exhaustive but represents most species renowned for their slave-making inclinations.

Darwin's Predation Hypothesis

On the basis of his observations of slave-raiding in the *Formica* (*Raptoformica*) *sanguinea* species group, Darwin (1859) first proposed that the ancestral "purpose" for raiding nests of other species was rapacious in nature, as pillaged brood was often consumed by the slave-raider. Excess brood that survived long enough to emerge in the predator's nest would accept the raider species as nestmates, if the pillaged species formed social attachments by learning nestmate cues during a critical period.

The facultative slave-makers, *Formica wheeleri* Creighton and *Formica sanguinea* Latreille, can certainly be considered reminiscent of Darwin's predatory species. According to Topoff and Zimmerli (1991) *F. wheeleri* is aggressive towards non-host species as well as towards their host species, and frequently captures stray individuals

Table 5.1. Queen and colony characteristics of Formicini & Formicoxenini slave-makers and their hosts.

/ = No
? = Unknown
F before letter = facultative

				MATING LOCATION			QUEEN & COLONY BEHAVIORS				COLONY CHARACTERISTICS					RECRUITMENT***		
		SLAVE-MAKER SPECIES	HOST SPECIES	Flight (F)	Nest (N)/ Ground (G)	Raid (R)	Return to Nest (RN)	Adopt (A)/ Reject (Rj)	Independent (I)/ Dependent (D) Invasion	Takeover Method**	Monogynous (M)/ Polygynous (P)	Haplometrotic (H)/ Pleometrotic (P)	Monodomous (M)/ Polydomous (P)	Bud Emigration (BE)/ Branch Colony (BC)	Slave-Raids	Food	Nest Emigration	Territorial Raids
Slave-Makers	Formicoxenini	***Harpagoxenus***																
		canadensis	*Lm*	F	Gc*	/	/	Rj	I	EQ-UB/W	M	H	M	BE	TR			
		sublaevis	*Lac, Lg, Lm*	F	Gc	/	/	Rj	I	ER-UB	M	H			TR			
		Leptothorax																
		duloticus	*Lam, Lc, Ll*	F	G	/	/	Rj	I	E-UB	M	?		BE	GR			TR
		Protomognathus																
		americanus	*Lam, Lc, Ll*	F	G	/	/	Rj	I	EQ-UB/W	M	?	P	BC	GR			
	Formicini	***Formica***																
		sanguinea	*Ff, Fr*	F		R	RN	PA/PR	FD	EQ-UB	M	FP	P	BE	GR		GR	TR
		wheeleri	*Ff,Fo*	F		?	RN	Rj	I	ER-UB	M	H			GR			TR
		Polyergus																
		breviceps	*Fg, Fo*	F	Gc	R	RN	Rj	D	KQ	M	H		BE	GR			
		lucidus	*Fa, Fs*	F	Gc	R	RN	Rj	D	KQ	M	H		BE	GR		GR	
		nigerrimus	*Fg*	?	?	?	?	?	D	KQ	M	?			GR			
		rufescens	*Fc, Fr*	F	Gc	R	RN	Rj	D	KQ	M	H			GR			
		samurai	*Fj*	F	Gc	R	RN	Rj	D (I)	KQ	M	?			GR			
Hosts	Formicoxenini	***Leptothorax***																
		acervorum (Lac)		F	Gc		RN	A			M/FP	H		BE		TR	TRy	
		ambiguus (Lam)			?		RN	A			M/FP	H/P	M/P	BE		TR		TRa
		curvispinosus (Lc)		F	?		RN	R/A			M/FP	H	M/P	BE		TR	TRy	TRa
		longispinosus (Ll)			?		RN	A			M/FP	H/P	M/P	BE		TR	TRy	TRa
		muscorum (Lm)			Gc						M/FP	H				TR	TRy	TRa
	Formicini	***Formica***																
		archiboldi (Fa)						?			P	P						Yes?
		cunicularia (Fc)						?					M/P					Yes?
		fusca (Ff)					R	A			M	H	FP				GR	No?
		gagates (Ft)																
		gnava (Fg)						?			M/FP	H/P?	M/P					Yes?
		japonica (Fj)		F			R	A			P	H/P	M/FP	Bud				No?
		occulta (Fo)						?			M/FP	H/P?	M/P					Yes?
		rufibarbis (Fr)						?				H	M					Yes?

* Gc = mating on ground with female calling

** E = evict Q = queen R = adult residents
U = usurp B = brood W = workers

*** GR = group/mass recruitment
TR = tandem running
y = with carry

of a variety of species, which are brought back to the nest and eaten. The transport of adults that are killed during raids back to the nests in addition to the retrieval of immatures has also been interpreted as evidence of their predatory nature (Topoff & Zimmerli 1991). Although not known to retrieve adults killed during raids, *F. sanguinea*, in the pine forests surrounding Tomsk, are equally as formidable. They prey on a variety of insects and a number of ant species, and quickly destroy the nests in the vicinity of their own (Marikovsky 1963a). Even the obligatory slave-makers show predacious habits, although they rarely, if at all, capture non-host species. As much as 74% of the brood captured by the obligate slave-maker *Polyergus lucidus* may be eaten both by their *Formica* slaves as well as by *P. lucidus* themselves (Goodloe *et al.* 1987; Kwait & Topoff 1983). Large piles of pupa exuviae outside nests of *Polyergus breviceps* on the day after a raid are evidence of the mass consumption of pupae by its members. When *P. breviceps* are artificially overfed, they stop their raiding activities (Topoff *et al.* 1985). This implies that 1) they are satiated, 2) raiding normally provides the means to satiate, and 3) slaves, which result because some of "the means to satiate" are left uneaten, are a consequence of predation. Nonetheless, the Predatory Hypothesis is rarely accepted as a suitable explanation for the evolution of slave-making behavior.

Slave-makers Versus Predators

One argument rebutting the Predatory Hypothesis has been that slavery is not found within predatory taxa (Hölldobler & Wilson 1990). The absence of slave-makers in predatory taxa may be due to the fact that predatory behavior has also evolved from territorial behavior. Unique predator morphologies, behaviors, and abilities (Fig. 5.1), however, may have determined the evolutionary path to exclusive predator as opposed to slave-maker with predatory tendencies. For example, some predatory taxa have mechanisms that prevent pillaged immatures from ever forming social attachments to their raiders. The myrmecophagous *Cerapachys turneri* Forel raid nests of *Pheidole* for larvae

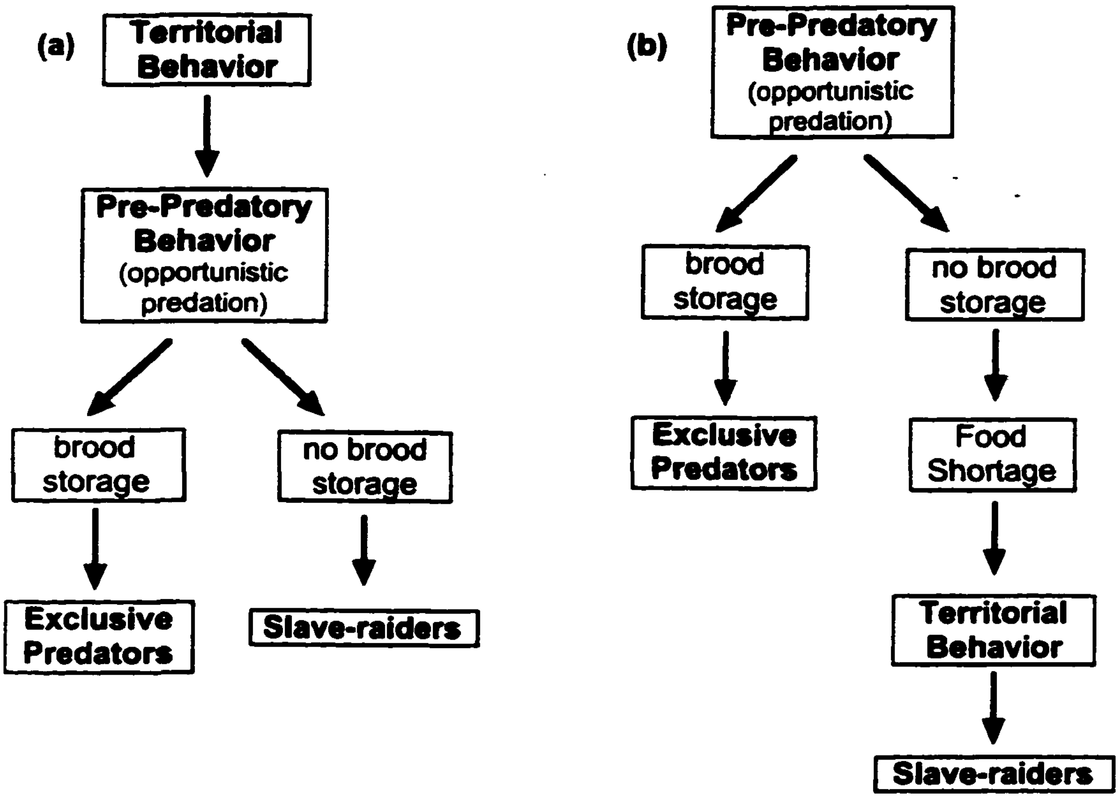

Figure 5.1. Two hypothetical pathways leading to either an exclusive predator or slave-raider outcome. **(a)** Ancestors conduct territorial raids and pillage brood, some of which is eaten. In taxa with a mechanism for brood storage, excess brood can be saved for later, there is little need to forage for alternative food, and exclusive predators evolve. In groups with no means to store excess brood, adults emerge and slave raiding evolves. **(b)** Ancestors prey on other species and brood storage again results in evolution of exclusive predators. Among groups with no means to store brood, when food is in short supply, territorial boundaries expand, tolerance decreases, and raids against territory intruders are conducted. Excess brood from raids matures and slave-raiders evolve.

and pupae; but, before the immatures are carried back to the *Cerapachys* nest, they are briefly stung by the raider (Hölldobler 1982). The paralytic substance appears to place larvae and pupae in metabolic shock, which allows them to be stored alive without developing further for two months or more. Eventually they are eaten, and any excess pillaged brood in these nests never develops into slaves.

If "freezer-storage" capability were a prerequisite for predatory behavior to evolve, slave-making is unlikely to have evolved from this type of exclusive predatory behavior. However, slave-making could and may have evolved from a pre-predatory state. The question to then ask becomes whether pre-predatory behavior evolved from territoriality or, if pre-predatory behavior was insufficient in providing the nutritional requirements of a colony because of the inability to store pillaged brood, whether territoriality evolved in attempt to secure other limited resources.

The answers may lie within the behaviors and chemistry of some slave-maker species. Both species of *Chalepoxenus muellerianus* (Finzi) and *Leptothorax duloticus* (Emery) sting the adults of their host species when raiding their nests and either paralyze them (*C. muellerianus* – [Schumann 1992]) or kill them (*L. duloticus* – [Wesson 1940; Alloway 1979]). Immatures, however, do not elicit a similar reaction. Why is there this differential response to immatures by slave-makers as compared to *Cerapachys* predators, when these slave-makers have the morphology and the ability? Do immatures of the host species lack the stimulus that induces raiders to sting? Are the contents of the Poison gland in slave-makers fatal? If we can determine whether constraints (if any) on brood storage ability is behavioral, chemical or both, we may be able to infer the evolutionary precursor to slave-making.

Slave-makers as Scavengers

A second, although not mutually exclusive, argument against the Predation Hypothesis is that most species acting as hosts to slave-raiders are scavengers (Topoff *et al.* 1987; Hölldobler & Wilson 1990) and, as closest relatives to slave-makers, slave-makers are likely to be scavengers as well (Topoff 1990). If this is the case, pillaged brood may be superfluous because it is a secondary, opportunistic food source from territorial raids. This may seem to contradict the results of the experiment involving over-feeding (Topoff *et al.* 1985) (Why would raiders stop raiding when "satiated" if raiding were not for the purpose of fulfilling nutritional needs?). However, if scouting is really a modification of scavenging (Topoff *et al.* 1987), and slave-raiding represents a modified response to a territory invader encountered during "scavenging," satiated colonies would be just as unlikely to scout, as they would be unlikely to scavenge (Fig. 5.2). The reduction in scavenging/scouting would, in turn, decrease encounters with territorial invaders during "foraging".

Topoff (personal communication 1999) and Johnson have independently observed that raiding by *P. breviceps* in the Chiricahua Mountains of Arizona substantially decreases when the seasonal monsoon rains are delayed and soil conditions are extremely dry. Nests of *Formica gnava* Buckley and *Formica occulta* Francouer, host species to *P. breviceps* Emery, seem to retreat deeper into the ground and become extremely difficult to find. While these are merely auxiliary observations, they do support the idea that raiding by *P. breviceps* is a response to a territorial invader encountered during "foraging" (see Independent Invasion/Olfactory Imprinting). If slave-makers were simply predators, we might expect attempts to prey on other available species. *Polyergus,* however, maintain their fidelity to raiding only their host species (see Independent Invasion Hypothesis) (Cool-Kwait 1982; Goodloe *et al.* 1987).

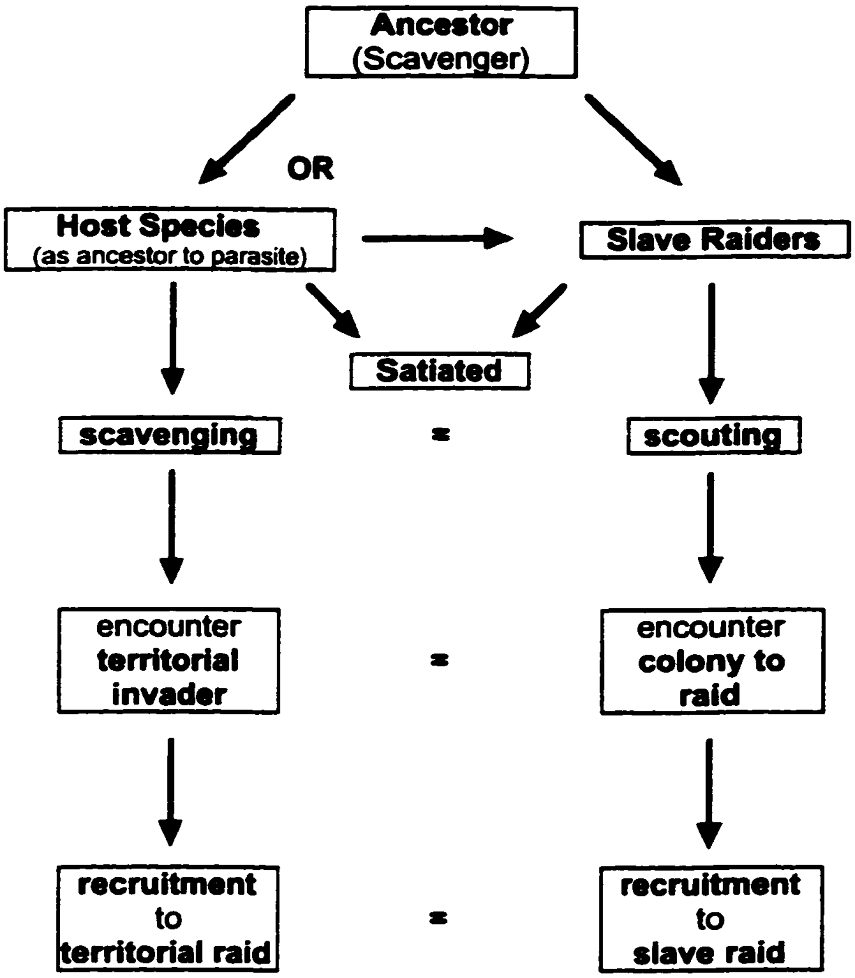

Figure 5.2. Cessation of raiding due to overfeeding may not implicate predatory behavior as a precursor to slave-raiding. If scouting by slave-raiders represents a modified form of scavenging, slave-maker colonies that are satiated may not scout/scavenge, reducing encounters with territorial invaders. Satiation may alternatively be an indication that resources are abundant, and territorial species may become more tolerant.

On the other hand, a satiated colony may perceive resources to be abundant and therefore become more tolerant of competing colonies (e.g., *Formica polyctena* Foerster - [Mabelis 1979]), just as they might become less tolerant when resources are scarce. In essence, territorial boundaries may wax and wane relative to available resources and colony needs. Without examining the modifications in raiding behavior of slave-makers when the number of colonies trespassing on slave-raider territories are manipulated along with available food supply, we are no closer to implicating predation as an evolutionary precursor to slave-raiding.

Survival of slave-maker queens is contingent on usurping a nest of workers (or brood that is soon to emerge), and mechanisms involved in colony establishment must be included in formulating any encompassing hypothesis. Hence, while pre-predatory and territorial behaviors present opportunities for slave-raiding to evolve, they unfortunately do not inherently account for the dependent mode of colony founding that is typical of slave-making species. The following four hypotheses attempt to incorporate the non-independent colony founding component.

Transport/Sympatric Speciation Hypothesis

The Transport Hypothesis was developed by Buschinger (1970, 1986, 1990) who recognized that most, if not all, host species to slave-makers have colonies that often consist of several nests (polydomous) among which brood is transferred, and that the association between slavery and polydomy is probably not fortuitous. He suggests that budding or fission, where a queen and group of workers break off from the main nest and form a peripheral "satellite" nest (Fig. 5.3), achieve the polydomous state of these colonies. As part of the colony, brood is transported to satellite nests from the central nest and vice versa, presumably as part of the natural shifting around of brood by

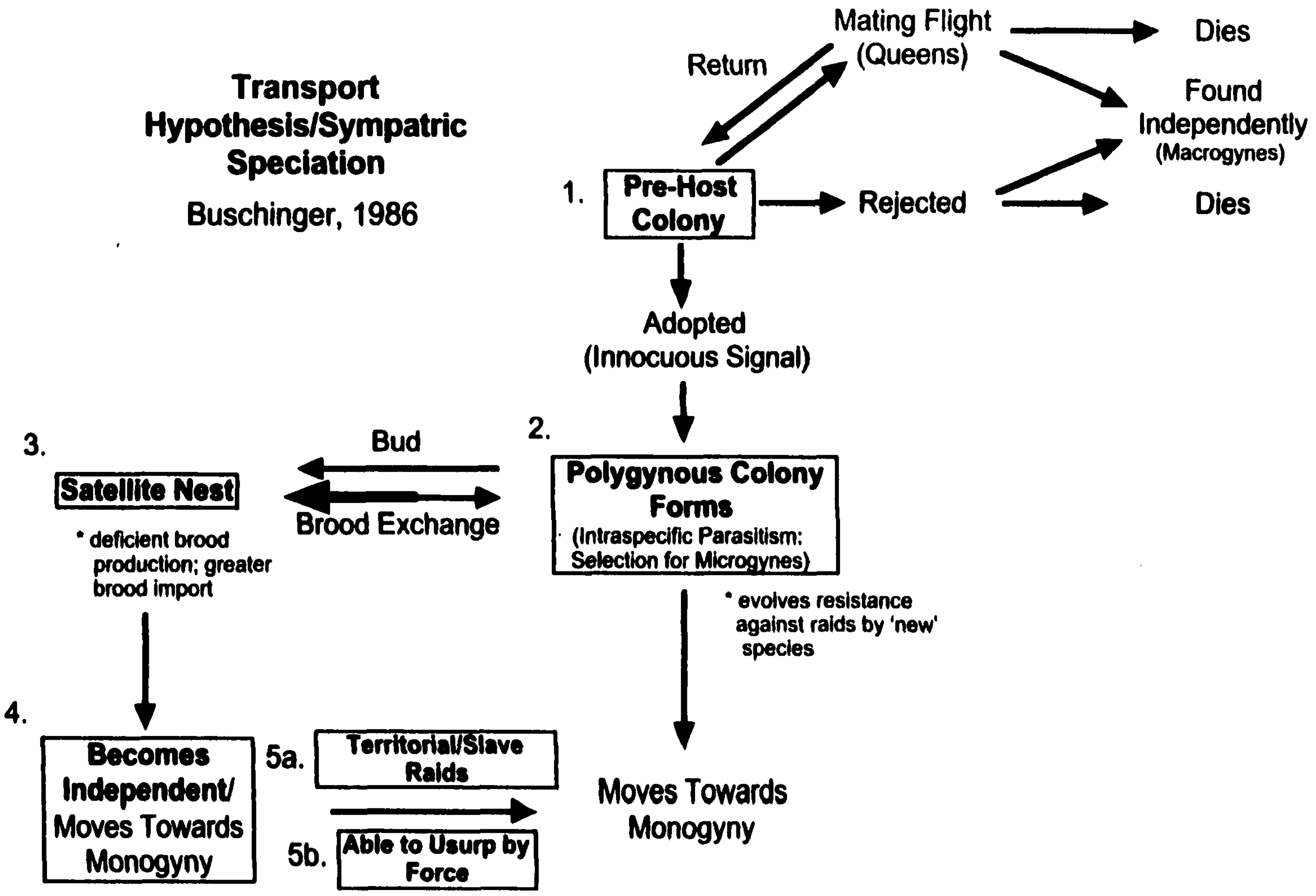

Figure 5.3. Pre-host colonies adopt newly mated queens and colonies become polygynous. New queens bud with a group of workers and form satellite nests, but they are reproductively deficient and require greater import of brood. As they gain independence, aggression between nests rises. Reproductive isolation leads to sympatric speciation and deficient nests become slave raiders of closely-related species.

workers (O'Neal & Markin 1973). If, however, a subpopulation of queens within the host species (or species ancestral to both host and parasite) are unable to produce sufficient worker numbers for their satellite nest, they may require a greater import of brood from the central nest. Should the satellite nest gain independence yet still require an import of workers, one or both nests may quickly become aggressive towards the other, manifesting in intra-specific territorial raids. If the central and satellite nests become reproductively isolated, founders of satellite nests might become specialized raiders on the ancestral polygynous colonies.

The appeal of Buschinger's idea is his suggestion that there are genetically distinct subpopulations. Rather than having subpopulations that differ in absolute fecundity as originally suggested (Buschinger 1970, 1986, 1990), these subpopulations may be genetically predisposed to respond differently to environmental conditions during dispersal and colony founding stages (Buschinger 1989). Genetic variation within a population resulting in the ability to exploit two or more niches by dispersing in alternative ways is likely to increase overall fitness, making such variation likely in general (Hamilton & May 1977). Producing *reproductive* offspring with reduced fecundity in addition to sterile offspring for the most part does not increase overall fitness[6]. Nonetheless, genetic divergence between subpopulations as a function of dispersal tendencies that have a genetic component could easily result in speciation if one subpopulation utilizing an alternative dispersal or founding strategy brings itself out of the mating range of the other. Mating could appear panmictic and non-assortative (Alloway 1980) while in fact the two subpopulations have become geographically separated as a consequence of their dispersal habits. New queens of *Leptothorax acervorum* Fabricius, a host species of the slave-maker *Harpagoxenus sublaevis* (Nylander), participate on mating swarms or

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[6] Subfertility [West-Eberhard 1978], however, probably played a role in the evolution of eusociality [Craig 1983].

mate intranidally. In effect, these divergent mating habits promote assortative mating (Douwes *et al.* 1987).

Variation in Dispersal and in Founding Strategies

All slave-maker host species in both Formicini and Formicoxenini form monogynous and facultatively polygynous nests (Buschinger 1968b, 1974). Monogyny in these species is probably achieved by new queens 1) dispersing from the mother nest and independently founding a new nest, 2) budding from a polygynous home nest (Möglich & Hölldobler 1974), or 3) reproductively outcompeting other queens in a polygynous nest, eventually leading to the ousting (or death) of subordinate queens. Polygynous nests, on the other hand, appear to be primarily formed through adoption of newly mated queens (Hölldobler & Wilson 1977; Heinze & Buschinger 1988; Rissing & Pollock 1988; Buschinger & Heinze 1992). The strategy (adoption/polygyny or dispersal/monogyny) queens select to found their colonies is undoubtedly influenced by certain ecological factors such as nest site availability, male availability, and/or weather conditions (Rosengren & Pamilo 1983; Herbers 1986, 1993; Elmes & Petal 1990). But, utilization of several tactics by one nest or species in response to these conditions implies a genetically mediated predisposition for some individuals to respond one way and other individuals to respond another way. Individuals may be "choosing" one founding strategy over the other, as a function of genetically influenced differential sensations and/or responses to available cues provided by environmental conditions. Or, reproductives (females and/or males) may merely be mating and dispersing differently relative to their genetic tendencies. Recently, variation in dispersal patterns of newly mated *Solenopsis invicta* Buren queens from polygynous colonies has been traced to genotypic variation at a single locus (DeHeer *et al.* 1999). Allelic differences between gynomorphic and intermorphic queens of *Leptothorax* sp. A also have been correlated with the respective dispersal tendencies (Heinze & Buschinger 1988, 1989).

Genetic polymorphisms, such as aptery, loss of ocelli, and reduced thorax size, have often been considered the cause of dispersal strategy variation (Heinze & Buschinger 1988): A wingless morph is unable to fly and disperse because she is wingless. However, polymorphisms may instead be evidence of genotypic variation underlying the variation in behavioral phenotypes (Heinze & Buschinger 1987; Buschinger & Heinze 1992): A morph is wingless because she does not fly and reduces the energy expended in producing wings and wing musculature by not producing them. The winged morph retains flight machinery because she continues to fly. Hence, the tendency to disperse (or not) may be the selective force governing queen morphology. Wingloss in the gynomorph queen caste of *Monomorium* has been attributed to electing adoption and budding instead of founding a colony independently (Bolton 1986). Similarly, the loss of nuptial flights in *Formica uralensis* Ruzsky is also considered function of behavior and not a result of wing loss (Rosengren & Pamilo 1983). We may, in fact, be able to infer genetic divergence by the occurrence of polymorphic reproductive males and females.

Polymorphisms and Genetic Divergence

The occurrence of polymorphic queens has been underestimated, and it is now becoming clear that queen polymorphism is correlated with dispersal and mating habits and that this does not necessarily signify reduced fecundity (Buschinger & Heinze 1992). Population studies on colony founding by queens of *Leptothorax rugatulus* Emery, a species of *Leptothorax* that is not parasitized, provide a nice correlation between size dimorphism and dispersal strategy (Rüppell *et al.* 1998), which may reflect genetic tendencies to found colonies in consistent, alternative ways. Microgynes, queens characterized by smaller body size on the basis of head width, thorax length, and thorax width index, were predominantly found in polygynous colonies (84.6%), whereas macrogynes were found in monogynous colonies (64.3%). Although it is unclear whether body size is a function of genetics, social effects, or both (Buschinger & Heinze 1992), the tendency for macrogynes to disperse and found nests independently and for microgynes to attempt adoption could reflect sympatric speciation in progress if dispersal strategy, or some aspect affecting dispersal strategy, is heritable. Direct evidence for such a scenario comes from studies with *Myrmica rubra* (Linnaeus), a species that also has macrogyne and microgyne queens. Microgynes are always found with macrogynes (although not vice versa), and both can only produce queens of the same type. Distributions of esterase allozymes indicate that microgynes have become genetically isolated from macrogynes and now belong to a separate species (Pearson & Child 1980; Pearson 1981), suggesting sympatric origination of the parasite (Brian & Brian 1955)[7]. In some species, however, winged dispersal may not endow individuals with greater reproductive success and therefore not contribute to genetic divergence. The development of winged and wingless *Harpagoxenus sublaevis* queens is also controlled by the allelic combination at a single locus. The winged genotype, however, is less successful than her wingless counterpart in surviving to reproduce and in reproduction (Winter & Buschinger 1986).

Polymorphism of reproductive colony members is not restricted only to females. In *Formica truncorum* Fabricius males exhibit wing dimorphism, and this is also correlated with dispersal phenotypes (Sundström 1995). Males from polygynous colonies have smaller wings relative to their head width than males from monogynous colonies, and tend not to disperse before mating whereas males from monogynous colonies disperse. Sundström (1995) suggests that the consistency with which non-dispersal and dispersal tactics are used relative to the colony type also indicates a genetic component.

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[7] It was pointed out that Pearson & Child (1980) and Pearson (1981) may have confused these traits as defined by Nixon & Wheeler with diagnostic characters (personal communication Carpenter).

Role of Chemical Signature

If queen and male polymorphisms evolve in response to divergent mating and dispersal strategies within a species, we might expect to find haplometrotic species that are not characterized by queen size and wing dimorphism but form both monogynous and polygynous colonies. Since monogyny in many cases is maintained by the rejection of queens attempting to be adopted, it follows that for polygynous colonies to form by welcoming returning queens (or not rejecting queens that mate intranidally), there must be some mechanism enabling queens to avoid rejection. One such mechanism may be the chemical cues of the newly mated queen. *Formica gnava*, a host species to *Polyergus breviceps*, does not readily exhibit queen dimorphism, but forms both monogynous and polygynous colonies. A recent experiment demonstrated that newly mated *F. gnava* queens do not induce the attack behaviors that *P. breviceps* queens typically exhibit towards *F. gnava* queens that have formed a colony (established queens) (Zimmerli & Topoff 1993). Aggression against newly mated queens does not occur until 190 days after mating, even though newly mated queens by this time have a small court of workers and brood! This suggested that the provoking stimulus might be cuticular chemicals (kairomones) associated with "mature" *F. gnava* queens. Analysis of principal components revealed that newly mated and established *F. gnava* queens could not be distinguished from each other on the basis of qualitative differences in cuticular hydrocarbons. Nor did there appear to be differences in the relative proportions of these hydrocarbons (see Chapter 1, Fig. 1.1), indicating that chemicals other than hydrocarbons probably produce this kairomonal effect. Nonetheless, if *P. breviceps* queens can distinguish *F. gnava* queens relative to how advanced they are in colony establishment, it is not unrealistic to presume that *F. gnava* workers are also able to distinguish *F. gnava* queens. Alternatively stated, *F. gnava* workers may be unable to distinguish newly mated *F. gnava* queens from alate females or worker nestmates. If newly mated queens don a non-threatening (reproductively competitive?) chemical signature, they could plausibly circumvent aggression and be accepted back into their natal colony.

When gynomorphs of *Leptothorax* sp. A, which tend to disperse on the wing and attempt independent colony foundation, were artificially placed back with their natal colonies, they were soon killed once they began developing eggs (Heinze & Buschinger 1988). Similarly, *Solenopsis invicta* queens from polygynous colonies that belonged to the heaviest weight class (Ross 1992) and carried the genotype for dispersal (DeHeer *et al.* 1999) were killed when they began egg laying or even before they left their nests to mate. These results suggest that a dominant or independent chemical signature is unacceptable and queens that are adopted are chemically different. Conversely, a new queen that bears the odor of an alpha queen may be able to redirect the worker attention away from the resident queen and towards herself (Elmes & Brian 1991). In either case, dispersal degenerate (wingless) queens, as in *Formica fusca* Linnaeus (Donisthorpe 1927), *Leptothorax rugatulus* (Rüppell *et al.* 1998) and *Myrmica rubra* (Brian & Brian 1955; Elmes 1976), could eventually evolve in addition to flight dispersing queens.

Differential Adoption

Stuart *et al.* (1993) observed colony responses by reintroducing newly mated *Leptothorax curvispinosus* Mayr queens to queenless, monogynous, or polygynous alien colonies and to queenless and monogynous natal colonies. Alien colonies almost completely rejected newly mated queens. Natal colonies, regardless of the number of queens, either consistently rejected or accepted the related newly mated queens. These findings suggest a genetic tendency for colonies to either accept or reject returning queens (Stuart *et al.* 1993), and that the involvement of a genetic component in seeking adoption (or not) and chemical signature is key (Herbers 1986; Stuart 1987; Stuart *et al.* 1993). Fortelius *et al.* (1993) found that unmated *Formica lugubris* Zetterstedt queens were adopted more frequently than mated females, and acceptance was particularly high when other alates were present in the test colony. If newly mated *Formica* queens maintain a signature similar to alates, their chances of being adopted may increase. Brückner (1982) found that virgin *Leptothorax acervorum* (Fabricius) queens were never accepted by any colony regardless of colony type. However, inseminated queens, particularly if they were the first queens received by the colony, were accepted (70%).

Topoff and Zimmerli (1993) had also introduced *F. gnava* queens to alien colonies but found that they were adopted, suggesting that even mature *F. gnava* queens can be transferred between alien conspecific colonies. However, they used dealate queens from polygynous nests. While *F. gnava* appear to form truly polygynous nests, I have collected numerous *F. gnava* and *Formica occulta* (an alternative host species of *P. breviceps*) nests with dealate queens that were not inseminated and did not induce aggressive behavior in *P. breviceps* queens during laboratory tests. In addition, within 24 hours after collection most alate queens had lost their wings even though they had not mated. Other polygynous *Formica* species have also been shown to maintain a small percentage of unmated queens in their nests (unpublished data Fortelius in Rosengren *et al.* 1993). Frequently queens of these *Formica* (*sensu stricto*) species dealate in the laboratory without mating (Fortelius 1987; unpublished data Fortelius in Rosengren *et al.* 1993). Colonies of *Formica lugubris* will accept unmated queens back into the nest to a greater degree than mated queens (Fortelius *et al.* 1991). Thus it is possible that the tests involving *F. gnava* queens reflect the ease with which unmated dealate *F. gnava* queens can be transferred across colonies. By introducing verified newly mated, pre-established and established *F. gnava* queens from monogynous and from polygynous nests into nests with one or many queens, we can determine whether these queens are differentially adopted. Analysis of queen chemicals could ascertain whether the queens are chemically distinct. These findings would allow us to generate a hypothesis on the correlation of a particular signal with dispersal strategy.

Some Problems

"Boomerang behavior" (returning to a nest), adoption, and sympatric speciation of a parasite from host species are, nonetheless, problematic in terms of providing a satisfactory scenario for current slave-maker species, although it is highly suggestive for the evolution of intraspecific parasites (see Bourke & Franks 1991). First, in current host populations, both adoption and independent colony founding are viable strategies. This would suggest that there are new genetic subpopulations that are independent of current slave-makers, and that among this new subpopulation a second parasitic species is evolving from the host (ancestral) species[8]. Second, if current slave-makers evolved from their host species (or a common ancestor) as a result of variation in dispersal strategies, this would suggest the following. First, the former intraspecific parasite (now interspecific parasite), which at one time could be readopted by conspecifics, has lost the ability to avoid aggression from conspecifics (parasite colonies are strictly monogynous) as well as from the species from which the parasite diverged. Second, the former intraspecific parasite (now interspecific parasite) has adopted new relatively aggressive tactics to take over a host colony, such as killing the host queen (*Polyergus*) or throwing out adult colony members (*Formica*, *Harpagoxenus, Leptothorax, Protomognathus*). Considering that some non-parasite *Formica* actually drag new queens back into the nest (Rosengren & Pamilo 1983), this seems unlikely. Third, different reproductive strategies alone do not account for the raiding of other nests, which may simply be an extension of territorial raids between closely related species (see Territorial Hypothesis).

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[8] Because some degree of dispersal is advantageous, boomerang behavior or intranidal mating are unlikely to become fixed strategies (Hamilton & May 1977; Rosengren & Pamilo 1983), and a new parasitic species may in actuality be evolving.

Other Considerations

Aggression between nestmates may increase when one group of nestmates residing with a queen has been separated for a time period from another queenless group of nestmates (Provost 1989). Satellite nests that become independent and separated from the main nest do have the potential to become aggressive towards former colony mates (and vice versa). However, interactions among conspecific colonies (Le Moli & Parmigiani 1982) or among colonies of closely related species are already likely to be aggressive (Jutsum 1979; Le Moli & Parmigiani 1981; Crosland 1989) since they frequently utilize similar resources (food, mates, nest sites) and compete for them (De Bruyn 1978; Hölldobler 1979). Territorial interactions often involve brood pillaging, making the splitting of a polydomous colony an unnecessary condition for brood raiding to evolve as the Transport Hypothesis suggests.

In order for the subpopulation of reproductively deficient queens to produce an adequate work force, satellite colony workers (either those that budded with the satellite queen or the offspring of the satellite queen) must aggressively acquire offspring by raiding the central nest and other unrelated nests. This implies that these workers have superior fighting abilities compared with those of the central nest. Considering the satellite queen is reproductively deficient, it would seem unlikely that her worker force could defeat the larger alpha colony.

Territorial Hypothesis

The Territorial Hypothesis, first proposed by Wilson (1971, 1975), identifies territorial interactions between conspecific colonies as precursor to interspecific slave raiding. These territorial raids terminate in the pillaging and transport of brood back to the nest, some of which matures and becomes part of the raider's colony. Eventually raids are conducted on closely related species. Alloway (1979, 1980), Hölldobler (1976), Stuart and Alloway (1982, 1983) advance the Territorial Hypothesis by suggesting 1) that raiders feed on pillaged brood to take advantage of a proteinaceous food source (and not because they are predatory) and 2) that dependent colony founding stems from the tendency of queens to move among nests of the polydomous colony of the pre-host/pre-parasitic species as this contributes to queens mistakenly entering foreign nests (Alloway 1980; Pollock & Rissing 1989).

Territorial Behavior in Host Species

Much of the supportive evidence stems from experiments and natural observations on species of *Leptothorax* that are hosts to the slave-makers *Harpagoxenus*, *Leptothorax*, and *Protomognathus*, which identify elements of host territorial behavior that are similar to slave-maker species, such as recruitment method, raiding, brood retrieval, and treatment of retrieved brood after raiding (Alloway 1980; Stuart & Alloway 1982, 1983). Colonies of conspecific or allospecific *Leptothorax* were placed in close proximity so as to encroach on presumed territorial boundaries. The resulting behaviors appeared territorial in nature and were extremely similar to the behaviors observed during slave-raids. A colony was encountered, nestmates recruited, and pillaged brood brought home. These behaviors exhibited by a non-slave-maker species revealed the potential for both brood raiding and slavery to evolve in a host species, as in some cases not all of the pillaged brood was destroyed. The raiding behavior of *Leptothorax muscorum* (Nylander) was almost identical to that of their enslaver, *Harpagoxenus canadensis* Smith, with the notable exception that free-living *L. muscorum* tended to feed the raided brood to their own larvae, whereas enslaved *L. muscorum* tended to care for the captured brood (Alloway 1980; Stuart & Alloway 1982, 1983). Moreover, the ability of hosts and parasites to respond to each other's alarm-recruitment signal further supports close phylogenetic association. In conjunction with the territorial behaviors observed in the host species, it is easy to deduce that with a reduced ability to discern captured brood, slave-making would evolve (Wilson 1975; Alloway 1979). The similarity of alarm-recruitment signals in *H. canadensis* during slave-raids and in a non-parasite *Leptothorax* during territorial raids provides an additional link between territorial raids and slave raids (Stuart & Alloway 1982, 1983). The slave-maker recruitment of nestmates to target nests appears to be a derivation of alarm-recruitment signals involved in territorial interactions of host or closely related non-host species (Stuart & Alloway 1982).

Wilson (1975) was able to stimulate raids by larger *Leptothorax curvispinosus* colonies on smaller *L. curvispinosus* colonies, as well as raids by *Leptothorax ambiguus* Emery on *L. curvispinosus.* Although *L. curvispinosus* adults emerged in both colonies, *L. ambiguus* destroyed adult *L. curvispinosus* after a few days. Alloway (1980), in a more comprehensive study, was similarly able to trigger attacks by *L. ambiguus, L. curvispinosus,* and *Leptothorax longispinosus* Roger on smaller colonies of the same species. Mixed colonies with all paired combinations formed except for *L. curvispinosus,* which did not raid and retain *L. curvispinosus* brood as slaves as they did in Wilson's (1975) study, providing further support that slavery may be a function of territorial tendencies in host species. Alloway's colonies, however, had been fed only the protein-deficient Bhatkar diet, which some have demonstrated can be insufficient for proper development (Porter 1989). Success of raid induction and the large number of brood consumed in Alloway's experiment may have been a consequence of insufficient nutrition in raiding colonies (Buschinger & Pfeifer 1988; Hölldobler & Wilson 1990). Accordingly, these results could instead be interpreted as reflecting opportunistic brood predation instead of typical territorial aggression. Alternatively, the deficient diet in this case might have led to increased intolerance of neighboring colonies utilizing similar resources, soliciting "dormant" behaviors that are typical during an environmental crisis, and the situation again becomes a question of whether territoriality precedes predatory behavior or vice versa (see Darwin's Predation Hypothesis). Although perhaps unintentionally, these results obviously reflect the degree of behavioral flexibility in a stressful circumstance. If the result is increased fitness, stressors such as over-crowding and fluctuations in food availability could catalyze a stable repertoire of alternative behaviors that may eventually lead to slave-making.

The greatest appeal of the territorial hypothesis is that brood pillaging in response to infringement on territorial boundaries of a colony can easily include opportunistic brood predation even though predation *per* se is not characteristic of slave-making taxa. The results from numerous experiments (Alloway 1980; Goodloe *et al.* 1987; Stuart & Alloway 1982, 1983; Topoff *et al.* 1984) most likely reflect opportunistic feeding as a consequence of intolerance for territory intruders versus strict predatory feeding. Opportunistic feeders would be unlikely to consume all of pillaged brood, whereas we would expect this of classified predators with no effective mechanism for delaying brood development. If the degree to which pillaged brood is consumed by predators and opportunistic feeders is a function of the nutritional needs of the colony (or individual), then during times when nutritious food (prey or scavenged items) is abundant, pillaged brood is more likely to be spared. This leaves a window of opportunity open for pillaged brood to mature in nests of raiders. Because predators rely solely on prey items and opportunistic feeders rely on a variety of food items, brood that has been pillaged during a territorial encounter is more likely to be spared than brood that has been pillaged solely as a food source. The nutritional needs of the scavengers are probably met primarily through scavenging whereas the predators needs are probably met primarily through consuming prey items like pillaged brood. Thus, the probability that slave-making will evolve in territorial scavengers as opposed to predators is greater, especially if the predator pathway has been selected by the abilities of a group to store prey items. Opportunistic predation is therefore quite consistent with the scavenging and foraging feeding strategies of both slave-makers and their hosts.

Some Problems

Discriminating between opportunistic predation and strict territorial behavior is still somewhat of a problem (see Darwin's Predation Hypothesis), but could be facilitated by establishing spatial relationships among conspecific and allospecific colonies relative to the available food supply and the nutrition requirements of a colony. At what distance will another conspecific or closely-related allospecific colony be tolerated? Does an increase/decrease in appropriate food supply affect the distance at which a colony is tolerated? If a colony that is not tolerated is continually brood depleted (for a period long enough that raiders would learn there is no brood), will raids continue on that colony? What effect does brood supply within a predator's nest or slave-maker's nest have on the frequency of raiding (Buschinger *et al.* 1980; Hölldobler 1982; Topoff *et al.* 1985) and is the frequency influenced by the species (conspecific or allospecific) of brood in the nest? If raiding is a function of intolerance for other conspecific and allospecific colonies, then slave-maker colonies well supplied with food should theoretically continue to raid nests invading slave-maker "space" even if they provide no reward.

Independent Invasion/Olfactory Imprinting Hypothesis

Topoff (1990) and Topoff and Zimmerli (1993) as well as others have observed that the hypotheses described above (predation, transport, territorial) do not adequately account for the dependent mode by which slave-makers (at least in Formicini) establish new mixed species colonies. They proposed an alternative scenario that nicely negates the need for an independent mechanism to account for brood raiding by making the first evolutionary step to slavery the usurpation of host colonies by newly mated queens (Fig. 5.4). Accordingly, pre-parasitic queens invade a nest of a closely related species, eventually killing the host queen and usurping the colony. Her offspring imprints onto the odor of host workers, and when pre-parasite workers encounter a colony of

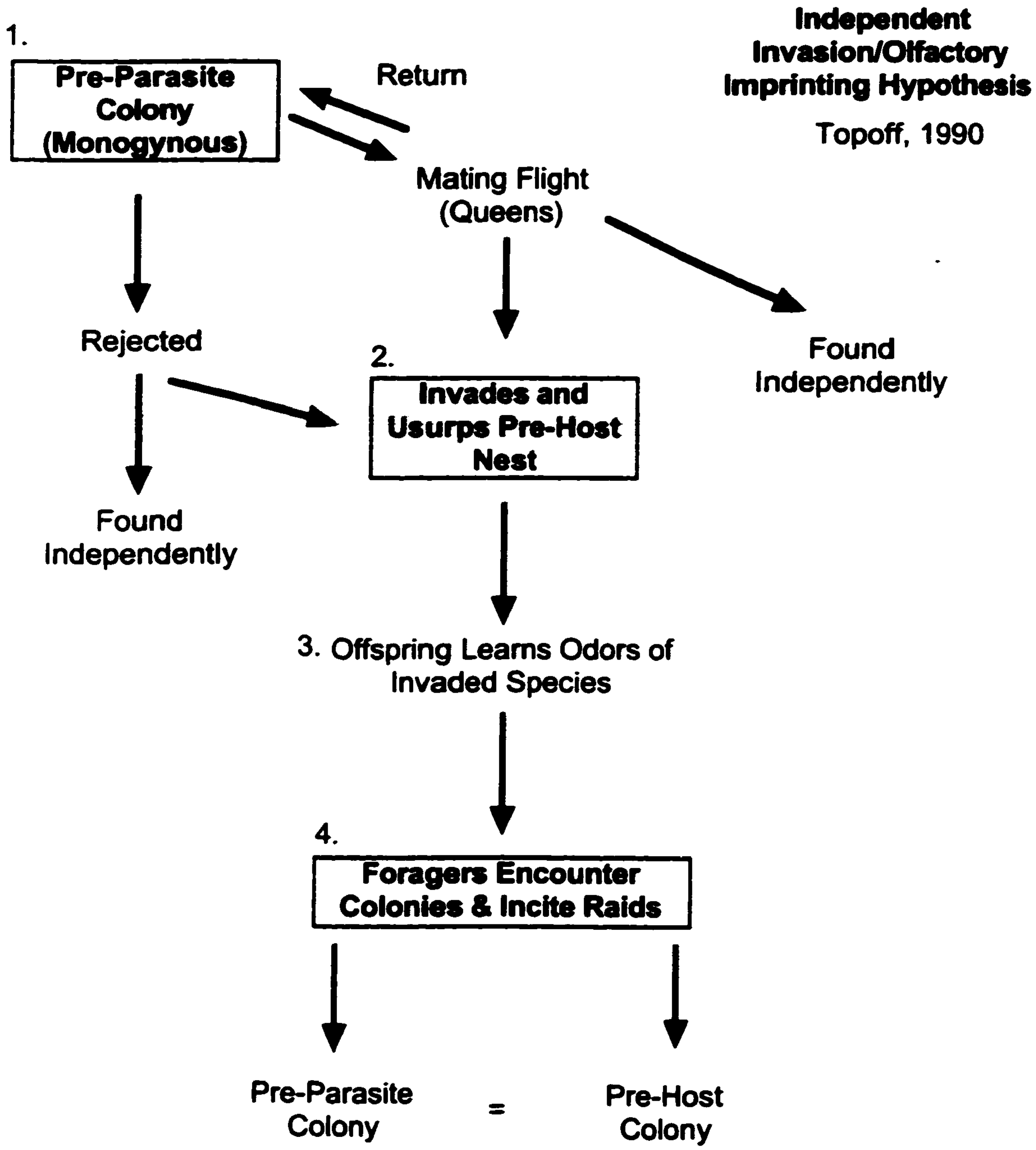

Figure 5.4. Newly mated pre-parasite queens independently invade and usurp pre-host nests. Interspecific bonds are formed between pre-host species and pre-parasite offspring through imprinting. Pre-parasite species incite territorial raids against pre-host nests in addition to conspecific nests, as pre-host species are now "perceived" as conspecific.

the pre-parasite or pre-host species in the field during foraging forays, a territorial raid is incited because both species (pre-parasite and pre-host) are regarded as conspecific. If the spared pupae provide greater benefits as slaves than as food source, the frequency of slavery spreads throughout the population.

Raiding Fidelity

This model is appealing because it accounts for the territorial reactions to the now-host species being raided, in addition to the expected territorial reactions toward conspecific colonies. Furthermore imprinting would also explain the fidelity to the species that rears *Polyergus* in raiding and usurping among potential host species. All species of *Polyergus* have only one species of host in their nest, although more than one potential host species is often available. In *Polyergus lucidus* Mayr, which nests among 4 potential host species of *Formica*, Goodloe *et al.* (1987) demonstrated that the particular species found in the mixed nest is maintained by raiding nests of only that species and not by raiding nests of numerous *Formica* species and then selectively culling captured brood. The same selectivity was observed when *P. lucidus* queens were given a choice between two species of potential host *Formica* queens (Goodloe & Sanwald 1985). The species of *Formica* queen attacked by the *P. lucidus* queens was the same species of *Formica* found in the natal nest, whereas the alternative species rarely elicited any aggressive behavior. *Polyergus breviceps* queens were found to be slightly less discriminating, sometimes attacking the queen of the alternative *Formica* host species (*F. gnava*, *F. occulta*), although this was clearly atypical (Topoff & Zimmerli 1993). These findings indicate that *Polyergus* territorial reactions towards a particular *Formica* species seem to be a consequence of early experience with the species of *Formica* found within the *Polyergus* nest (Topoff 1990; Topoff & Zimmerli 1993; Mori *et al.* 1995). The ultimate test of course would involve cross-fostering *Polyergus* brood with other *Formica* species or even with other genera in Formicini to determine whether they would incite raids against nests of the same species in their foster nests. In *Harpagoxenus sublaevis*, the queens seem to have a genetically fixed preference for the species of host nest they choose to invade, but their choice can be moderately influenced by experience with the species they are reared with (Buschinger 1991).

Independent Invasion

Although some have argued that invading a colony alone is precarious (Buschinger 1986), some species of slave-maker queens do usurp nests independently. Individual newly mated queens of *Harpagoxenus, Leptothorax*, and *Protomognathus* (Buschinger *et al.* 1980; Stuart pers com) slave-makers search out a host nest to usurp after they have departed their natal nest by flight. These queens take over nests by indiscriminately throwing workers out of the nest and appropriating whatever brood was not saved by fleeing workers (*Harpagoxenus canadensis* - Stuart 1984; *H. sublaevis* - Buschinger 1968a, 1974; Viehmeyer 1921; *Protomognathus americanus* - Creighton 1927, 1929; Sturtevant 1927; Wesson, 1939). According to descriptions by Viehmeyer (1908) and Sturtevant (1927) of takeovers by *H. sublaevis* queens, the process appears similar to takeovers by queens of the slave-maker, *Formica sanguinea*: initially the workers attack the parasite queen, but she eventually kills them (*Leptothorax*) or drives them away (*Leptothorax*, *Formica*). In either instance, the parasite queen does not need to kill the host queen in order to appropriate the brood. Queens of *Protomognathus americanus* also drive workers and the queen to leave the nest by regularly nipping at them, often fatally injuring them (Sturtevant 1927). Creighton (1929) and later Wesson (1939), however, found that worker number could be a limiting factor in the ability of *P. americanus* queens to overtake a nest. Unless a colony consists of only 10 - 15 workers, a *P. americanus* queens could not defend herself against attacking workers and was unsuccessful in her usurpation attempt. *Harpagoxenus sublaevis* also appear limited in their ability to take over host colonies with a large number of workers, and often are themselves overcome by workers and killed (Buschinger 1974). In addition to this commonly used "active strategy", Stuart (1984) discovered that *H. canadensis* queens also utilize a passive strategy when taking over a nest. During passive usurpation, the parasite queen moves about the workers slowly, seizing and evicting only those who attack. Eventually the *Leptothorax* workers accept the parasite queen and ignore their own queen, which subsequently dies of starvation (Creighton 1929).

Newly mated queens of *F. wheeleri* also appear to be independent usurpers. In contrast to the more active eviction by the Formicoxenini queens, laboratory tests indicate that a newly mated *F. wheeleri* queen achieves resident abandonment of a host species nest by merely walking into the nest chamber (Topoff *et al.* 1990), allowing her to stake claim to the forsaken brood. Unlike other Formicini that are obligate slave-makers, newly mated *F. wheeleri* queens are frequently found crawling on the ground alone, but are rarely, if ever, seen following raids in the Chiricahua mountains of Coronado National Forest in Arizona (Topoff *et al.* 1990; personal observation). Thus, it seems that they may use independent invasion techniques exclusively.

Host Nest Location

Topoff (1990) formulated the Independent Invasion/Olfactory Imprinting hypothesis based on observations of *Polyergus*, but evidence that *Polyergus* can or could successfully find and invade a *Formica* nest independently is lacking. *Polyergus* queens typically find host nests by following homocolonial raiders after they have mated by flying *en masse* from their nest, on the ground near their nest, or while following homocolonial workers on a raid (Table 5.1). Occasionally, I have found single dealate *P. breviceps* queens crawling around alone, and others (Mori *et al.* 1994; Cool-Kwait & Topoff 1984) have reported finding lone dealate *Polyergus rufescens* Latreille and *P. lucidus* following routes of previous raids. In an attempt to determine what the fate of these lone queens might be - could they find *Formica* nests on their own? Were they using chemical cues of previous raids? - I collected newly mated *P. breviceps* queens, marked them with fluorescent paint, and released them the following day at the time they were collected, approximately 1 meter from their home nest. Of the 24 queens released, I was able to follow four for more than four hours. The other 20 queens were lost, usually within the first 40 minutes. Once placed on the ground, 86% of the 24 queens ran in a single compass direction. Those queens that were not lost ran an average of 58.29 meters at a rate of approximately 1.3 meters per minute, before they slowed their pace and began a "search/probe" phase (Fig. 5.5). In an area of about 2.5 meters in diameter, queens continually touched the ground with their antennae. Two of the four queens were within 1 meter of a *Formica gnava* nest. Although these two queens made antennal contact with *Formica* workers outside the nest, there was no obvious external change in the behaviors of the *Polyergus* queens. The *Formica* workers on the other hand increased their rate of movement and, in three instances, returned to the nest. Neither of the two queens followed the workers. Instead, all four queens eventually ceased probing and crawled under a leaf where they remained for at least one hour. Attempts to restrict movement of the queens, after they crawled under the leaf, during the night were unsuccessful, and the queens could not be re-located the following morning. Complete excavations of the two *Formica* nests, however, did not reveal any *P. breviceps* queens. Interestingly, when four alate females were released, all ran around in a small area and died within an hour, suggesting that the process of mating and/or hormones involved in dealation may also stimulate ground dispersal.

What do these findings have to offer? Certainly any implications are quite speculative. On one hand, the results of these four trials could reflect the inability of newly mated *P. breviceps* queens to find a *Formica* nest, at least during the first 6 hrs after mating. Alternatively, the two queens within 1 meter of a *Formica* nest may have located

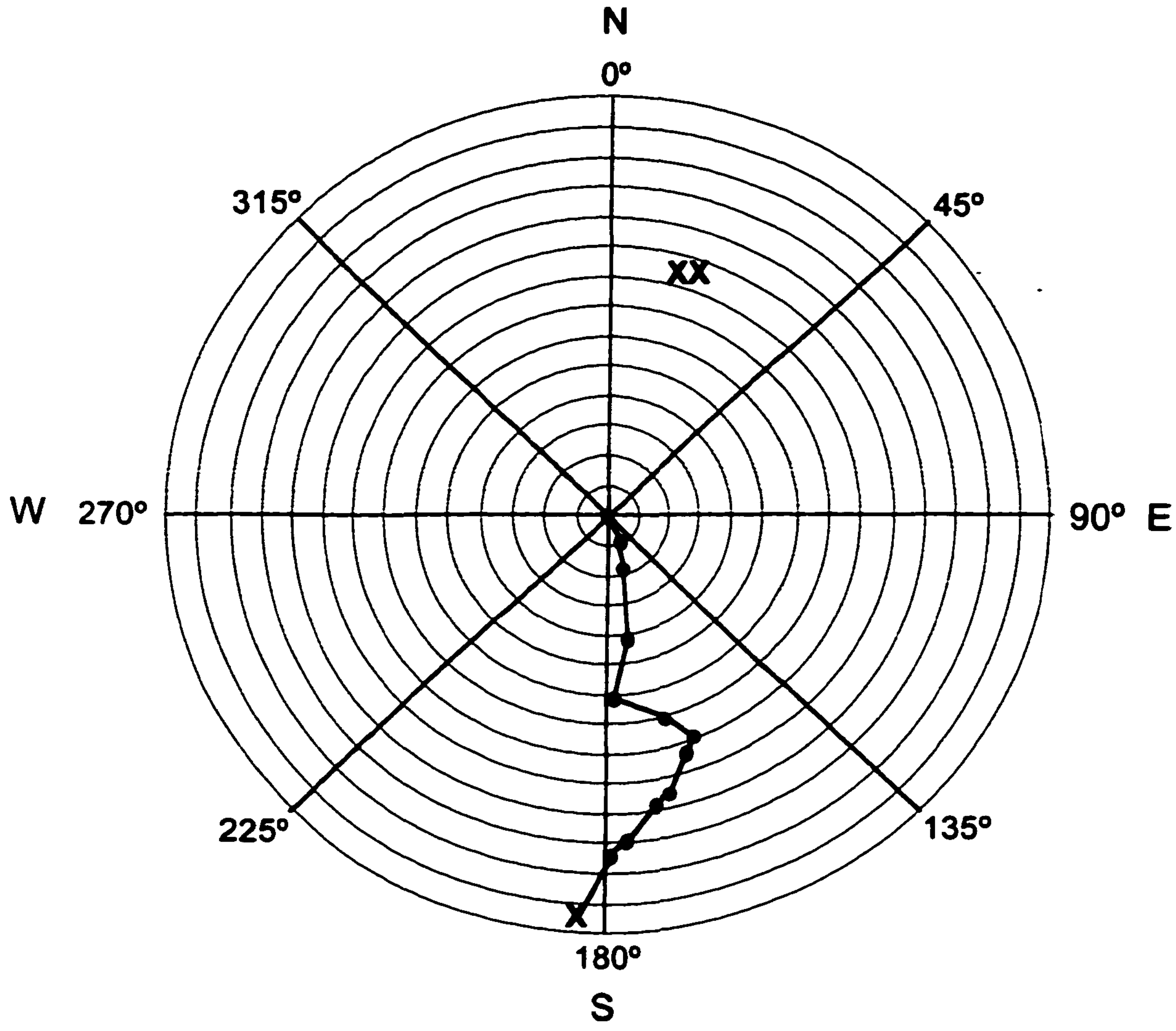

Figure 5.5. General path taken by one of the newly mated *P. breviceps* queens that was released at her home nest, 24 hrs after collection. Each concentric circle represents 5 m from the last. **X** = point where queen stopped moving; **XX** = *Formica* colony raided previous day.

the nest during the night but were greeted by aggressive, rejecting *Formica* and were unable to enter. This biphasic pattern may in fact increase a queen's chances of encountering a *Formica* nest, possibly fostering invasion. Further experiments utilizing techniques that allow for long-term tracking are necessary to establish conclusively the capability of a *Polyergus* queen to find a host nest on her own.

The running pattern probably reflects an ancestral, though not lost, dispersal mechanism after mating and appears not to currently benefit *P. breviceps* queens. Among *Leptothorax* species that are host to formicoxenini slave-makers, newly mated queens are frequently adopted back into their home nest and hibernate during their first winter. When spring arrives, however, many of the queens disperse from the colony alone or with a group of workers to found their own nests (Buschinger & Winter 1978; Alloway *et al.* 1982; Stuart 1985; Herbers & Tucker 1986; Heinze & Buschinger 1988). Aggressive behaviors by the dominant queen are often the cause of new queens leaving (Ortius 1997). If *Formica* species that are hosts to Polyergus similarly seek readoption, are rejected, and subsequently ground disperse, and if the dispersal mechanism resembles the biphasic pattern observed in *Polyergus,* another link between host and parasite behavior could be established. In fact, Rosengren & Pamilo (1983) report observing lone *Formica* queens running in a straight line along the ground.

This biphasic pattern of running and then probing is identical to the pattern observed in *P. breviceps* workers scouting for a host nest, except that the straight run and the probed area of the scout averaged 35 meters and 140 m^2 respectively (Topoff *et al.* 1987). The formicine *Cataglyphis bicolor* (Fabricius) also exhibits a similar biphasic pattern during foraging (Harkness & Maroudas 1985). It would be interesting to ascertain whether *C. bicolor* queens, which are not social parasites, disperse in a similar manner.

Some Problems

Assuming that this running pattern by *P. breviceps* queens reflects the ability to find a *Formica* nest, this unfortunately does not provide information on the ability of queens to usurp a *Formica* nest independently. According to laboratory observations, colonies of *Polyergus* are founded when newly mated queens enter a *Formica* nest that has just been raided by their non-reproductive nestmates, and fatally attack the resident queen (Wheeler 1906; Topoff *et al.* 1988). *Formica* workers, which typically assault any invading *Polyergus* queen, immediately begin grooming her after she ceases attacking a *Formica* queen of their (the workers) species. Although *Formica* workers may adopt *Polyergus* queens that have not attacked a host queen (85.7% in queenless or queenright colonies of *P. rufescens* - [Mori *et al.* 1995]), laboratory studies suggest that aggressive attacks on host queens seem to be commonplace and necessary. Aggression by *Formica* workers against *P. breviceps* queens observed in the laboratory tests is intense. Even during raids, large colonies of *Formica* can stage counterattacks against the more formidable *Polyergus* workers (Topoff *et al.* 1985). Adoption of a *Polyergus* queen by *Formica* workers increases dramatically when the *Polyergus* queen has killed a *Formica* queen that is the same species as the adopting workers (from 12% to 79% of tests - [Topoff *et al.* 1988]; 38% to 54% - [Topoff *et al.* 1990]; Topoff & Zimmerli 1993; Zaayer 1967) or when the queen of the host species has been removed for a period of time (Zaayer 1967). Queens that are denied the opportunity of killing a host queen, tend to be killed by host workers (e.g., D'Ettorre *et al.* 1997; Topoff *et al.* 1988). And even when *Formica* queens are available, invading *Polyergus* queens are not always successful at taking over a nest (Topoff *et al.* 1988; Topoff *et al.* 1990; Topoff & Zimmerli 1993), especially when experimental setups include a second chamber where host species can "escape" (D'Ettorre *et al.* 1997). Excavations of two *Formica* field nests in which newly mated *P. breviceps* queens were seen entering during a raid produced no live *P. breviceps* queens and numerous dead *P. breviceps* queens (*M* = 7) (unpublished data). Thus, while it seems that slave-maker queens in Formicoxenini and *F. wheeleri* queens are able to find and invade a host species colony alone, an alternative scenario is likely for *Polyergus*.

Chance Encounter Hypothesis

After mating on mass nuptial flights (*P. lucidus*, *P. rufescens*, *P. samurai* Yano), on the ground near their nest (*P. rufescens*) or while following homocolonial workers on a raid (*P. rufescens*, *P. breviceps*), newly mated *Polyergus* queens often return to their home nest (or sometimes an unrelated nest) and follow homocolonial workers advancing towards a *Formica* nest. The queens enter the raided nest and attempt to take it over (Creighton 1950; Talbot 1968; Marlin 1968, 1971; Cool-Kwait & Topoff 1984; Goodloe & Sanwald 1985; Topoff & Greenberg 1988; Topoff *et al.* 1985; Topoff *et al.* 1988; Mori *et al.* 1991; Hasegawa & Yamaguchi 1994; Mori *et al.* 1994). The use of slave raids by slave-maker queens to mate and to find a host nest to invade is commonly assumed to be an adaptive, evolved alternative to mating flights and independent invasion of host colonies (Talbot 1968; Topoff & Greenberg 1988; Topoff 1990; Topoff & Mendez 1990; Mori *et al.* 1995). Utilizing slave raids, however, requires that queens either return to a parasite nest after a mating flight or forgo mating flights altogether, traits which are currently found in at least six of the slave-maker host species (Table 5.1). Thus, the act of returning to a nest ("boomerang behavior") could be considered a retained behavior in *Polyergus* slave-maker (but lost in Formicoxenini) and most likely pre-dates nest invasion in this group, significantly weakening the above assumption. Instead, the ancestral trait might have contributed to the evolution of nest invasion from pre-existing slave (or territorial) raiding behavior.

Newly mated queens that return to their natal colony find themselves in a physically uncharacteristic position of the nest with respect to "normal" colony structure. Rather than being among brood tenders and nurse workers deep within the nest, they are above ground, outside the nest among the class of foragers and nest defenders (and/or patrollers) that frequently recruit other foragers to food and other defenders to territorial intruders. Returning newly mated queens, thus, are at risk of exposure to recruitment signals and, if vulnerable (receptive), may be induced into performing atypical behaviors. Assuming that slave-raiding evolved from territorial interactions between conspecific colonies and between colonies of closely related allospecifics, non-independent colony founding in *Polyergus* may be derived from newly mated queens intercepting signals recruiting nestmates to a slave raid/ territorial interaction (Fig. 5.6).

Hypothetical Scenario

Foraging/territorial encounters, mating flights, and possibly nest emigrations overlap temporally and seasonally. Newly mated queens return to their home nest and attempt to enter while foragers and patrollers are coming and going. They are forcibly rejected, but remain in the vicinity of the nest for an undetermined amount of time before setting off to found a colony independently. A forager/patroller returns home after encountering a territory invader and incites nestmates to raid. The group of workers near the entrance of the nest detects the signal and responds. If signals recruiting to a new nest site resemble signals recruiting to a territory invader, queens on the nest exterior (newly mated queens that have returned and/or unmated queens waiting to fly) also respond and accompany the raiders to the intruding nest. But because queens differ from other caste members (physiologically, physically, and behaviorally), once they reach the raided.nest, they may respond differently to the available cues from non-reproductive raiders. Instead of entering the raided nest, picking up pupae and larvae, and returning home like the territorial raiders, queens enter and are "drawn" to the remaining brood pile (e.g.,

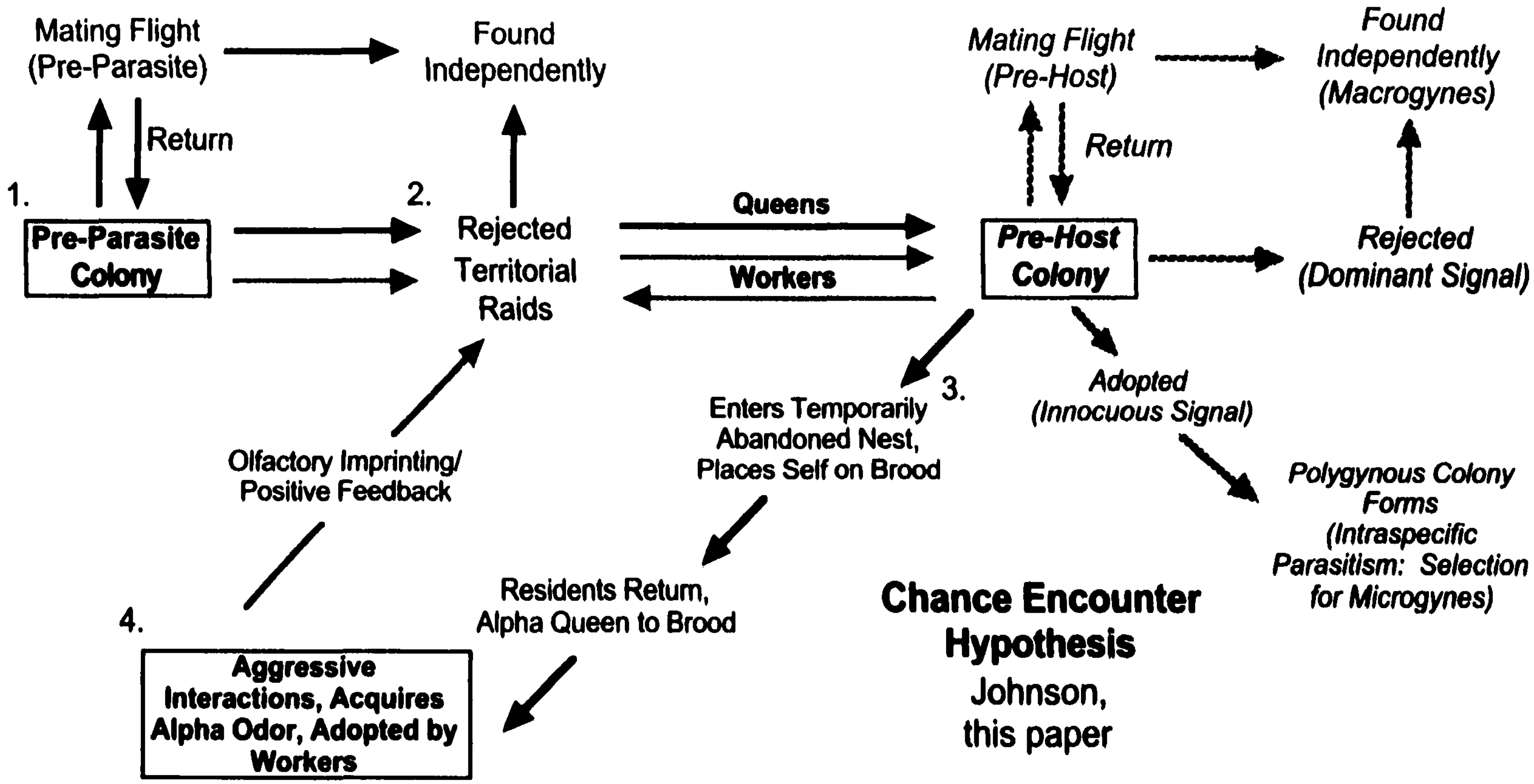

Figure 5.6. Pre-parasite queens return to nest after mating flight. Strictly monogynous, their colonies reject them. Recruitment to a territorial/slave raid, however, coincides with their return. Recruitment signal resembles signal for emigration. Newly mated queens respond, follow raiders, and enter temporarily abandoned *Formica* nest as if new nest site. Raiders return home with pillaged brood (signal not mistaken); *Formica* residents return to their nest, the resident alpha queen goes to remaining brood pile. Pre-parasite queen and alpha resident queen fight, host odor is acquired, and pre-parasite queen is adopted. Offspring imprint onto Formica, strengthening allospecific raiding tendencies.

H. sublaevis - [Sturtevant 1927]; *P. americanus* - [Creighton 1929]; *P. breviceps* - [Topoff *et al.* 1988]). Responding as many inseminated female reproductives do, they place themselves on top of the brood pile.

Requirement 1: *Overlap of mating flights and slave (territorial) raids*

If newly mated queens are to respond to a signal recruiting nestmates to a territorial raid, they must be exposed to signalers. It is therefore essential that mating flights (or the time period during which queens return to the nest) and slave (territorial) raids overlap in time. The daily hours during which raids, mating flights, and emigrations for slave-makers occur are presented in Table 5.2, along with whether they overlap seasonally. In all species of Formicini and in *H. sublaevis* and *P. americanus* mating occurs at some point during the raiding season, and daily raiding times coincide with the times during which alates are either preparing to fly or returning from flight. Nuptial gatherings of *Polyergus* may be concentrated into a few days (*P. lucidus* - Marlin 1971; Talbot 1968; *P. rufescens* - Mori *et al.* 1991; *P. samurai* - Hasegawa & Yamaguchi 1994) or reproductives emerge in smaller group numbers over a period of 40 days or more (*P. breviceps* - unpublished data Johnson) during the raiding season. Newly mated *P. rufescens* queens participate on mass mating flights much earlier than the average onset time for raids, but they return to the nest and are present when raids occur (Mori *et al.* 1991). *Polyergus lucidus* queens also participate on mass flights much earlier than the time when raiding starts, but appear at the mixed nest often 2 hours before raiding begins and continue to arrive during and after raiding (Kwait 1982; Cool-Kwait & Topoff 1984).

It is also necessary that newly mated queens are not adopted back into their colony. Consistently, all obligatory slave-makers are monogynous and there is no evidence that slave-maker queens are accepted back into a mixed nest (natal or alien), even though many of the host species commonly adopt newly mated queens (Buschinger & Alloway

Table 5.2

Slave-raid and mating flight times of slave-makers in Formicini and Formicoxenini, and whether slave-raids and mating flights overlap in season

	TIME		SEASON	
	Raids	**Matings**	**Raids**	**Matings**
Harpagoxneus				
canadensis	?	?	?	?
sublaevis	20:31 - 21:05	20:15 - 21:30	Y	Y
Leptothorax				
duloticus	14:35	15:30	Y	Y
Protomognathus				
americanus	Later than mating flight	Early a.m.	Y	Y
Formica				
sanguinea	15:30 - 19:00	7:00 - 11:00	Y	Y
wheeleri	12:15 - 16:12 8:04 - 14:40	12:00 - 16:00	Y	Y
Polyergus				
breviceps /gnava	15:30 -19:00	15:30 - 17:00	Y	Y
breviceps / occulta	12:00 - 16:00	12:00 - 16:00	Y	Y
lucidus	15:30 - 17:50	return 2h before/during raiding	Y	Y
nigerrimus	?	?	?	?
rufescens	16:55 - 17.27	13:30 - 14:47	Y	Y
samurai	13:00 - 16:30	10:00 - 11:30	Y	Y

Y = overlap, ? = unknown

1978; Rissing & Pollock 1988; Stuart *et al.* 1993). Host and sometimes parasite workers of mixed species nests attack and drag away slave-maker queens attempting to enter (Talbot 1968; Goodloe & Sanwald 1985; Hasegawa & Yamaguchi 1994; Topoff *et al.* 1985; Topoff 1990; Mori *et al.* 1994). Dealate *P. lucidus* queens following homocolonial workers on a raid will also elicit aggression from raiders (Talbot 1968; Cool-Kwait & Topoff 1984). In colonies of *H. sublaevis*, younger queens are driven from the natal nest by an aggressive older queen, even if the younger queens have not yet mated (Buschinger 1974). Mori *et al.* (1994) and Cool-Kwait and Topoff (1984) have independently observed that rejected newly mated *P. rufescens* and *P. lucidus* queens will sometimes "wait" for several days in the vicinity of the mixed nest for a raid. These dealates may have attempted reintegration among former nestmates but were rejected.

The overlap with raiding naturally implies my assumption that raids (territorial or slave) precede nest invasion and non-independent colony founding by queens. Territorial behavior *per se* (as compared to predation - see Predatory Hypothesis) is difficult to demonstrate conclusively in slave-makers. We may, however, presume slave-makers to be guilty (of territorial behavior) based on their close phylogenetic association with 1) some of their host species (*L. muscorum* - Stuart & Alloway 1982, 1983), 2) facultative slave-makers (*F. sanguinea* - [Möglich & Hölldobler 1974; Levings & Traniello 1981]) and 3) other generic sister taxa (*Formica polyctena* - [Möglich & Hölldobler 1974]; monodomous populations of *Formica lugubris*, *Formica pratensis* Retzius, *Formica rufa* Linnaeus - [Levings & Traniello 1981]) that are territorial (see Territorial Hypothesis and Independent Invasion/Olfactory Imprinting Hypothesis), and accept slave raids as evolved manifestations of territorial defense.

Requirement 2: *Similarity of different recruitment signals*

It is not uncommon for different behavioral sequences with similar behavioral components to be induced by chemical and/or behavioral signals that vary little, if at all. Subsequent behaviors may then be determined by the context in which the signal was received (where the individual is: outside the nest, near the nest surface, deep within the nest), the amount of signal received (which could be influenced by where the receiver is relative to the emitter), if the signal is received in conjunction with other signals, or the receptive abilities of the individual involved (individual or between caste differences) (Hölldobler & Wilson 1990; e.g., Cassill & Tschinkel 1999). Hence, an individual may or may not respond to a signal based merely on proximity to the emitter and not because the signal constituents differ. If signals are specified based on proximity (absolute amounts sensed), they may be subject to misinterpretation. This could be the case for returning newly mated *Polyergus* queens. Recruitment of "established" queens during nest emigrations (personal observation) but not to territorial events could suggest that the respective recruitment signals differ. However the fact that newly mated *Polyergus* queens follow nestmates raiding clearly indicates responsiveness to signals recruiting to raids and suggests that raiding signals may resemble emigration signals. It also suggests that queens may not typically respond to raiding signals when within the nest, because they are not exposed to the signal (proximity to emitter). Thus, a circumstance in which raiders and non-raiders are coincidentally exposed to a generalized recruitment signal can result in the induction of a similar initial sequence of behaviors. If encounters with additional stimuli, such as brood, solicit different responses, the terminal behaviors will differ respectively.

Among Formicoxenini, active female reproductives concomitant with raiders also revealed an equivocal function of a recruitment signal, although it does not appear to be involved in the evolution of nest invasion. The sex pheromone of *H. sublaevis* females, a secretion from the poison gland (Buschinger & Alloway 1979), also appears to act as the recruiting pheromone used by workers to recruit to a raid (Buschinger 1983), and can stimulate *H. canadensis* males to approach (or vice versa). Copulation between species is prevented by aggression from female *H. sublaevis* against approaching male *H. canadensis*, and by female *H. canadensis* fleeing approaching male *H. sublaevis*.

Group Recruitment Versus Tandem Running

In addition to the similarity of signals recruiting to different events, it seems that the type of recruitment signal may also be significant in contributing to the susceptibility of mistaking a signal (Fig. 5.7). All Formicini slave-makers (Buschinger *et al.* 1980; Talbot 1967; Topoff *et al.* 1985; Mori *et al.* 1991) and two species of Formicoxenini slave-makers, *Leptothorax duloticus* Wesson and *Protomognathus americanus* (Wesson 1939), use group or mass recruitment, a method in which the recruiter signals to numerous individuals simultaneously (Hölldobler & Wilson 1990). Of the group/mass recruiters, those species in which mating flights overlap in season and time with raiding, all have queens that use slave raids to invade nests. The group recruiters *Leptothorax duloticus* and *P. americanus* do not use slave raids for invasion, but they also do not return to their nest after mating. *Protomognathus americanus* do, however, form branch colonies (Wesson 1939). Branch colonies consist of raiders that remain in the nest raided forming queenless, relatively defunct colony (except for the production of males and the occasional female that eclosed), rather than returning to their home nest (Buschinger & Alloway 1977). They differ from bud emigrations in that budding involves the exodus of a queen and a group of workers from a polygynous colony, which form viable colonies. Some speculate that the function of branch colonies may be to provide surplus males or to extend the range of activities of the primary colony (Buschinger & Alloway 1977). They do not appear to result from overcrowding of the primary colony (Wesson 1939) and are seen as a product of slave-raiding at the end of the season (Wesson 1939).

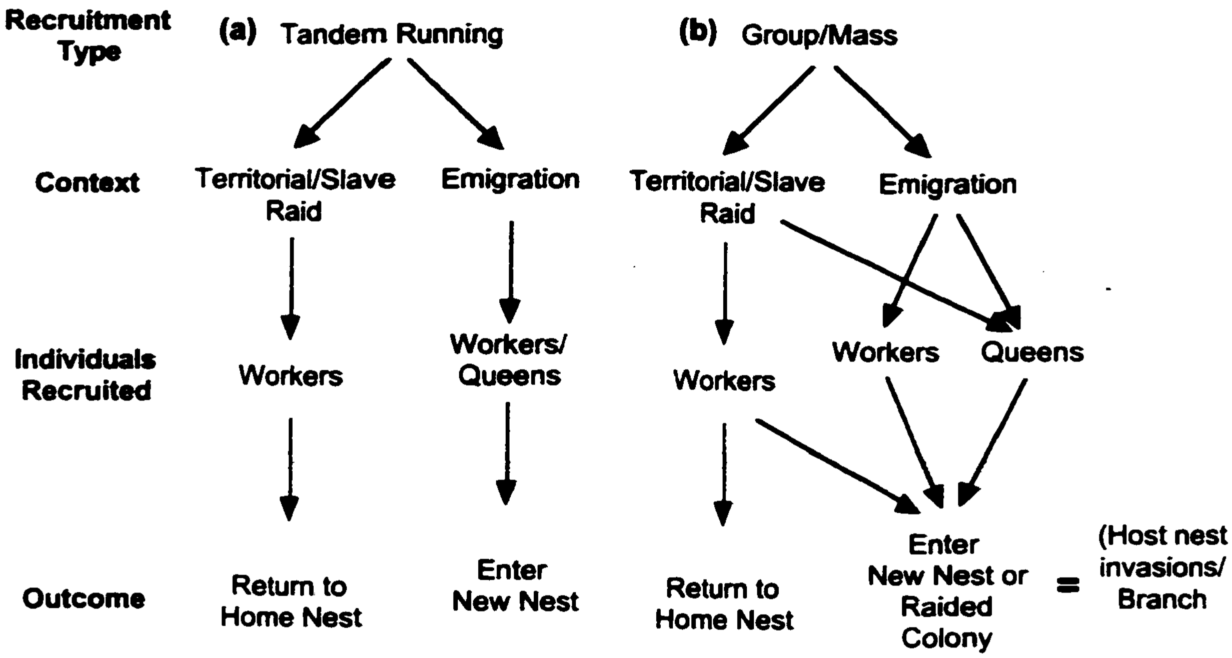

Figure 5.7. The type of signal used in recruitment may correctly lead individuals to an intended target or, if received by unintended receivers, may also incorrectly lead individuals to an intended target. **(a)** Tandem running involves an intimate one-on-one interaction that requires almost continual contact between recruiter and recruitee. An individual following a recruiter most likely will not mistake the signal nor be led to an inappropriate target. **(b)** Group and mass recruitment signals on the other hand induce response in several to many individuals simultaneously. The specificity of the signal is obtained by additional information, leaving an opportunity for error. For example, queens exposed to a signal recruiting to a raid may respond to the signal if the signal resembles the signal for emigration. However the target they are recruited to is not a new nest site but a territory invader. Because queens are not raiders, their response at the target is to enter the nest as during emigration. Invasion of a raided nest by *Polyergus* and other slave-makers may reflect this. Similarly, workers that mistake the signal for emigration will also respond at the target nest as they would during emigration. The formation of branch colonies in *Protomognathus americanus* may be evidence of a such a mistaken signal.

Of the species of slave-makers and hosts that use tandem running as primary means to recruit nestmates, there is no evidence of branch colonies (Stuart & Alloway 1983) and queens, whose mating flights overlap in time with raiding, do not invaded raided nests. Why? Tandem running, which functions in Formicoxenini to recruit nestmates to food (Möglich *et al.* 1974), new nest sites (Möglich 1978), territory defense (Stuart & Alloway 1982, 1983), and slave raids (Buschinger *et al.* 1980; Stuart & Alloway 1983), involves a relatively intimate one-on-one interaction. The leader recruits an individual nestmate who in order to be recruited must continually maintain antennal contact with the leader or remain in close proximity relative to the distance at which more volatile pheromones can be detected (Wilson 1971). When *L. duloticus* slave-raiders and slaves lose touch with the leader, they return home to their nest (Wesson 1940). In comparison with group and mass recruitment, a recruit of tandem running is less likely to mistake the "purpose" of the recruitment signal because the follower must be led all the way to the target and because the recruiter is unlikely to recruit an inappropriate individual, such as a queen. Signals from group and mass recruiters, however, are able to reach inappropriate individuals, and thus inappropriate targets, because of the signals effect on the group. And, more "mistakes" in group- and mass-recruiters are expected, especially if there is a potential for inappropriate individuals to be exposed to such a signal. Branch colonies or invasion of raided nests by newly mated queens may be the errors that result from inappropriate exposure to recruitment signals.

Recruitment by host species of slave-makers to food (Buschinger & Winter 1977) or by slave-makers of host species to slave raids (Wesson 1940; Alloway 1979; Winter 1979; Stuart & Alloway 1982, 1983) using the tandem running methods may arguably also be mistakes. However, the mistakes are limited in magnitude because of the nature of tandem running recruitment. First, a slave-maker that is recruited to food by a slave will not respond to the target by recruiting other slave-makers or slaves (Buschinger & Winter

1977), and the mistake leads nowhere. Second, a slave that is recruited to a slave-raid will respond as it would against a territory invader. Third, discrimination between caste members that are recruited is retained; queens are not actively recruited to either food or territorial interaction.

In sum, non-independent colony founding could have evolved in *Polyergus* because newly mated queens, upon returning to their nests, encounter nestmates recruiting *en masse* to a territory invader using a signal that potentially resembles the signal to emigrate.

This hypothesis is attractive because it provides a logical alternative to single queens entering a colony by happenstance and being able to overtake the colonies with such success that the behavior becomes the norm. It may be argued that the ability of slave-making queens in Formicoxenini to independently invade nests of other species is evidence to the contrary. However, differences in general ecology between the two groups suggest that, although territorial raiding probably preceded slave-raiding, the pathway leading to invasion by slave-maker queens differed. First, even small nests of *Formica* have greater worker numbers than the average *Leptothorax* nest, and can be extremely aggressive (even towards researchers digging them up) making invasion of *Formica* nests by lone queens unlikely. Larger *Leptothorax* nests have also proven to be difficult for at least *P. americanus* queens to usurp (Wesson 1939; Creighton 1929), and this may also be true for other formicoxenine slave-makers. Second, nests of *Leptothorax* tend to be clumped (Heinze *et al.* 1996; Foitzik & Heinze 1998) with distances between nests significantly shorter than between nests of *Formica*. Although Formicoxenini slave-makers are substantially smaller and slower than Formicini slave-makers, the greater distance a formicine queen would need to travel to locate a nest would increase her chances of encountering predators and decrease her chances of finding a nest, if not survival. In fact, limitations on the availability of nest sites may even lead to nest usurpation and temporary intraspecific parasitism in the non-parasitic *Leptothorax nylanderi* (Foerster) (Foitzik & Heinze 1998).

The overlap in time of mating and raiding, in conjunction with vulnerability to recruitment signals, on the other hand, is all that is needed to provide *Polyergus* queens with an edge over the invaded colony. An invading queen is easily placed in a situation that provides the necessary opportunities for full integration among host species (see *Power Seat* below). Once usurpation takes place, the propensity to raid host species increases as slave-maker offspring experience the odor of the host species and treat alien colonies as conspecific (Topoff 1990).

Power Seat

Newly mated queens have entered the raided nest from which the residents have bolted (Topoff *et al.* 1985) as if they were entering a nest during emigration and have most likely placed themselves on the brood pile. Although observations underground have not yet been made, in laboratory tests, soon after the death of the host queen, *Polyergus* queens are found sitting on the brood pile (Topoff *et al.* 1988; Topoff 1993). If residents of the raided nest did not return, queens could fight amongst themselves for reproductive rights. Residents, however, do return and newly mated queens are attacked. But, because the pre-parasite queen is deeper within the nest among the brood, her chances of withstanding attacks may be greater than if approaching a fully occupied nest. First, workers returning to the brood pile are probably brood and nurse workers, the less formidable and less aggressive members of the colony. The pre-parasite queen is presumably more able to defend herself against brood tenders than against nest defenders, and this might allow her either to be adopted by *Formica* workers or to proceed with other behaviors that facilitate adoption. Second, she will most assuredly encounter the resident queen who is also likely to return to the brood pile. Encounters with resident queens may result in aggressive interactions, particularly if the resident queen attempts to defend her brood. Aggressive interactions, in turn, may result in the death of one or the other queen. If the parasite is killed, the *Formica* colony will continue to grow as a *Formica* colony. Elimination of the resident queen, however, will present a new set of possibilities that may lead to takeover by the slave-maker queen. When resident queens are experimentally removed from nests, aggressive tendencies of workers tend to diminish (Vander Meer personal communication) possibly allowing the slave-maker queen to eventually be adopted. *Formica fusca* workers were shown to adopt a *Polyergus rufescens* queen after the resident *F. fusca* queen had been removed from the nest for a period of time (Zaayer 1967). Similarly, reduction in worker aggression may have contributed to the successful adoptions of *P. rufescens* queens, as Mori *et al.* (1995) placed test workers in their queenless condition 48 hours prior to testing. On the other hand, the lipid layer of the cuticle is prone to absorbing other lipid soluble compounds (Soroker *et al.* 1994, 1995; Vienne *et al.* 1995). If the pre-parasite queen acquires chemicals superficially from the resident queen during fighting, she may be sufficiently camouflaged or attractive to be adopted by workers of the queen (Topoff & Zimmerli 1993; D'Ettorre *et al.* 1998).

Chemical Camouflage

Currently, established *Formica* queens are the recipients of fatal attacks by newly mated *Polyergus* queens attempting to takeover a host nest (Wheeler 1906; Topoff *et al.* 1988). Although Mori *et al.* (1995) demonstrated that host workers adopted *P. rufescens* queens that had not killed a host queen, the killing process appears to be a necessary component in colony takeovers. Aggressive interactions seem to endow *Polyergus* queens with chemicals from the *Formica* queen that increase her chances of being adopted by adult *Formica* workers (Zaayer 1967; Topoff *et al.* 1988; Topoff & Zimmerli 1993). The rate of success increases dramatically when a *Formica* queen of the same species as the adopting workers has been killed (Topoff *et al.* 1988; Topoff & Zimmerli 1993; Topoff *et al.* 1990).

D'Ettorre *et al.* (1998) examined the cuticular hydrocarbons from queens of the European slave-maker *P. rufescens*. They found that few chemical peaks comprised the newly dealate profiles, but the profiles of queens that had attacked a *Formica cunicularia* queen were more complex and resembled the profiles of *F. cunicularia* queens. Analysis of cuticular hydrocarbons from *P. breviceps* queens revealed similar findings (see Chapter 2). I was further able to demonstrate that the change in profile of *P. breviceps* queens was specific to the species of queen killed, because hydrocarbons from two species of host queens and from *P. breviceps* queens that attacked the two host species queens were examined as well. Hydrocarbon profiles of newly mated *P. breviceps* queens were simple (Figs. 2.1a & 2.2a), whereas profiles of killer queens changed qualitatively and contained peaks found in the queen profiles of the respective *Formica* species killed (Figs. 2.1b,c & 2.2b,c). Principal component analysis on cuticular hydrocarbon extracts revealed that *P. breviceps* queens clustered with the species of queen killed. This specific change in profiles suggests that chemicals of the *Formica* queen are indeed absorbed by the slave-maker. In combination with previous behavioral data (Topoff *et al.* 1988; Topoff *et al.* 1990) this suggests that the chemical change may increase the slave-maker queen's attractiveness to *Formica* workers, allowing her adoption. This is further supported by preliminary examinations of cuticular hydrocarbon profiles from *F. wheeleri* queens. Queens of *F. wheeleri,* a facultative slave-maker that also uses *F. occulta* as their host species, do not kill the host queen when taking over a nest. Instead they scatter all adults and appropriate the abandoned brood. The profiles of newly mated *F. wheeleri* (Fig. 2.3a) do not resemble the profiles of either newly mated *F. occulta* queens nor established *F. occulta* queens (Fig. 2.3c), even after living with *F. occulta* for a year (Fig. 2.3b). Considering that a *F. wheeleri* queen usurps brood and not adults, it is unnecessary for her to resemble the slave species queen since newly emerged workers will learn her odor.

Attacks on Host Queens

Selection for aggressive reactions by *Polyergus* to established *Formica* queens was probably driven by primordial interactions involving established *Formica* queens versus unmated queens or workers as a consequence of the position of a pre-parasite queen in a recently raided nest. This is supported by the fact that attacks are directed only at established *Formica* queens (see Chapter 1 & Transport Hypothesis, this chapter). While *Polyergus* queens could have run the gamut, attacking workers, alates, or newly mated queens before finally finding success in attacking established queens, this progression, given the situation, is somewhat unparsimonious. Nonetheless, camouflage of the slave-maker queen with chemicals from the *Formica* queen during attack, does not suffice as an explanation for the tendency of naïve adult allospecific workers to care for slave-maker offspring.

Brood Care

It is often assumed that slave-maker offspring will be reared by host workers after the slave-maker queen has successfully taken over a nest. Some have suggested that the slave-makers (*Protomognathus americanus*) themselves contribute to the degree with which their host species accept allospecific pupae (Alloway 1982). Others have suggested that pupae and larvae of slave-making species are attractive to their slave species, soliciting brood rearing behaviors from heterospecific workers (Alloway & Hare

1989; Zimmerli & Mori 1993). Conversely, social experience appears to be fundamental in the adoption of slave-maker pupae by some of the host species (Le Moli & Mori 1987).

To ascertain whether conserved chemical characteristics or generalized brood-tending pheromones are involved in the adoption of slave-maker pupae, I presented pupae of *P. breviceps* and pupae of two of their host species (*F. gnava* and *F. occulta*) to groups of free-living and enslaved *F. gnava* and *F. occulta* workers. The numbers of pupae that were found alive inside the nests were counted daily for 10 days (Fig. 3.5). I found that neither conserved chemicals nor a generalized pheromone could completely explain the results I obtained. Both species of free-living *Formica* adopted almost all conspecific pupae but only a few allospecific *Formica*. On the other hand, slave-maker pupae were adopted to a greater degree by enslaved host workers than free-living workers, although free-living workers did adopt more slave-maker pupae than allospecific *Formica* pupae. And, slave-maker pupae were adopted to a greater degree if they had been reared originally by workers that were conspecific to workers used in the experiments. These variable results suggested that the "odor" of slave-maker pupae was influenced by three factors: species specific (genetic) odors, a "generalized" or generic brood odor, and the host odor imposed by tending workers (Fig. 3.6). This also suggested that using pupae in adoption tests does not necessarily reflect the mechanisms involved when newly enslaved workers adopt eggs of the slave-maker queen, and to test these mechanisms appropriately, it was necessary to use eggs.

Naïve Host Workers and Slave-maker Eggs

If we assume that the energy invested in offspring increases as they mature, than we can assume that destruction of offspring at later stages of development is more costly (Bourke & Franks 1995). It might be worthwhile, therefore, for workers to accept or reject immatures at the earliest stage of development, and not to reject immatures at later stages of development. This may be the case even though the exclusive system of rejecting chemically incompatible individuals limits the probability heterospecific eggs will be found in a nest and, thus, the selection for the ability to discriminate eggs. Because the egg develops with the queen, the egg odor is likely to contain queen odors (Schal *et al.* 1998) and is, therefore, likely to be species-specific. While there may not be active selection to discriminate eggs, because eggs have species specific odors, workers may be able to discriminate eggs because eggs have species specific odors via the queen. Specificity of recognition odors may be achieved by modifications in quality or in relative proportions of chemical characteristics. The increased complexity of a specific profile may make the receiver ultimately prone to errors in sensation and related offspring may sometimes be misidentified and destroyed. To reduce incorrect destruction of immatures as they develop, there may be selection for odors that are less likely to be rejected - a generalized or generic brood rearing pheromone - which may eventually be lost once newly eclosed individuals are integrated into the adult colony and mobility again necessitates specificity.

To determine whether host workers would adopt eggs of slave making queens, I presented groups of 35 *Formica* workers, free-living and newly enslaved, with clumps (5 - 8) of conspecific eggs, allospecific *Formica* eggs, or *P. breviceps* eggs associated with a host species either conspecific or heterospecific to experimental workers. Nests were censused daily for the presence or absence of egg clumps, and once eggs had developed into larvae, the number of immatures alive were counted until they reached callowhood. Free-living *Formica* reared only 4.8% of allospecific *Formica* eggs to adulthood, but 51% of conspecific *Formica* eggs to adulthood (Fig. 3.6). Newly enslaved *Formica* workers rejected *P. breviceps* eggs regardless of whether they were from nests with workers conspecific (2.2%) or heterospecific (0%) to workers used in tests (Fig. 3.6). Although numbers of individuals that survived declined at each stage of development, the majority of the rejection took place at the egg stage. Eggs were either left outside the nest or brought into the nest and eaten. Eventually, after approximately 5-6 months of living with a *P. breviceps* queen, newly enslaved workers began adopting *P. breviceps* eggs. By this point, however, my colonies, not provided with conditions to diapause, were very "weak" and testing ended. Cuticular hydrocarbons profiles of *F. gnava* and *F. occulta* eggs were quantitatively distinct (Fig. 4.4) and differed qualitatively from pupae profiles (Fig. 4.6). The hydrocarbon profiles of *F. gnava*, *F. occulta*, and *P. breviceps* pupae, although separated on the basis of their principal components, were all dominated by two common peaks (see Chapter 4).

The results of these behavioral tests suggest that eggs are not easily transferable across species even if they are congeneric. Adoption of parasite offspring may ultimately involve time-linked modification of worker recognition templates through continual interactions with the parasite queen. This may be due in part to differences in relative proportions of hydrocarbons. However, the rejection of pupae, even though their hydrocarbon signatures were similar, suggests that other chemicals that are physiochemically linked to hydrocarbons may be involved in nestmate recognition. The search for chemical signals that mediate nestmate recognition should include other chemicals. Although quite a body of work exists on cuticular hydrocarbons, the exact role that hydrocarbons play in nestmate recognition is nonetheless unclear. We seem to be only scratching the surface quite literally by looking at cuticular hydrocarbons.

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