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The behavioral ecology and intraspecific interactions of socially parasitic dulotic ants

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THE BEHAVIORAL ECOLOGY AND INTRASPECIFIC INTERACTIONS OF SOCIALLY PARASITIC DULOTIC ANTS

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by

Joseph R. Sapp

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ABSTRACT

THE BEHAVIORAL ECOLOGY AND INTRASPECIFIC INTERACTIONS OF SOCIALLY PARASITIC DULOTIC ANTS

Joseph Sapp

I investigate a social parasitism by examining the lives the parasite involved. I use a variety of techniques to understand the population genetics, host acquisition behaviors, and space-use of an obligate parasitic ant (*Polyergus mexicanus)* that conducts raids to steal the brood of its host species (several *Formica* spp.) and makes them “slave” workers in the parasite’s nest. I consider the interacting roles of intraspecific competition, kinship, and host specialization on each of these topics.

In Chapter 1, I use microsatellite loci to characterize the genetic structure of the parasite population at three scales: subpopulations, neighboring nests, and among nestmates. I find that there are three very distinct subpopulations that often predict host-use patterns, but not always. Neighboring nests are sometimes closely related, enabling potential kin selection. Lastly, parasite nestmates are overwhelmingly full siblings, yet there were numerous nestmates that must have come from different parents, suggesting that raiding parasites may also steal conspecific brood for enslavement.

In Chapter 2, I characterize raids from an optimal foraging perspective. I found that parasitic colonies that send more raiders further distances obtain more brood on their raids. Nests that are capable of larger raids in general had a higher success rate, and more variability in raid sizes, but not distances. These findings suggest that raids are tailored to the expected quality of the host nest target, and that larger nests have more flexibility in their raiding tactics.

In Chapter 3, I investigate intraspecific competition, aggression, and territoriality among parasites by looking at the spatial arrangement of *P. mexicanus* nests and raids relative to their conspecific neighbors and their host species. I found that nests were often overdispersed, a classic sign of intraspecific competition. However, contrary to my predictions, raids from neighboring nests that shared host species overlapped more than those that used different host species. Overall, these spatial results suggest that competition among parasites may play out at different life stages, and that the specific identity of host species might determine the nature of conspecific parasitic interactions.

This work documents the complexity and far-reaching implications of parasite-parasite interactions.

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INTRODUCTION

The topics addressed in this thesis are very different than the topics that first attracted me to the study of dulotic ants. Initially, I was interested in how the socially parasitic lifestyle of ants, in which most tasks are accomplished via a deceived host, affects reproductive conflict between queens and their daughters; and how this in turn affects colony and population sex-ratios. Though this is a fascinating topic that is more than worthy of many theses, within days of visiting Sagehen Creek Field Station (SCFS), the natural history I saw compelled my interests to change. I noticed three salient features of the ecology of *P. mexicanus* there: the high density of *P. mexicanus* nests, the great diversity of raids, and the apparent similarities of raids from the same nest. Fortunately, the questions these observations generated connected me to a more neglected ecological aspect of parasitic systems that is germane to central questions in ecology, social parasitism, and parasitism in general: the ecological interactions among parasites.

The interactions among parasites may have strong effects on the ecology of hosts and parasites, both in classic endo- and ecto-parasites, as well as social parasites (Schmid-Hempel 2011, Spottiswoode 2013). However, the ways in which intraspecific parasitic interactions affect parasitism can be complex and are understudied, especially for social parasites. The interactions between parasite ecology (e.g. competition), genetic structure (e.g. relatedness), and host ecology can all affect fundamental aspects of the parasitism, e.g. virulence. The central aim of this thesis is to understand the interactions and mechanisms of host exploitation in parasitic ants (*Polyergus mexicanus*) that steal nests and workers from their host (several species of ant in the genus *Formica*). I do this by focusing on features of the parasite's ecology and intraspecific interactions of parasites. I examine the genetic relationships of these parasitic ants with their own nestmates and with potential conspecific rivals for the hosts they exploit. I connect their genetic population structure to their use of three distinct host species. I then assess the connections between the raids they use to capture their hosts, the success of those raids, the condition of the raiding parasitic nest, and the environment. Finally, I consider what the geography of parasitic nests and raids can tell us about the nature of intraspecific interactions in this parasite.

Dulotic ants are social parasites that exploit their host at two key stages of their life cycle. First, a newly fertilized dulotic queens starts her nest parasitically by finding suitable free-living nests of her host species and assassinating the resident host species queen. She uses chemical deception to induce the dead queen's worker daughters to take care of her, and her brood. The dulotic queen's brood will be raised into dulotic workers whose primary task is the second stage of parasitism: dulotic raids. Dulotic raids are the way a dulotic colony maintains its population of host workers. Dulotic worker scouts find neighboring free-living host species nests, and recruit hundreds of their nestmates to attack these host nests and steal larvae and pupae from them ("brood"). The stolen brood are raised by existing adult host workers in the dulotic nest to become new host workers. Raids allow a dulotic nest to sustain a sufficient number of host workers despite the absence of a host queen in the nest to produce more.

Chapter 1: genetics of dulotic ants

In this chapter, I use microsatellite loci developed by Bono et al.(2007) to understand three key features of *P. mexicanus* ecology: 1) Genetic subpopulation structure and its relationship to host specificity, 2) kinship among neighboring *P. mexicanus* nests, and 3) kinship within *P. mexicanus* nests.

The genetic structure of a population both influences and is influenced by the interactions of its members with each other and their ecological environment. Various aspects of a species' ecology and social life, such as mating preferences, mating systems, migration, among others, shape genetic population structure (Loveless and Hamrick 1984). In turn, population structure itself can have profound effects on the evolution of altruism (Keller and Ross 1998), sexual selection (Pryke et al. 2001), and sibling conflict (Caro et al. 2016), via kinship.

The importance of kinship in shaping ant societies has been recognized and rigorously studied since Hamilton's original observation that individuals should be selected to help their genes, no matter which body they occur in (Hamilton 1964a), and that the haplodiploid genetic systems of hymenopterans might make this observation especially relevant to the evolution of eusocial insect societies (Hamilton 1964b). In dulotic ants, workers from the same colony are typically full siblings (Bono et al. 2007). The genetic relatedness of interacting conspecifics may influence the level of cooperation or conflict they exhibit towards each other. I discovered that while most nestmates are likely full siblings as expected, 24% of nests have nestmates that could not have shared both parents. I think the most likely explanation for this is the capture of conspecific brood in addition to host brood during intraspecific dulotic raids, thus providing genetic evidence to support what has been observed in closely related dulotic ants(Le Moli et al. 1993).

Many studies have explored the role of relatedness within the nest, but here I also consider the effect of relatedness among neighboring nests that are potentially rivals. If nests are closely related, as queen dispersal patterns would suggest, then neighboring nests may tolerate each other's presence instead of destroying them, as I have observed directly and as has been reported in other dulotic ant populations (Topoff et al. 1984). I find that among neighboring nests, a minority of pairs of nests are close relatives, in contrast to what I would expect given queen dispersal patterns and prevailing wisdom about aggression towards neighbors. Nevertheless, the mix of neighboring nests that are close kin with neighboring nests that are not close kin means the potential exists for *P. mexicanus* nests to favor related nests in their competitive interactions.

Host specialization by parasites is a major focus of research, especially for social parasites, because it can tell us about both the co-evolutionary forces occurring between host and parasite, and the competitive forces at play among parasites. Host specialization may be associated with partial or complete genetic isolation, leading to the formation of host races (Marchetti 1998). Recent work has used genetic information to reveal the existence of host races in *Polyergus mexicanus* (Torres 2012). Here, I detect three distinct subpopulations (a number which matches the number of known host species) and contrast nests from each subpopulation with their use of one of the three *Formica* host-species to find that genetic subpopulation is related to host species use, but imperfectly: some subpopulations use multiple hosts and some hosts are used by multiple subpopulations.

Chapter 2: raid characteristics and outcomes

Ants are model systems for studying central-place foraging (Hölldobler and Wilson 1990). Because ant colonies are typically highly organized societies of closely related, highly cooperating individuals, the colony as a whole is often considered the logical unit of foraging behavior, though many excellent studies have examined the behaviors individual foraging ants as well, often linking them back to colony-level emergent properties (Greene and Gordon 2006, Robinson et al. 2009). As a group, ants are phenomenally successful foragers, due in part to the emergent evolutionary innovations of their colonies: trail maintenance, chemical communication, information sharing, and recruitment, for example. For dulotic ants, parasitic brood raids on nests of the host species are fundamentally a specialized kind of foraging trip. Because of this, dulotic ants combine aspects of foraging ecology with parasite ecology. Many studies have brilliantly elucidated how several aspects of raids work, but to date none have considered such a large population of raids from interacting or potentially interacting nests in a natural environment in the level of detail I present here.

Some of the best work linking optimal foraging concepts with parasite host acquisition comes from work on parasitoid wasps. Dulotic ant raids are likely shaped by many of the same ecological forces that influence the behavior of parasitoids: host quality, parasite condition, and environmental constraint (Vet et al. 2002). In chapter 2, I use a combination of videos and direct field observations to understand how the characteristics and outcomes of dulotic raids reflect these three forces. I create a method for efficiently estimating the size of raids and amount of brood captured from video footage and use these estimates to assess the foraging patterns of many interacting nests at two sites. I use optimal foraging theory to infer host quality by quantifying several key characteristics and the two main outcomes of raids and exploring the interactions between these characteristics and outcomes. I assess parasite condition by considering the role of two key nest characteristics (nest size and genetic subpopulation of raiding colonies as determined in Chapter 1) in influencing raid characteristics and outcomes. I build mixed models to identify which raid characteristics most influence raid quality. I find that raids are constrained by their abiotic environment and the size of the raiding nest, but still vary enormously in many of their key characteristics (size, distance) and their outcomes (success, quantity of brood in successful raids). While I am able to describe clear relationships between nest traits, raid characteristics, and raid outcomes, I suggest that much of the remaining unexplained variation in raid characteristics and outcomes is likely related to variation in host nests. However, the variation in raid outcomes for raids with similar characteristics cannot be explained by differences in host nests. Instead, I suggest that raiding parasites may have imperfect information about the host resource they intend to exploit, yet are still able to approximate optimal foraging decisions.

Chapter 3: spatial signatures of nests and raids

Competition is a prominent feature of ant ecology and competition among parasites can have strong effects on parasitic interactions. Despite this, there are only a few examples of studies that consider how parasites interact with each other, especially compared to the amount of work devoted to understanding host-parasite interactions. Intraspecific interference or exploitation competition are both likely to have strong effects on the behavior of parasites that cascade to the population and community ecology of both hosts and parasites.

In Chapter 3 I examine the spatial signatures of intraspecific interactions between parasites. Specifically, I look for spatial overdispersion of dulotic ant nests and overlap in raiding areas. Overdispersion—the regular spacing of nests (Lach et al. 2010)—is a classic indicator of territoriality in ants that is frequently linked to interference competition, but the pattern can arise for many reasons such as the pre-existing geography of suitable nest sites, resources, and exploitation competition (Adler and Gordon 2003). Interestingly, because dulotic nests are always at the site of a former host nest, the location of dulotic ant nests in the present may be both directly affected by their own social interactions, as well as indirectly affected by host decisions and interactions in the past.

Raiding areas that overlap with neighbors are rare at other sites even when nests are dense, possibly because raids are directionally biased to avoid costly confrontations with conspecifics (Bono et al. 2006). However, I frequently observed extensive overlap in our study area. This observation is puzzling given the well-documented intense conspecific aggressive interactions of neighboring dulotic ant nests (Topoff et al. 1984). One possible solution to this puzzle is the role of host-specificity: if hosts are the resource that drives competition, then parasites should only compete with conspecifics that specialize on the same host species as they do. I identified the host species used at each dulotic nest I studied and assessed the effect of host species both on nest spacing and raid area overlap.

A major challenge to interpreting overlap of raiding areas is that it is dependent on two factors: nest spacing and maximum distance a nest can feasibly raid. Dulotic nests that are far away from each other or can only raid short distances may have no overlap simply because they are far apart, not because raids are directionally biased. To address this issue, I created a spatial simulation where each nest raids in random directions, while retaining their real observed number of raids and real distance of each raid. By comparing the overlap of these simulated raiding areas between pairs of neighboring nests to the real overlap I observed between them, I was able to assess the raid overlap in a way that minimizes the biasing effect of nest spacing—the other spatial pattern I examine in this chapter.

My spatial analysis revealed consistent evidence for the effect competition at two levels: for the spacing of nests, which is influenced during nest establishment and persistence of the nest; and for the patterns of raid overlap which is determined by the raiding decisions of individual nests. For nest spacing, nests are often overdispersed and never clustered on the landscape, irrespective of which host species they use. For raid overlap, most pairs of nests have less overlap in raiding area than random expectation, but the identity of the host species may affect the details of this pattern. Counter to expectation from competition, pairs of nests that had the same host species were more likely to have raids that overlapped than those that had different host species. I also found a relationship between nest spacing and raid overlap by nests sharing specific host species: nests that were most consistently overdispersed were also most likely to share raiding space, while nests that were never overdispersed shared space less often than any other pair of nests using the same host species. This indicates that host species identity itself seems to affect how parasites occupy space, and colony use of space at one phase of its life cycle might affect how it uses space at a subsequent phase. Our results indicate that dulotic ant nests are aware of their conspecific neighbors and avoid them, but share space when they forage.

Taken together, the findings I present here point to the importance of considering the lives of parasites for understanding all forms of parasitism. Parasites are complex in their genetics, behavior, and intraspecific interactions. Considering parasites from a behavioral ecology perspective—i.e., one that highlights the differences among parasites and asks why, in an evolutionary context, those differences exist—produced the results I present here. I’m convinced that this framework can continue to provide us with many more insights about the nature of parasitism, and I propose some specific lines of future research in the concluding chapter.

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CHAPTER 1

MICROSATELLITES REVEAL POPULATION STRUCTURE, VISCOSITY, AND WORKERS OF MIXED PARENTAGE IN A SOCIALLY PARASITIC DULOTIC ANT (*POLYERGUS MEXICANUS*)

INTRODUCTION

Social parasites exploit the social behaviors of their hosts for their own fitness benefit. Interactions between social parasites and their hosts often present striking scenes of cognitive deception of the host by the parasite, and offer useful systems for studying fundamental questions about costs of social behaviors (Fischer and Foitzik 2004, Grim et al. 2009, Cini et al. 2011, Guillem et al. 2014). Social parasites occur in many species but are perhaps most elaborate in social insects and best studied in brood parasitic birds. Most work on social parasites has focused on host-parasite dynamics, but it has long been recognized that parasite-parasite interactions are likely to have profound effects on how parasitic relationships evolve (Davies and Brooke 1988, Spottiswoode 2013). Parasites may compete with each other for hosts (Brooker and Brooker 1990, Mideo 2009). It is difficult to predict the outcomes of competition among parasites on the parasitism because models are sensitive to the ecology, natural history, and genetic relationships of the parasites (Schmid-Hempel 2011).

The genetic structure of parasite populations may be fundamentally important for understanding socially parasitic systems and is hardly ever characterized. Population structure can reveal social and ecological interactions among parasites

(Sugg et al. 1996, Sacks et al. 2005, Andrews et al. 2010, Sanches et al. 2012, Hindrikson et al. 2013) such as competition, as well as evolutionary processes like speciation. As a practical matter, any inferences about relatedness will be fundamentally flawed without correctly estimating the boundaries of randomly mating populations.

Kinship among parasites is generally predicted to reduce virulence in the host relative to unrelated parasites in a single host (in which case virulence is expected to increase due to competition)(Schmid-Hempel 2011). However, these predictions are sensitive to the ecological and evolutionary assumptions of the model. For example, if parasites have the capacity for interference competition, or produce and share a costly public good that facilitates host exploitation, then relatedness may increase virulence. Though we do not address virulence directly here, this example highlights the broader relevance of parasite-parasite interactions: the kinship dynamics among parasites have implications for their ecology, their interaction with their hosts, and the ecology of the host species

The role of kinship has received some attention for some parasitic interactions (Schmid-Hempel 2011), but is largely missing from studies on social parasites. In epidemiology, the theoretical and empirical work on multiple infections (i.e. two strains of the same species of parasite in one host) suggests the consequences of kinship among pathogens on both pathogens and their hosts are poorly understood and theoretical predictions are complex.

In contrast to epidemiological parasitology, kinship among social parasites is seldom if ever even characterized. However, social parasite systems may provide unique advantages to answer questions about the effects of parasite kinship on host-parasite interactions. Many of the analogous quantities of interest are directly observable or do not require a laboratory assay, e.g. virulence, mechanisms of “infection”, host fitness, and parasite fitness. Lastly, though analogous in many ways, social parasitism differs from epidemiology because of their special relevance to behavioral ecology, where we would like to understand the costs of societies and altruism.

Ants (Hymenoptera: Formicidae) are an ideal taxonomic group for examining the genetics of social parasitism because they have some of the most complex societies of any animals (Hölldobler and Wilson 1990), with numerous examples of social parasitism (Stuart 2002). Ant ins the genus *Polyergus* (Trager 2013) are obligate social parasites of species of ants in the genus *Formica*. The central feature of their life history is a form of social parasitism known as dulosis (often referred to as “slave-making” in past literature but see Herbers 2007). Dulosis is characterized by two essential features: (1) newly-fertilized dulotic queens parasitize an intact host species nest by killing the resident queen and usurping her workers; and (2) the dulotic queen’s sterile daughters (dulotic workers), which are reared to adulthood by the dead host queen’s workers, conduct raids on neighboring *Formica* nests in which they steal brood (larvae and pupae). Existing adult *Formica* ants present in the dulotic (i.e.; already parasitized) nest raise the purloined brood to become the next generation of host workers. As a result, all *Polyergus* nests are mixed-species, comprising a parasitic *Polyergus* queen and her descendants, and the stolen workers of numerous nearby host *Formica* spp. nests. Dulosis has evolved at least 10 times in ants (D'Ettorre and Heinze 2001, Beibl et al. 2005).

Most research on parasitism has involved the interaction between the parasite and their host species, but the parasites also interact with other parasites (Davies and Brooke 1988, Brooker and Brooker 1990, Kilner 2005, Spottiswoode 2013). Parasite-parasite interactions play a central role in the ecology and evolution of virulence in both traditional parasites(Gandon 2004, Massey et al. 2004, Schmid-Hempel 2011) as well as many social parasites (Kilner 2005, Bono et al. 2006a, Johnson and Herbers 2006). Conspecific interactions are probably common, since dulotic ant nests send out scouts to find nests to raid (Topoff et al. 1987, Le Moli et al. 1994, Pohl and Foitzik 2013). These scouts undoubtedly encounter conspecific nests, many of which are well within typical raiding distance of each other. In general, *Polyergus* nests are thought to be intolerant of each other (Topoff et al. 1984, Mori et al. 2001, Bono et al. 2006b, Trager 2013). This is supported by the occurrence of intraspecific raids in which one *Polyergus* nest raids and—in contrast to raids on their host species—completely destroys a rival neighboring *Polyergus* nest, and the generally accepted best hypothesis that territorial intraspecific raids among non-parasitic ants were the evolutionary precursor to dulotic raids (Topoff 1990, Bourke and Franks 1995). Previous research has suggested such conflict influences the direction and timing of raids from neighboring nests (Bono et al. 2006b). Despite these documented conspecific interactions, our observations indicate that *Polyergus* ants from different nests are not universally aggressive towards each other. Nests manage to persist much closer to each other than the typical raid distance (personal observations). Moreover, the same areas can be raided by several different nests, and raids occasionally even cross each other at the same time on their way to their respective target *Formica* nests with no apparent aggressive interaction, which has also been documented in *P. breviceps* raids (Trager 2013).

Understanding the genetic context of the interactions may provide key insights on why and when dulotic nests exhibit conspecific hostility, tolerance, or altruism. Here we investigate three levels of the genetics of a dulotic ant population that could influence the nature of the social interactions between nests: genetically differentiated subpopulations of parasites, kinship between parasitic ant nests, and kinship among parasitic nestmates within a parasitic nest.

The existence of genetic population structure may indicate that the parasite population is divided into host races (Fanelli et al. 2005, Torres 2012). Previous work supports the existence of host races at our site, where a single *Polyergus* species parasitizes multiple species of host ants in the genus *Formica*, but individual nests typically specialize only on a single host species (Goodloe and Sanwald 1985, Goodloe et al. 1987, Schumann and Buschinger 1994, 1995, Bono et al. 2007, Torres 2012). Furthermore, this preference is thought to be passed from queens to their daughters, and thus influences the host species choice both of new foundresses and the workers who conduct raids. *Polyergus* males may drive the evolution of host races if they also inherit a preference for unmated queens who share their natal *Formica* species (Mori et al. 1994, Mori and Le Moli 1998, D'Ettorre and Heinze 2001, Beibl et al. 2007, Torres 2012). If both male and female *Polyergus* share a preference for mates reared by the same conspecific *Formica* host, the resulting assortative mating patterns should lead to genetically isolated subpopulations, and—if host switching was relatively rare—incipient species, either of which would correspond with host species preferences. However, it is also possible that males mate randomly, which would erode the genetic isolation conferred by the host specialization of female *Polyergus* queens and workers. In this scenario, the existence of host races in *Polyergus* would be similar to the gentes of common cuckoos: host races of female parasites that persist despite random mating with males(Marchetti 1998, Gibbs et al. 2000, Fossøy et al. 2016). Since the genus *Polyergus* has variable mating behavior at a species and a population level (Trager 2013), the details of male mate choice for any given population cannot be presumed from past work elsewhere, but these details have important consequences for kinship and population structure among dulotic nests, and the resultant social interactions among nests and individuals.

By using microsatellite genetic structure to determine subpopulation boundaries and comparing them to patterns of host *Formica* species use, we can assess the extent to which host races exist in this population of dulotic ants. There are two additional reasons to assess the subpopulation structure of *P. mexicanus*. First, good estimates of relatedness require knowledge of the subpopulation membership of each individual, since relatedness models presume Hardy-Weinberg equilibrium within the populations where relatedness is measured. Since relatedness among nests and nestmates within nests is a major focus of this study, it is essential that we establish subpopulation boundaries as a precursor step. Second, recent work within the same population we study here, using the same microsatellite loci, has already indicated that the *Polyergus* population clusters into three subpopulations corresponding to their host *Formica* species (Torres 2012). Our study has the potential to independently verify this work with a much larger sample of nests (84, up from 10) over a much larger geographic area. This broader sample may clarify previous findings of some evidence for a fourth subpopulation cluster (Torres 2012). Since all the members of this cluster were *P. mexicanus* workers from a single nest, this finding may have been driven by rare alleles present in this single family.

The details of queen mating behavior suggest viscosity, the likelihood that related individuals have behavioral interactions, may be high for *Polyergus* populations. Though mating behavior varies across the genus *Polyergus,* at our field site, all our observations are consistent with the "female calling syndrome" (see pp. 144-146 of Hölldobler and Wilson 1990): new queens forgo flying and instead attract males via pheromones. Consequently, mating and parasitic nest founding happen close to new queens' maternal nests.

This difference between male and female dispersal suggests that the relationship between relatedness of nests and spatial distance may be different at different spatial scales. The mechanics of queen dispersal suggest that many neighboring nests will be close relatives, with relatedness decreasing with the distance between nests. However, male dispersal by flight could reverse any such negative relationship between distance at greater distances. We will measure the relationship between relatedness of pairs of nests and their distance from each other at scales

The degree of population viscosity could also be affected by relatedness within nests: the higher the within-nest relatedness, the higher potential viscosity. Two factors ultimately affect within-nest relatedness in ant nests: the number of queens in the nest, and the number of males that queens mates with. Monogyny (where nests have only one queen) and monogamy (where the queen mates with a single male) together produce nests with uniformly highly related females because all females are full sisters and half of their genes are an identical set inherited from their haploid father. The high relatedness produced by monogamy and monogyny was likely an important precursor for evolution of eusociality and extreme within-nest cooperation (Hughes et al. 2008), despite recent high-profile assertions to the contrary (Wilson and Wilson 2007). In combination with local queen dispersal, monogamy and monogyny could also influence among-nest relatedness. Monogamous queens in monogynous nests seems to be a general feature of all *Polyergus* and appears to be common among dulotic ants regardless of phylogenetic proximity (Le Moli et al. 1993, Foitzik and Herbers 2001, Brunner et al. 2005, Bono et al. 2007).

In this study, we use microsatellite loci to characterize the population structure and relatedness values of interacting *Polyergus mexicanus* nests to understand parasitic interactions occurring at a population, local, and nest level. At a population level, we quantify the population structure present in a spatially contiguous range of *P. mexicanus* nests and contrast this population structure with nest-level host-species preferences. For the local and nest level, we make use of the results of our population level study to determine genetic subpopulations in Hardy-Weinberg Equilibrium, and calculate relatedness values of ants within each subpopulation. At the local level, we evaluate the potential for viscosity by examining relatedness among workers from different nests as a function of their distance from each other. We consider the contrasting effects of male versus female (queen) dispersal by first including all possible nest pairs (which includes nests > 4 km away from each other, a reasonable spatial scale for male dispersal), then again using only nest pairs within two times the maximum observed raiding distance of each other (155 meters). At the level of individual nests, we assess whether *P. mexicanus* workers in each nest are full siblings, as is generally assumed for dulotic ants. We also quantify the relatedness among *P. mexicanus* nestmates.

We make several predictions based on what is known of *Polyergus* natural history at our site and other sites. First, genetic subpopulations of *P. mexicanus* should be congruent with host-species preferences, as past work has shown (Torres 2012). Thus, we predict that there will be three subpopulations of *P. mexicanus* that correspond to *Formica accreta*, *F. fusca*, and *F. argentea* host species. Second, we predict that *P. mexicanus* from the same nest will share both parents, resulting in no more than two unique maternal alleles plus one paternal allele that is shared by all nestmates (because males are haploid). This has been documented in other *Polyergus* populations (Bono et al. 2007) and other populations of dulotic ants (Pamilo 1981, Buschinger 1989, Bourke and Franks 1995, Foitzik and Herbers 2001, Brunner et al. 2005). Since we believe nests consist of simple families, we also predict that nestmates will be highly related to each other, close to the expected 0.75 relatedness of full-sibling sisters.

MATERIAL & METHODS

Study site and subjects

We studied a population of *Polyergus mexicanus* in the eastern Sierra Nevada Mountains near Truckee, CA at the Sagehen Creek Field Station (“SCFS", a University of California Natural Reserve; 39.432181, -120.241263, ~ 2000 m elevation). Note that *P. mexicanus* at this site were previously segregated as *P. umbratus*, which are characterized by a long and often convex mesonotum compared to *P. mexicanus*. Though *P. umbratus* was recently synonymized with *P. mexicanus* (Trager 2013), recent genetic work indicates that *P. umbratus* is actually a distinct species, so the name may soon be resurrected (Trager, personal communication).

The site comprises a variety of habitats, but nests were typically found within 200 meters of dirt roads in disturbed (mechanically thinned for fire control) mixed-conifer forest on the south-facing slope of the Sagehen Creek drainage basin. Nests were often found associated with downed tree trunks, stumps of harvested trees, or the root structure of common understory plants such as *Ceanothus prostratus* and *Wyethia mollis*.

The elevation of study populations ranged from 1,931 to 2,124 meters over a contiguous area of approximately 9 km^2. We estimate a density of *P. mexicanus* nests at 8.4 per 100 m^2, which is greater than any we are aware of elsewhere in the literature for any species of *Polyergus*. While *P. mexicanus* nests at SCFS are only known to parasitize *F. accreta, F. argentea,* and *F. fusca*, there are approximately 20 species of *Formica* at our site, many of which are quite similar in habitat and appearance to the three most common host species.

Field sampling methods and design

From 2008 to 2010, we searched for raids and nests on two 10,000 m^2 study plots. To better characterize the genetic diversity of the population of *P. mexicanus,* we also searched an ~15 km^2 area for nests along roads and trails throughout the reserve in 2010. In 2011, we conducted daily observations on four additional smaller (2,500 m^2) focal plots to provide more independent observations of unique pairs of interacting nests for behavioral studies. Daily visits to these smaller plots in 2011 yielded the discovery of several more *P. mexicanus* nests for our genetic samples presented here. All plots were centered on a *P. mexicanus* nest and were chosen because of the high density of surrounding *P. mexicanus* nests in the area as revealed through preliminary pilot searches for nests. Our observations and collection of specimens were not limited to plot boundaries: when we detected raids and nests near but outside plot boundaries, we included them in our study.

We searched for raids at these plots daily for at least a month during the peak of the raiding season (typically during July) and used the conspicuous raids to locate both the mixed-species *P. mexicanus* nests and the nests of their host *Formica* species, which are inconspicuous. We collected between 1-12 individuals from 84 nests for a total of 391 *P. mexicanus* female workers and 6 males for genomic DNA extraction. Live ants were frozen and preserved in 95% ethanol.

To assess host species identity, we collected host *Formica* workers either from the *P. mexicanus* nest or from a *Formica* nest raided by the *P. mexicanus* nest. For each nest, we attempted to collect three host *Formica* workers, which we mounted according to museum standards for species identification. We used the dichotomous keys developed by Francœur (1973) as well as several characters known to be diagnostic for the different local populations of host species (Phillip Ward and Candice Torres, personal communications) to determine the species of these *Formica* host workers. However, characters were sometimes ambiguous and *Formica* species within the sub-generic *fusca*-group (as are the three host species at SCFS) are notoriously difficult to identify (Mackay and Mackay 2002, Fisher and Cover 2007, Glasier et al. 2013). To avoid making classification errors in some ambiguous cases we assigned individuals from some colonies as "*Formica* cf. *fusca*" and "*Formica* cf. *argentea*". For all analyses, we verified that the inclusion or exclusion of these ambiguous individuals did not qualitatively change the results.

Microsatellite protocol

We extracted genomic DNA from the collected *P. mexicanus* workers and six males using either Qiagen DNEasy Kits or Quick-gDNA MiniPrep Zymo kits according to the manufacturer's instructions. We amplified the DNA with PCR using 6 primers developed by Bono et al. (2007): Pol1, Pol2, Pol3, Pol4, Pol5, and Pol12. We modified these original primers to use an M-13 dye-tagging protocol (Schuelke 2000). Each PCR was labeled with one of Applied Biosystems DS-33 dyes (LIZ, 6-FAM, VIC, NED, and PET).

The amplification process for all six loci differed only in annealing temperature. For all loci, extracted DNA was initially denatured at 95˚C for five minutes, then run through 36 cycles, each of which consisted of additional denaturing at 95˚C for 30 seconds, 30 seconds at one of two annealing temperatures, and 30 seconds of extension. After these 36 cycles, there was a final extension step of five minutes at 72˚C before samples were stored at 4˚C. The annealing temperature was 58˚C for Pol1, Pol4, and Pol12, and 53˚C for Pol 2, Pol 3, and Pol 5. Amplified DNA was preserved in highly deionized ("HiDi") formamide and sent to the University of California at Berkeley Sequencing Facility for microsatellite fragment size analysis using Applied Biosystems 3730XL DNA Analyzers and LIZ size standard. We determined peaks and bins of each locus on the resulting electropherograms using GENEIOUS version 6.0 (Kearse et al. 2012).

Estimating population structure

To estimate population assignment of individuals, we used STRUCTURE (Pritchard et al. 2000), which uses Bayesian techniques to form clusters of individuals that best meet the assumptions of Hardy-Weinberg equilibrium and linkage disequilibrium. We tested hypotheses for K=2-10 populations with 50 independent runs for each hypothesized K value (400 total runs). We used a burn-in length of 10,000 followed by 100,000 Markov Chain Monte Carlo (MCMC) repetitions, and left all other parameters in their default state. We used the online tool, STRUCTURE HARVESTER (Earl and vonHoldt 2012), to collate these results to determine which hypothesized K was most likely, using the Evanno (2005) method. We then used CLUMPP (Jakobsson and Rosenberg 2007) to create a consensus dataset that utilized the data from all the structure simulations of the selected K value. We analyzed the goodness of fit between our estimated genetic subpopulations and host-species groupings we described based on the morphology of host species for each *P. mexicanus* nest. Finally, we used the graphical software DISTRUCT (Rosenberg 2004) to visually contrast these genetic populations with the three host-species groupings.

Since males are haploid, we coded their genotypes as missing a second allele at each locus for the STRUCTURE analysis, as per instructions in the STRUCTURE software manual. While we used all 397 samples for our initial estimates of population structure, we subsequently removed all six haploid males as well as three other individuals where <50% of the loci successfully amplified from all subsequent analysis. Because we were primarily interested in relatedness values within subpopulations and not gene flow between subpopulations, we also removed 9 individuals whose cluster assignment was less than 80%, i.e. putative hybrid individuals that we could not clearly assign to one population.

We used the STRUCTURE estimated subpopulations to identify null alleles, allelic dropout, and stutter using MICROCHECKER (Van Oosterhout et al. 2004), which compares molecular data to Hardy Weinberg expectations and thus requires an estimate of population boundaries among samples.

Because MICROCHECKER assumes that samples within a population are from unrelated individuals, we randomly chose only one individual per sampled nest for the MICROCHECKER dataset. However, we found that this method missed seven rare alleles that we knew were present, so we also added six individuals from nests that were already represented to ensure that these alleles were included in our analyses. In other words, we tolerated slight violations of the software's assumption regarding relatedness of samples in favor of including all alleles. We found evidence of null alleles for only one locus (Pol 1) in one of our estimated subpopulations (Population 3), and we used the "Brookfield 2" corrected allele frequency provided by MICROCHECKER for subsequent relatedness analysis (Brookfield 1996).

Comparing population structure to host species preference

We compared the number of genetic subpopulations detected above to three; the number of host species known to be parasitized by *P. mexicanus* at SCFS. To assess the relationship between host *Formica* species use and genetic subpopulation, we first calculated a nest-level subpopulation assignment score for each nest by averaging the scores of nestmates. We excluded any nests whose nest-level assignment score was less than 0.80 from subsequent analysis, and assigned the remaining nests to their most probably subpopulation. We then conducted a contingency test to assess to relationship between host species and genetic subpopulation. Lastly, we tested the goodness of fit of the observed host *Formica* species used to a model based on proportions on nests assigned to genetic subpopulations. That is, we used the proportion of nests assigned to each genetic subpopulation as the null hypothesis for the distribution of nests using distinct slave species. A significant result in the contingency test would indicate that genetic subpopulations and host species use are not independent, while the contingency test determines if host nest use differs from genetic subpopulation.

Testing for simple families

Because all ants are haplodiploid, if all workers share the same two parents (here referred to as “simple families” after Bono 2006), then they all must share one identical paternal allele and the other allele at each locus must be one of two maternal alleles. Thus, at a nest level only three alleles are expected at each locus and all individuals must share one allele. We wrote a script for R version 3.4.2 (R Core Team 2017) to verify that this expected pattern was supported by our microsatellite data for all nests where we had molecular data from more than one worker. The script compares all nestmates and returns a “True” value if alleles in a nest are consistent with simple families or “False” value if they are not, for each locus. Just one “False” value per nest is sufficient to reject the simple family model, but we tallied the total number out of six possible loci to assess the strength of the evidence given the potential alternative hypothesis that a genotyping error caused the discrepancy. Higher proportions of “False” values, then, signify greater certainty that nestmates do not share parents.

Estimating relatedness

We used KINGROUP (Konovalov et al. 2004, Konovalov and Heg 2008) to measure the pairwise relatedness among all individual workers in each of our estimated genetic subpopulations. We summarized these relatedness values into within-nest relatedness (i.e. the average pairwise relatedness of all combinations of workers in the same nest) and between nest pairs (i.e. the average pairwise relatedness of all combinations of workers from one nest to another nest).

Correlations between distance between nests and nest relatedness

We calculated the Pearson product-moment correlation for the pairwise relatedness of nests and the distance between nests for all pairs of nests. Because we were primarily interested in the effects of female dispersal (not male) and inter-nest interactions (such as intraspecific raids), we repeated this analysis with only nest pairs that were less than twice the maximum observed distance of host raids, 155 m. We analyzed each genetic subpopulation separately as well as pooled together for both distance ranges.

RESULTS

Population structure

We found the strongest evidence for three subpopulations (Delta K = 864 for K = 3). These subpopulations were very distinct, with most individuals clearly assigned to one of the three populations (Figure 1.1a). Evidence for gene flow between subpopulations was minimal: we detected only nine individuals out of 391 sampled whose membership coefficients were less than 0.8 for all three clusters. We omitted these putative hybrids from the relatedness analysis discussed below.

The number of genetic subpopulations estimated by our population structure analysis (three) equaled the number of known host *Formica* species that *P. mexicanus* parasitizes at SCFS. While there was correspondence between genetic subpopulations of *P. mexicanus* and their observed host *Formica* species (Contingency test, $\chi^2 = 57.1$, df = 4, $p < 0.001$), genetic subpopulation was a poor predictor of host *Formica* species overall (Goodness of fit, $\chi^2 = 22.82$, df = 2, $p < 0.001$). This poor fit was largely driven by nests using *F. fusca* host species which contained most members of Subpopulation 2, but also many from 1 and 3 (Figure 1.1b): when we excluded nests using *F. fusca* or assigned to Subpopulation 2, populations 1 and 3 were a good fit for host species *F. argentea* and *F. accreta*, respectively (Goodness of fit, $\chi^2 = 0.86$, df = 1, $p < 0.35$).

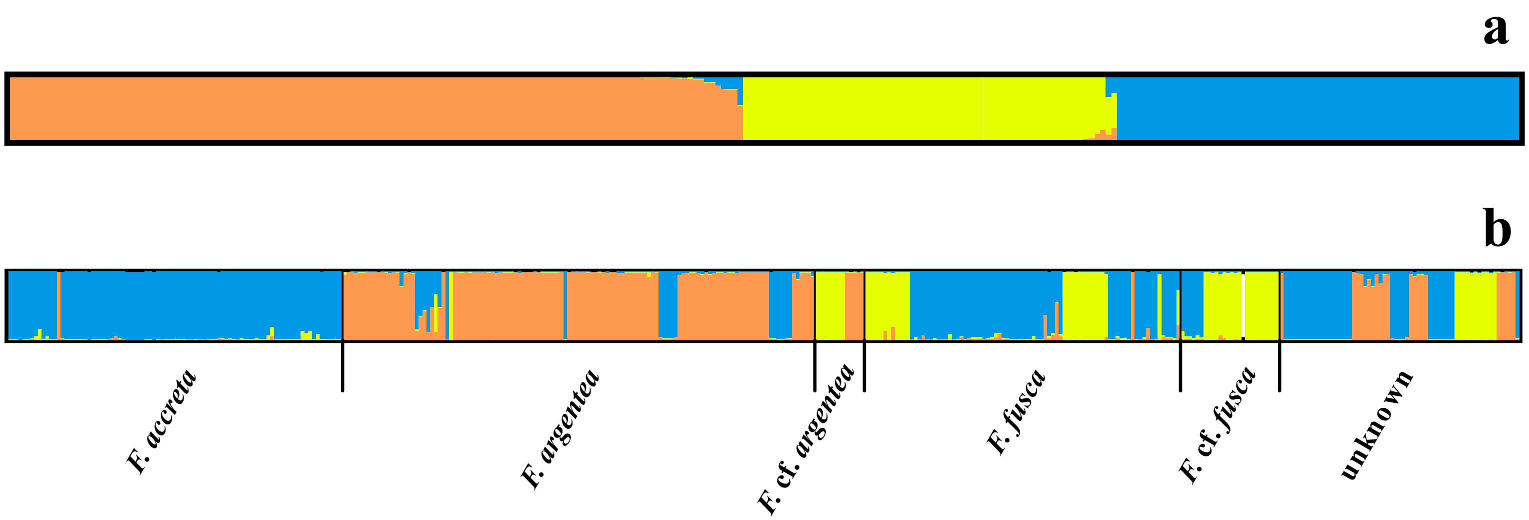

Figure 1.1: Genetic subpopulation structure for *Polyergus mexicanus* individuals. Vertical bars represent individual ants. Colors represent population cluster assignments. Orange is Subpopulation 1, yellow is Subpopulation 2, and blue is Subpopulation 3. (a) All individuals sorted by their assignment to each subpopulation. (b) All individuals sorted by the host *Formica* species present in their nest.

Relatedness between nests

The global average relatedness of all non-nestmates was close to zero (*R* ± *SD* = 0.03 ± 0.27; n = 26,410 pairs in 85 nests, indicating that most nests are not closely related to each other. However, 75 nests pairs (out of 1363) had an average relatedness equal to or greater than 0.375; the relatedness expected between workers of one nest and workers of a new queen arising from that nest (Figure 1.2a). There were 130 unique pairs of nests within 155 meters of each other. Of these, eleven nest pairs had a relatedness value of 0.375 or higher (Figure 1.2b).

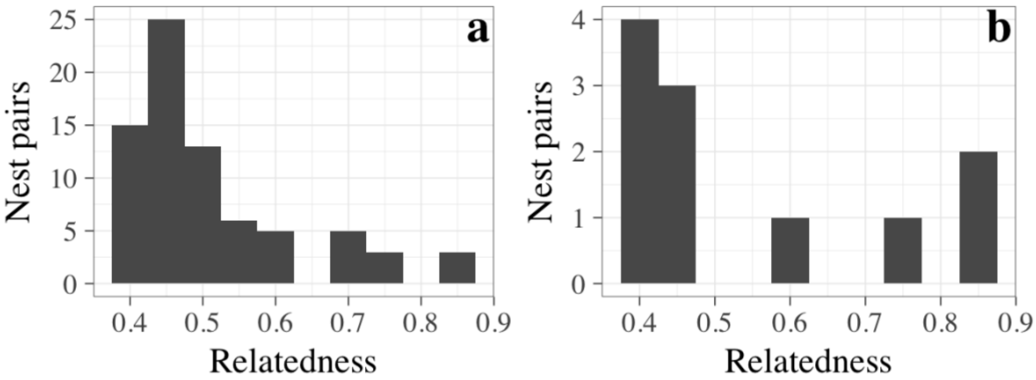

Figure 1.2: Histograms of average relatedness of pairs of nests for highly related nests at two spatial scales. Only nest pairs with average R > 0.375 are included. (a) All possible pairs of nests at our study site, no matter how far. (b) Pairs of nests < 155 m from each other**.**

There was a weak but significant negative correlation between relatedness of nests and their distance from each other over local distances (up to 155 m, the maximum distance between two raiding nests) (Table 1.1). However, there was no such correlation in an analysis that included pairwise comparisons at all distances (Table 1.1). Separate analyses of each subpopulation revealed that only Subpopulation 1 had a significantly negative correlation between distance and relatedness (Table 1.1), and that this correlation existed even when all distances were considered.

Table 1.1: Correlations between relatedness and distance for different subpopulations at two spatial scales. Statistics shown are Pearson product moment correlations for unique pairs of nests. "Pop" is genetic subpopulation. "df" is degrees of freedom. "p" is p-value. "t" is Pearson test statistic.

Distances	Pop	Sample size	df	p	t	correlation
all	all	1782	1780	0.06	-1.88	-0.04
< 155 m	all	134	132	0.04	-2.08	-0.18
all	1	627	625	< 0.01	-5.02	-0.20
all	2	104	102	0.12	1.59	0.16
all	3	1051	1049	0.38	0.87	0.03
< 155 m	1	48	46	0.02	-2.47	-0.34
< 155 m	2	12	10	0.22	-1.30	-0.38
< 155 m	3	74	72	0.76	-0.30	-0.04

Relatedness within nests and simple families

The global average pairwise relatedness of nestmates was still close to the 0.75 value expected for full-sibling sisters ($R \pm SD = 0.71 \pm 0.24$, $n = 939$ pairwise contrasts from 62 nests). Of the 66 *P. mexicanus* nests where we obtained samples for at least two workers, 16 nests (24%) contained workers that could not have been from the same two parents.

DISCUSSION

By analyzing microsatellite loci at three biologically important levels, we reveal many important features of the lives of socially parasitic ants. At the population level, our data support the role of specialization on host species in restricting gene flow, but fail to identify clear host races. At a local level, the relatedness patterns we observe between pairs of nests demonstrate that both conspecific competition and viscosity are possible: most neighboring nests of the same subpopulation are not close relatives, but there are nests that are both closely related and spatially close enough to interact with each other. Despite this, the expected relationship between distance and relatedness between pairs of nests within raiding distance of each other was weaker than we expected from our observations of the mating behavior of *P. mexicanus* queens at SCFS. Within *P. mexicanus* nests, our data indicate that while most *P. mexicanus* workers within a nest are full siblings, for some nests the allelic diversity present among *P. mexicanus* workers proves that they did not have the same parents.

While genetic subpopulations 1 and 3 supported the existence of *P. mexicanus* host races specializing on *F. argentea* and *F. accreta*, respectively, Subpopulation 2 did not appear to be a *F. fusca* host race of *P. mexicanus*. To the contrary, *P. mexicanus* nests that used *F. fusca* hosts were more often members of Subpopulation 3 (13 of 22 nests with *F. fusca* hosts) than Subpopulation 2 (8 of 22 nests with *F. fusca* hosts).

The relationship between genetic subpopulation and host *Formica* species does not describe a clear case of host races, but does provide insight into host-use by *P. mexicanus* nests. Our data indicate that Subpopulation 3 may be more generalist than the other two subpopulations and thus able to use both *F. accreta* and *F. fusca* as hosts. Though this explanation conflicts with the hypothesis that host specialization alone drives assortative mating and subsequent genetic isolation, it is possible that the cues used for mate selection differ from those used for host selection, at least for

Subpopulation 2. Additionally, the subpopulation boundaries may indicate past host specialization that differs from present ecological pressures. Some combination of the following three conditions could give rise to the relationships we found among nests using *F. fusca* hosts, nests from subpopulation 2, and nests from subpopulation 3: First, Subpopulation 3 might numerically dominant over Subpopulation 2. Second, *F. accreta* nests may be rare while *F. fusca* nests are relatively abundant. Third, *F. fusca* could be a competent (if suboptimal) host for *P. mexicanus* nests in Subpopulation 3. Alternatively, the inconsistent match between genetic subpopulations and host species use may be attributable to hybridization among the closely related *Formica* host species, or the existence of cryptic *Formica* host species.

Nests within raiding distance of each other (<155 meters) had a wide range of relatedness values (-0.62 – 0.82), with about 8.5% (11 out of 130) of nest pairs more related than 0.375. This means that a sufficient diversity of kinship relationships exists among neighboring nests to behave altruistically towards related nests or aggressively towards nonrelated nests. Whether such interactions occur is contingent on the ecology of the system. The costs and benefits of altruism and territoriality vary, but there is at least the genetic background present for such interaction to occur based on kinship or lack thereof. Future work should examine the ecological costs and benefits of inter-nest social behaviors of *P. mexicanus* to determine if and when altruism or local kin-competition dominate the system.

Relatedness between pairs of nests did decrease with distance over the probable range of *P. mexicanus* queen dispersal, but very weakly. Strangely, this pattern seems driven by only Subpopulation 1, and the relationship persisted when all distances were considered. We have no a priori reason to suspect that one subpopulation would have different dispersal or mating behavior than the others, even if we assume subpopulations represent host races.

Queen dispersal on foot should tend to increase the relatedness of neighboring nests, but it is not the only ecological force that may have shaped the relatedness patterns we observe among neighboring nests. Competition past may have already led to the extermination of neighboring nests, and the intensity of competition may make kinship irrelevant to neighboring nests. Unlike females, males disperse by flight, further complicating spatial relationships and nest pair relatedness. We have no *a priori* reason to expect the correlation between distance and relatedness between nests for Subpopulation 1 to differ from the other two populations. Future work should document the dispersal distances of females versus males, the altruistic and competitive interactions of neighboring nests, and the fitness consequences of such interactions.

Nestmates were as related to each other as full siblings in the overwhelming majority of cases. This high proportion of shared genetic interest implies that cooperation among workers is high as predicted by Hamilton's rule (Hamilton 1964a, 1964b). It also diminishes the likelihood of conflict over reproduction by workers versus queens and suggests that there is only one *P. mexicanus* queen, thus no conflict over which queen's brood to favor. Because brood care is performed by host *Formica* workers, *P. mexicanus* workers have little influence over these within-nest dynamics, but their high level of relatedness may influence their raiding behavior: If nestmates are closely related to each other, there may be less conflict in raiding decisions, especially intraspecific raids motivated by intraspecific competition.

Our finding that *P. mexicanus* nestmates are not always from the same mother and father counters conclusions from previous studies on dulotic ants, including species both closely and distantly related to *P. mexicanus*. There are three possible explanations for the lack of simple families. First, rather than the monogamous mating pattern long assumed for most dulotic ants (Buschinger et al. 1980, Buschinger 1989), some parasitic queens at our nest may mate with more than one male such that nests contain half-siblings. Our genetic data do not have the resolution to reliably distinguish half-siblings from full, but, other studies on *Polyergus* and our direct observations of matings are consistent with monogamous queens: mating is easily observed, occurring above ground near the new queens' maternal nest, yet all our observations and those of other studies report only one mating per queen.

Complex families might also be generated if nests were founded by or contained more than one queen (polygyny, e.g. if daughters returned to their natal nest after fertilization). However, polygynous nests have never been reported for the genus *Polyergus* to our knowledge, despite laboratory-based studies of colonies (Le Moli et al. 2001, Johnson 2002) and field observations of colony usurpation by *Polyergus* queens (Topoff and Mendez 1990), though they are known for some facultatively dulotic ants (Mori et al. 2001).

The third possibility is that the lack of simple families is generated by the capturing of *P. mexicanus* brood during intraspecific raids (Pamilo and Seppä 1994) Intraspecific raids do occasionally occur in many dulotic ants (Topoff et al. 1984, Trager 2013)—these raids target other dulotic ant nests of the same species, not host nests. It is likely that dulotic raiding workers steal *Polyergus* worker brood from nests they raid. If raiders take both host (*Formica*) and parasite (*P. mexicanus)* brood, this would lead to nests of mixed parentage. Topoff (1990) pointed out that parasitic worker ants probably do not have a cognitive distinction between host *Formica* and nestmates of their own species, and brood may have a reduced or generalized chemical profile in general among ants (Fouks et al. 2011), so it is likely that raiders would take conspecific brood should they encounter it during an intraspecific raid. The relative rareness of mixed parentage *P. mexicanus* nests (24%) is congruent with the rareness of intraspecific raids observed at our site and in other *Polyergus* populations.

Our work shows that the social parasite *P. mexicanus* has population structure and a complex pattern of relatedness within and among nests. These discoveries suggest a potential unique consequence of the parasitic behavior: that dulotic raiders may also socially parasitize other dulotic raiders. They also provide a genetic context for future studies on conspecific social interactions among these *P. mexicanus* nests. Future work should look at the use of space and the extent of host *Formica* nest sharing by neighboring raiding *P. mexicanus* nests. Connecting the population genetic insights here to the social and behavioral aspects of the parasitism may help us understand crucial features of the parasitism such as virulence, host mortality, and the evolution of hosts and parasites.

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CHAPTER 2

OPTIMAL FORAGING IN PARASITIC SLAVE-MAKING ANT RAIDS

INTRODUCTION

The mechanisms by which parasites find and parasitize their hosts are intimately linked to parasite fitness. Because host-acquisition is shaped by natural selection, the principals optimal foraging theory can be used to understand and predict the behavior of parasites. Optimal foraging theory (optimal foraging theory) can be applied to males' search for mates (Louâpre et al. 2015), and for host selection and exploitation in parasitoids (Vet et al. 2002, Boivin et al. 2004). Optimal foraging theory assumes that organisms are shaped by natural selection to maximize some currency related to fitness. It uses this assumption to understand how foragers respond to environmental constraints, and the fitness trade-offs foragers make to optimally acquire resources. Work on other foragers and parasitoids has shown that parasite condition, environmental constraints, and host quality all can affect forager behavior. Little work with social parasites has considered parasite behavior as a foraging problem.

While brood parasitism among birds is the most well-known example of social parasitism, ants (Formicidae) are unrivaled in the abundance and diversity of ways they exploit other societies of ants (Topoff 1977, Stuart 2002). Obligate dulosis, often and formerly always called "slave-making"(Herbers 2006, 2007, Trager 2013), is a form of social parasitism in ants in which host-acquisition is especially similar to foraging tasks in non-parasitic animals. Adult dulotic workers find and raid neighboring nests of their host species. They steal larvae and pupae (henceforth, ‘brood’) which are reared to adulthood by adult host workers that were previously captured, and then act as workers in their captor’s nest, presumably because they imprint on colony scent after eclosion but not before (Trager 2013).

Dulotic ant colonies are formed when a dulotic queen kills the resident host queen of a free-living host nest and is subsequently treated as the resident by the host workers (i.e., the recently dead queen’s sterile daughters) via chemical deception. These deceived host workers raise the dulotic queen’s eggs to produce dulotic worker adults, which conduct the raids described above. The dulotic queen needs the scent of the killed queen to successfully usurp a new host. It is this killing of the resident queen that necessitates the raids performed by the dulotic queen’s offspring. Dulotic workers must raid to maintain the population of enslaved host workers in the absence of a host queen. The result is a mixed-species nest where host workers work for the benefit of their parasitic queen and her workers instead of their own mother and sisters. Dulosis has evolved 10 times independently (Beibl et al. 2005).

Like all members of its genus, *Polyergus mexicanus* is an obligate dulotic species that is entirely dependent on captured host *Formica* ants to do all the foraging for food, brood care (both of *Polyergus* brood and newly captured *Formica* brood), and nest maintenance (Trager 2013). *Polyergus* workers are nearly helpless and participate in none of the typical tasks of their resident stolen host *Formica*. Instead, *Polyergus* workers are highly specialized for one task: finding and raiding host *Formica* nests.

Polyergus raids are initiated by scouts, worker ants that leave the colony hours before a raid in search of *Formica* nests to potentially raid (Topoff et al. 1984, 1987, Dobrzańska and Dobrzański 1989, Le Moli et al. 1994, Visicchio and Castracani 2003). When scouts return, they recruit up to thousands of *Polyergus* workers to conduct a raid on a host nest sometimes over 50 meters from the *Polyergus* colony (Hasegawa and Yamaguchi 1995, Le Moli et al. 1994, Topoff et al. 1984, Trager 2013). Raids always take place in the summer months (corresponding to brood production of their host *Formica* species) (Cool-Kwait and Topoff 1984) in the late afternoon.

Foraging by social parasites for their hosts consists of three phases, and each can be linked to a specific part of the dulotic ant life cycle (Stuart 2002): finding host species habitat, finding hosts, and accepting hosts. A dulotic ant society uses queens, males, scouting workers, and raiding workers to accomplish all three of these tasks during host nest usurpation and subsequent raids. However, the focus of this study is limited to observations of raids that form upon the return of a scout. This means that we are considering only the final phase of accepting hosts, and raiding workers are the only dulotic ants involved.

To understand dulotic raids, we borrow from a framework used in optimal foraging theory studies on parasitoids that identifies three main factors that influence foraging decisions: the quality of the host, the condition of the parasite, and environmental constraints. In this study, we describe several characteristics of dulotic raids, their outcomes, and the environment that represent each of these factors, and assess their relationship via optimal foraging theory.

For *P. mexicanus*, the quality of the host nest is likely determined primarily by host colony sociometry, i.e.; the physical and numerical attributes of social insect colonies and their inhabitants (Tschinkel 1991, 2010). The presence and quantity of brood, and the defensive abilities and numbers of defending *Formica* workers are sociometric characteristics likely to affect foraging decisions of dulotic raiders. None of these factors is easily measured because *Formica* host species nests at our site are cryptic, the soil is rocky, and accurate measurements of colony sociometry would require careful excavation of many *Formica* nests at multiple time points. By contrast, the distance of a host nest from the raiding *Polyergus* colony is an easily observed and measured aspect of host quality that we assess in this study. Raiders should always prefer a closer host nest, all things being equal, and the distance raiders are willing to travel to a host nest should be positively correlated with the amount of brood raiders can capture there.

Previous work with laboratory colonies has elucidated how *Polyergus* raiders respond to other aspects of host quality. When the amount of brood was experimentally manipulated in laboratory colonies of *F. cunicularia*, *P. rufescens* raiders sent more raiders to brood-heavy *F. cunicularia* nests (Visicchio and Castracani 2003), and suffered less raider mortality per brood captured at raids of *F. cunicularia* nests with high amounts of brood. Surprisingly, *P. rufescens* nests preferred *F. cunicularia* nests where scouts had been attacked more, suggesting that scouts use *Formica* worker aggression as a proxy for the amount of brood present.

Formica host species identity is a fundamentally important aspect of host quality for Polyergus. Individual *Polyergus* nests typically specialize on a single species of *Formica* host (Goodloe and Sanwald 1985, Goodloe et al. 1987, Mori et al. 1994, Schumann and Buschinger 1994, 1995, D'Ettorre and Heinze 2001, Beibl et al. 2007, King and Trager 2007, Bono et al. 2007). Moreover, both theory (Sudd and Franks 1987, Buschinger 2009) and empirical work (Torres 2012) suggests that host preference is maternally inherited, which would result in limited gene flow among *Polyergus* nests specializing on different hosts.

While species-specific differences in chemical signals and cues are the primary reason dulotic ants are thought to specialize on specific host species (D'Ettorre and Heinze 2001), hosts species may also differ in other aspects of their biology that are relevant to the foraging decisions of dulotic ants. The density of host nests, the ratio of workers to brood at the host nest, the aggressiveness of host workers, and the fecundity of the host nest could affect the distance, size, frequency, and outcome of raids.

At our study site, we have previously established that there are three primary hosts that are closely related—*F. fusca, F. argentea,* and *F. accreta*—and that there are three genetic subpopulations of *P. mexicanus* that map onto these host species reasonably well (Chapter 1, Torres 2012). For this study, we assume that the genetic subpopulations we established represent true host races. We examine the effect of host species preferences (as inferred by genetic subpopulation) on the characteristics and outcomes of *P. mexicanus* slave raids.

The optimal foraging decision for a parasite is often contingent on their individual condition. Parasitoid wasps calibrate their decisions on whether or not to oviposit and how many eggs to oviposit in in a given host based their expected survival, and will accept poorer hosts when their own condition is worse (Vet et al. 2002). For ants, foraging decisions are influenced by the internal condition of the forager, their nestmate-encounter rate, and the condition of the colony as a whole (Greene and Gordon 2006, Robinson et al. 2009).

Colony size is another aspect of parasite condition that can affect host foraging strategies. Ant colonies exhibit distinct foraging strategies contingent on colony size (Gordon 1995, Gordon and Kulig 1996). For dulotic ants, colony size should be especially important because of the highly synchronized nature of dulotic raids. For a series of experimental *Polyergus* raids on *Formica* nest fragments in which the adult *Formica* worker numbers were held constant but the amount of brood was varied, a nearly constant number of raiding *Polyergus* workers was killed in each raid, though the size of the raid and brood captured was directly related to the amount of brood present in the raided *Formica* colony. This suggests that the size of a dulotic raid is under strong selection. Although the authors held defending *Formica* worker numbers constant, if we assume a linear relationship of raiding *Polyergus* death and number of defending *Formica* workers, then only larger nests could mount raids big enough to reap the rewards (more brood) and pay the costs (raider death from defending *Formica* workers) of raiding larger nests.

Foraging decisions are subject to environmental constraints. In this study, we assess these constraints by measuring the time of day that raids occurred, and the date of occurrence of raids throughout the summer, when raiding activity occurs. Note that the timing of the onset of raids in the year is most likely caused by environmental constraints on the host *Formica* population, but raiding *Polyergus* perceive it as host quality, not environmental constraint. *Formica* brood development is probably driven by environmental seasonality, which *Polyergus* scouts track by assessing the presence of brood in target *Formica* nests. In contrast to the seasonal onset of raids in the year, the start time of raids is most likely influenced by air and ground temperature, which has strong effects on a wide range of foraging ants (Traniello et al. 1984, Traniello 1989). Throughout its Holarctic distribution, the genus *Polyergus* raids almost exclusively in the late afternoon in the summer, when the ground is warm but air temperatures are declining from their peak (Trager 2013), and *Formica* nests are full of brood.

Both the characteristics of raids and their outcomes vary considerably. Raids often fail i.e., the *Polyergus* raiders return without any brood (Wheeler 1916, Talbot 1967, Cool-Kwait and Topoff 1984, Hasegawa and Yamaguchi 1995), either because they couldn’t successfully find or infiltrate the host *Formica* nest and retrieve brood. How do differences in raid characteristics contribute to a raid’s chances for success? Raids vary in the time of day, date, size (number of raiders), and the distance they travel to the host *Formica* nest. As obligate parasites, the fitness of a *Polyergus* colony is intimately and inextricably linked to its ability to obtain host *Formica* workers, so variation in raid outcome and raid characteristics should have important consequences for *Polyergus* nests.

In this study, we measure and compare several aspects of naturally occurring *P. mexicanus* raids to understand how host quality, parasite condition, and environmental constraints interact to shape variation in raiding behavior. Over the course of 4 consecutive summers, we measured the distance traveled, start time, and date of raids of a population of *P. mexicanus* colonies on three *Formica* host species. We present a method for efficiently recording raids on video and subsampling the recorded raids to estimate the number of *P. mexicanus* raiders and the quantity of brood they captured. We make use of the genetic data obtained from our previous work (Chapter 1) to assign *Polyergus* colonies to one of three genetic subpopulations which we here use to represent host *Formica* species preference.

We combine these field, video, and laboratory data in mixed models to determine both the factors that lead to successful raids, and the factors that best predict the amount of brood captured during successful raids. We interpret these model results using optimal foraging theory to infer the constraints, currency, and fitness trade-offs imposed on raiding *P. mexicanus* colonies.

METHODS

Field methods

We studied *Polyergus mexicanus* raids at Sagehen Creek Field Station (SCFS) in the Eastern Sierra Nevada Mountains (39.432181, -120.241263, ~ 2000 m elevation). Note that *P. mexicanus* at this site were previously segregated as *P. umbratus*, which are characterized by a long and often convex mesonotum compared to *P. mexicanus*. Though *P. umbratus* was recently synonymized with *P. mexicanus* (Trager 2013), recent genetic work indicates that *P. umbratus* is actually a distinct species, so the name may soon be resurrected (Trager, personal communication).

We searched for *P. mexicanus* nests within 200 meters of trails and dirt roads, in thinned montane mixed coniferous forest. *P. mexicanus* nests were often found in stumps of harvested conifers, downed logs, or associated with the root structure of perennial forbs such as *Ceanothus prostratus* and *Wyethia mollis*. Our previous work has estimated *Polyergus* nest densities of 8.4/ m^2 (Chapter 1), to our knowledge the highest reported for any *Polyergus* population. *Polyergus mexicanus* raids occur during the late afternoon of summer months. Raids consist of tens to hundreds of *P. mexicanus* workers moving in a nearly straight line towards their target. They return along the same path, many carrying brood if they have successfully infiltrated a *Formica* nest.

We searched ~100 m^2 areas for new *P. mexicanus* nests and raids at seven different sites at SCFS. During the raiding period (approximately 16:00 - 20:00), we continuously checked known *P. mexicanus* nests for raids or signs of imminent raids and walked the site in search of raids from unknown nests or for the unknown nests themselves, in both cases using above ground ant activity for detection. When we discovered a nest, we flagged it and checked it daily along with the previously known *P. mexicanus* nests. We marked each *P. mexicanus* nest and each raided *Formica* spp. nest with a unique three-digit number on a permanent metal tag. For each nest, we noted the site, coordinates, and later determined the genetic subpopulation by microsatellite loci from previous work (Chapter 1). We also include 24 raids not associated with one of our seven focal sites that we found walking to or from one of our focal sites.

We observed 812 raids from 87 *P. mexicanus* nests between 2008-2011, but we did not collect all possible data for all 812 raids. Whenever possible, we assigned (if newly discovered) or recorded the nest ID of the raiding *P. mexicanus* nest (all raids), raid distance (775 raids), start time (544 raids), and date (all raids). For a subset of these raids (146 raids from 20 nests, all from sites A and B), we filmed all returning raiders of successful raids to estimate the number of participating raiders and the amount of brood they captured. Videos were recorded on Hi8 Sony Handicams.

Video subsampling protocol

To accurately compare our large sample of video recorded raids efficiently, we developed a standardized subsampling protocol. For 25 randomly chosen raids, we counted the number of all returning *P. mexicanus* raiders in an entire filmed raid.

We regressed these “full” counts of all returning raiders against counts of all returning raiders present in every tenth minute of the same recorded raid (i.e. one minute count completed every ten minutes). For both counting methods, we categorized returning raiders as either “with brood”, i.e. carrying larvae or pupae, or “without brood”, i.e., carrying nothing. We created separate regressions for these two classes of raiders because of the possibility that video resolution, background complexity, lighting, and raider movement might bias estimates of raiders with brood relative to those without. We used the resulting regression equations to convert subsamples of the other 118 recorded raids into estimates of the number of returning *P. mexicanus* raiders both with and without brood.

Statistical analysis

All statistical comparisons were conducted using R statistical software (R Core Team 2017). We first described several prominent characteristics of raids: the daily and seasonal distribution of raids, and the duration of raids. Using the video subsampling method described above, we calculated the estimated number of raiders and estimated amount of brood captured for 146 of the 812 raids, and used these estimates to compare brood per raid (total estimated brood in each raid), raid size (total estimated raiders in each raid), and brood per raider (per capita brood captured, i.e. brood per raid divided by raid size) to the other raid characteristics described above.

For interactions among nest level characteristics such as average raid size of nests, success rate of raids, and nest genetic subpopulation membership, we omitted nests where fewer than 5 raids were observed. This left 9 nests: 2 in subpopulation 1, 4 in subpopulation 2, and 3 in subpopulation 3 (see Chapter 1 for details).

Effect of subpopulation

We examined the effect of genetic subpopulation using 6 separate linear mixed models (LMMs) that all included nest ID, site, and year as random effects, via the lme4 package in R (Bates et al. 2015). Specifically, we tested the effect of genetic subpopulation on (1) the season day of raid onset, (2) the time of day of raid onset, (3) the size of raids, (4) the distance of raids, (5) the amount brood captured per raid, and (6) the amount of brood captured per raider. For each model, we conducted an analysis of variance (ANOVA) using type III sum of squares and a Satterthwaite approximation of degrees of freedom (Satterthwaite 1946, Welch 1947).

Effect of max raid index

Because it was not possible to obtain nest size data for this study, we assumed nest size was related to the maximum raid size we observed for each nest. We tested the effect of this “max raid index” on four response variables using separate LMMs with raiding nest ID as a random effect: (1) raid size with nest ID as a random effect, (2) raid distance, (3) total brood captured per raid, and (4) brood captured per raider per raid. We dropped the largest observed raids from each nest from these analyses to avoid spurious correlations since our max raid index is equal to the size of the largest observed raid for each nest.

We also assessed the role of the max raid index on the variation of the above raid characteristics within nests and the nest-level raid success rate (i.e. the proportion of observed raids that were successful) using linear models. Because variation in a raid character is a nest-level characteristic, each nest was included only once so nest ID was not included as a random effect. We constructed five separate linear models regressing max raid index against: (1) standard deviation of raid size, (2) standard deviation of raid distance, (3) standard deviation of brood captured per raid, and (4) standard deviation of brood captured per raider, and (5) the success rate for each nest. For success rate, we limited the analysis to nests where we had observed at least 4 raids to increase the accuracy of success rate estimates.

To determine how the size and success of raids varies as proportion of the maximum capacity of a nest, we calculated “proportional raid size” by dividing the size of raids by max raid index. We tested the effects of max raid index on proportional raid size in a LMM with nest ID as a random effect.

Predictors of raid success

Many raids fail to obtain any brood, so we constructed a Generalized Linear Mixed Model (GLMM) to determine whether certain raid characters could explain why some raids are successful (i.e. some brood captured) while others failed (i.e. no brood captured). This model included raid distance, raid start time, and raid date as potential predictors of raid success. For this model, we excluded raid size and brood per raid because we consider them later in a more fine-grained analysis on brood quantity (described below) and because it enabled us to consider a much larger dataset of raids (464 raids from 47 nests with relevant field observations compared to 117 raids from 19 nests for which raid size and brood were estimated via recordings). Each of these predictor variables was scaled as per the software package's suggestion by subtracting the mean from each value, then dividing the resulting difference by the standard deviation. We compared this model to three simpler models that excluded distance, date, and time; respectively using likelihood ratio tests to determine the importance of each predictor variable on raid success.

Predictors of raid quality

To investigate raid optimization, we constructed a linear mixed model to determine which raid characteristics best predicted the amount of brood returned in successful raids. We included raid characteristics likely to be influential: number of raiders, time of day, raid distance, and subpopulation. We log transformed variables that deviated from normality (number of raiders, amount of brood, raid distance). Numeric variables (number of raiders, amount of brood, and raid distance) were scaled prior to model fitting by subtracting the mean from each value, then dividing by the standard deviation. We used corrected Aikaike Information Criterion for small sample sizes (AICc) to compare models and model averaging to generate a final model that included information from all statistically equivalent models (i.e., those with a delta AICc < 2.0).

RESULTS

Video recorded raids regression

Our subsampling protocol allowed us to accurately estimate the number of raiding ants and brood returning from recorded raids. For raiders with brood, the total count of returning ants was equal to 8.59 multiplied by the sum of all subsampled counts of returning ants plus 84.47 (adjusted $R^2=0.77$, $P < 0.0001$). For raiders without brood, the total count of returning ants was equal to 8.44 multiplied by the sum of all subsampled counts of returning ants plus 106.10 ($R^2=0.96$, $P<0.0001$). We used these linear relationships to estimate raid size and amount of brood captured for the other 122 raids that we video recorded.

Raid characteristics

We observed raids as early as June 25 and as late as September 2 during our study years (Figure 2.1a). Across years, the highest frequency of observed raids occurred from late July to early August.

Raids always occurred in the late afternoon and occasionally persisted until after sunset. Raids began within a nearly 5-hour window (range = 15:00 - 19:42, mean = 17:24, n = 544, Figure 2.1b) and ended within a nearly 4-hour window (range = 16:43 - 20:30, mean = 18:19, n = 149).

The distance of raids was highly variable (range = 1 – 78 meters, mean = 19 meters, n = 775, Figure 2.1c). Raids were also quite variable in their estimated size (51 – 4216 ants, mean ± SD = 1297 ± 1013.41 ants, median: 951 ants, Figure 2.1d) and the estimated brood captured (0-3666, mean ± SD = 433 ± 538.45, median = 269, Figure 1e).

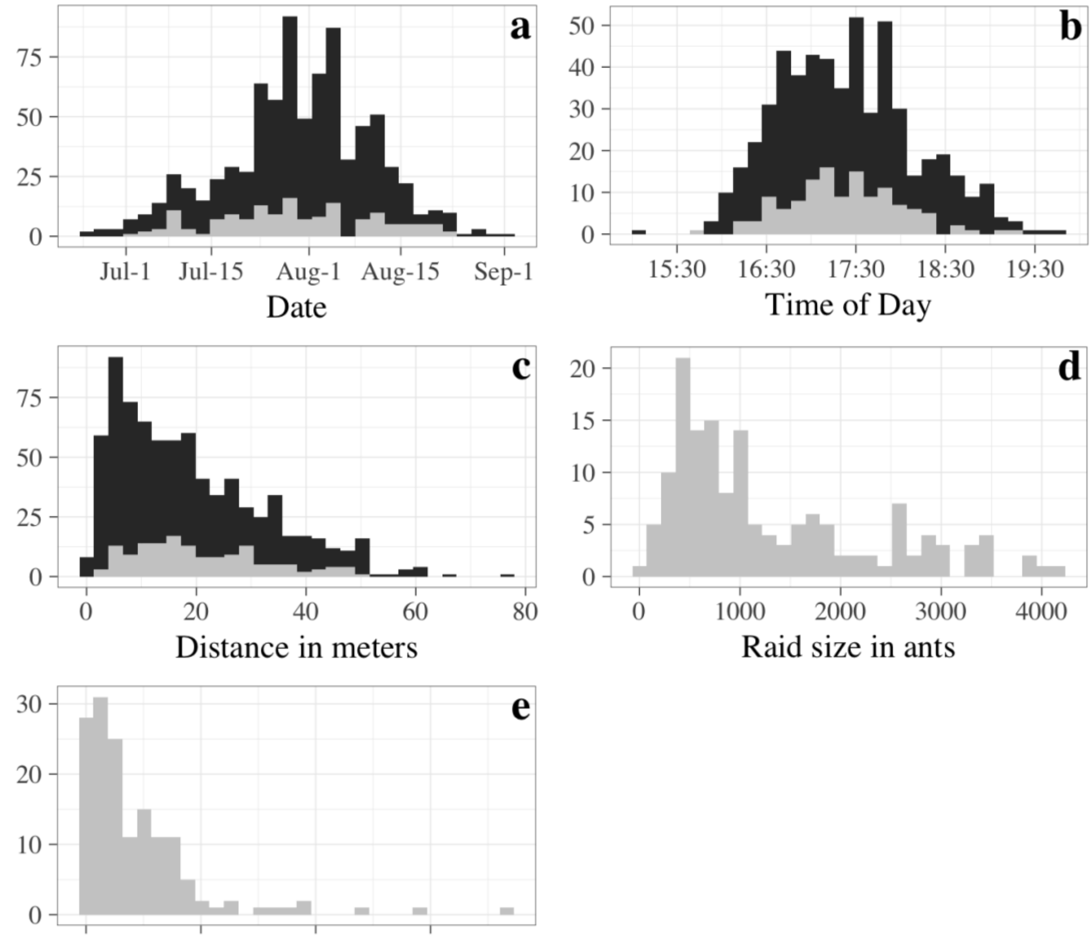

Figure 2.2: Histograms of raids by raid characteristics. Grey portions of bars represent raids that were video recorded; black portions of bars are all other raids. For all panels, Y-axis is the number of raids. (a) Month and day of raids (all years combined). (b) Start time of raids. (c) Distance of raids. (d) Raid size in number of raiding ants. (e) Brood captured per raid in combined number of larvae and pupae.

When we ignore the role of nest membership of raids, the success rate of all raids measured was 72% (n = 779 raids where we definitively knew their success or failure from field observations). Individual *Polyergus* nests also averaged a success rate of 72%, but these nest-level success rates varied widely (mean ± SD = 72% ± 18%, range = 22% - 100%, n = 35 nests where the success status of ≥ 4 raids was known).

Some measured raid characteristics covaried significantly, but in no case was this correlation stronger than ±0.5 (Pearson's r). Raid date and size were negatively correlated (Pearson's r = -0.33, $p < 0.001$). Two raid characteristics; size (Pearson's r = 0.49, $p < 0.001$, Figure 2.2a), and distance (Pearson's r = 0.29, $p = 0.002$, Figure 2.2b) were correlated with brood per raid.

Effect of subpopulation

Raids from the three genetic subpopulations did not differ in their raiding dates, start times, size (Figure 2.3a), distance, brood captured per raid (Figure 2.3b), or brood captured per raider (all ANOVA results summarized in Table 2.1).

Table 2.1: Effects of genetic subpopulation on raid characteristics. All results from linear mixed model with genetic subpopulation as a fixed effect, and nest identity; year; and site as random effects.

Response variable	Sum of squares	Degrees of Freedom	Denominator df	F value	p value
Date	206.21	2	39.582	1.39	0.26
Start time	< 0.001	2	27.255	0.28	0.76
size	729311	2	17.327	0.68	0.52
brood per raid	5.2306	2	13.335	3.02	0.08
brood per raider	3.8163	2	12.902	2.09	0.16
distance	3.8146	2	36.834	3.03	0.06

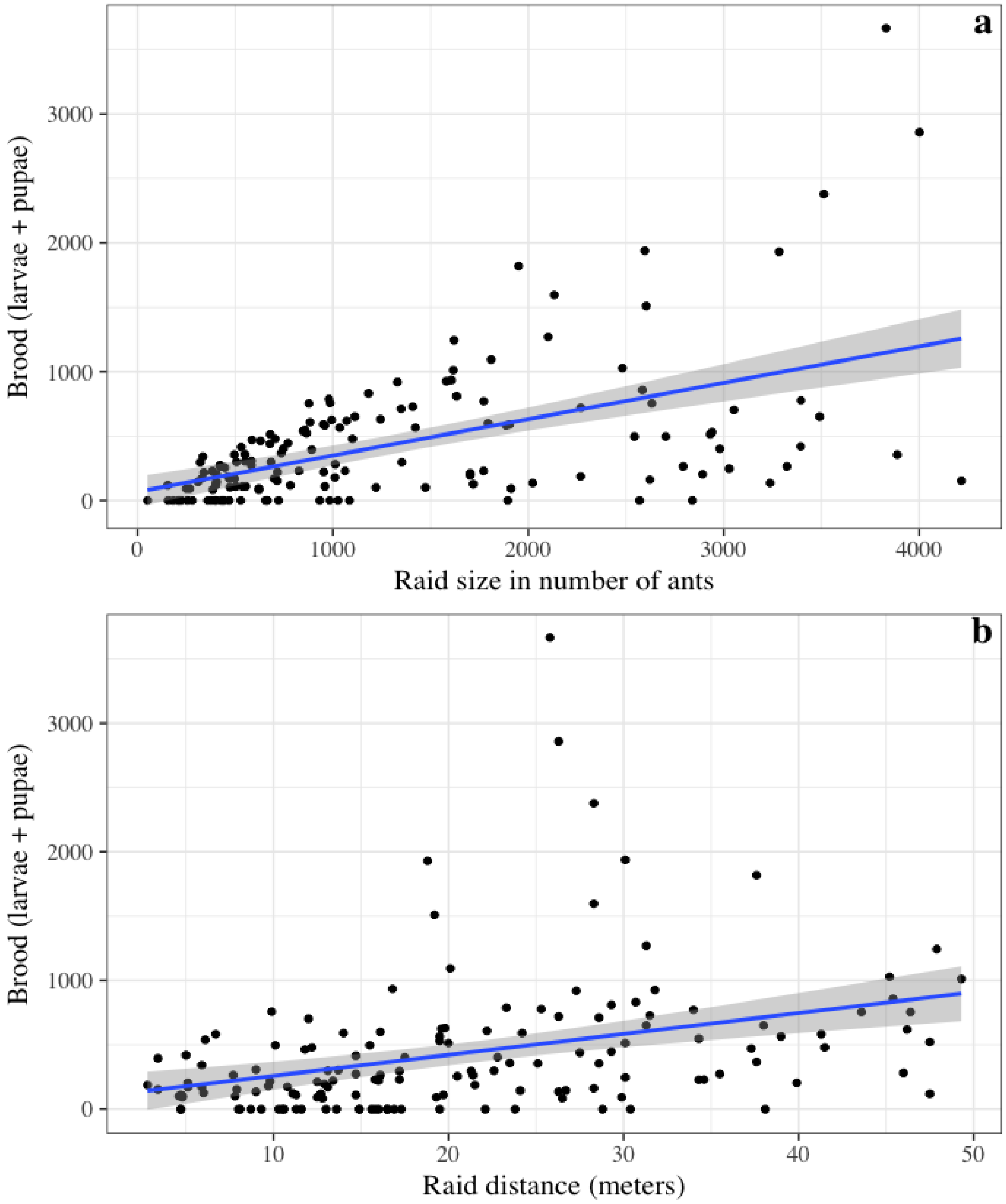

Figure 3.2: Correlates of brood per raid. Linear trend (lines) and 95% confidence intervals (shading) showing relationship of brood captured per raid with (a) size and (b) distance. Points are individual raids.

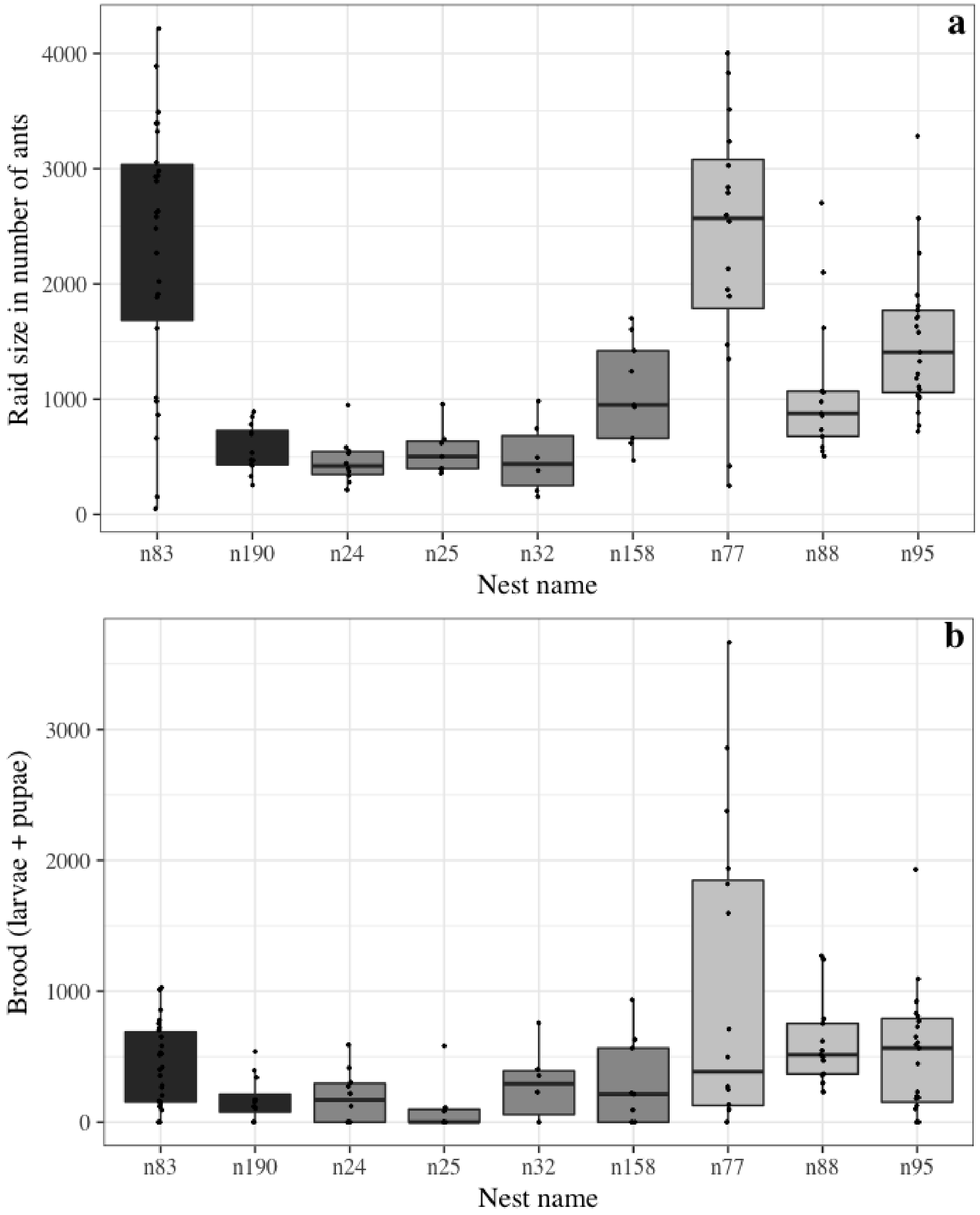

Figure 2.4: Variation in (a) raid sizes and (b) brood captured per raid within nests and subpopulations for nine nests with > 4 raids observed. Boxplots show median and quartiles, whiskers extend to furthers observations within 1.5 times interquartile range. Dots represent individual raids. Shading indicates genetic subpopulation membership of each nest. Black is Subpopulation 1, dark grey is Subpopulation 2, and light grey is Subpopulation 3.

Effect of max raid index

Max raid index affected raid size, distance, amount of brood captured, and success rate. Nests with larger maximum raids had larger raids overall (with largest raid excluded, LMM t = 2.8, estimated df = 15.58, $p = 0.013$, Figure 2.4a) and more variation in raid sizes (LM t = 14.18, df = 16, adjusted $r^2 = 0.92$, $p < 0.001$, Figure 2.4b). Nests with high max raid index values also raided further distances (LMM t = 2.8, estimated df = 15.58, $p = 0.013$, Figure 2.4c), but the variation of raid distance did not increase with increasing max raid index (LM t = 1.62, df = 17, adjusted $r^2 = 0.083$, p = 0.12). Raids from nests with larger maximum raids captured more brood per raid (LMM t = 3.69, estimated df = 15.19, $p = 0.002$, Figure 2.4d), but not more brood per raider (LMM t = 0.031, estimated df = 13.3, $p = 0.98$): the average brood per raider across all successful raids was 43% (n = 122), regardless of raid size. Raids from nests with larger maximum raids also had more variation in the amount of brood captured per raid (LM t = 4.094, df = 16, adjusted $r^2 = 0.48$, $p < 0.001$, Figure 2.4e), but again not in variation in brood captured per raider (LM t = 0.30, df = 16, $r^2 = -0.06$, $p = 0.77$). Max raid index had no effect on the proportional raid size. The average raid size was typically 57% of Max raid index (i.e. the maximum observed raid), regardless of max raid index. Nests with larger maximum raids had a higher proportion of successful raids (LM t = 2.57, df = 7, adjusted $r^2 = 0.41$, $p = 0.037$, Figure 2.4f).

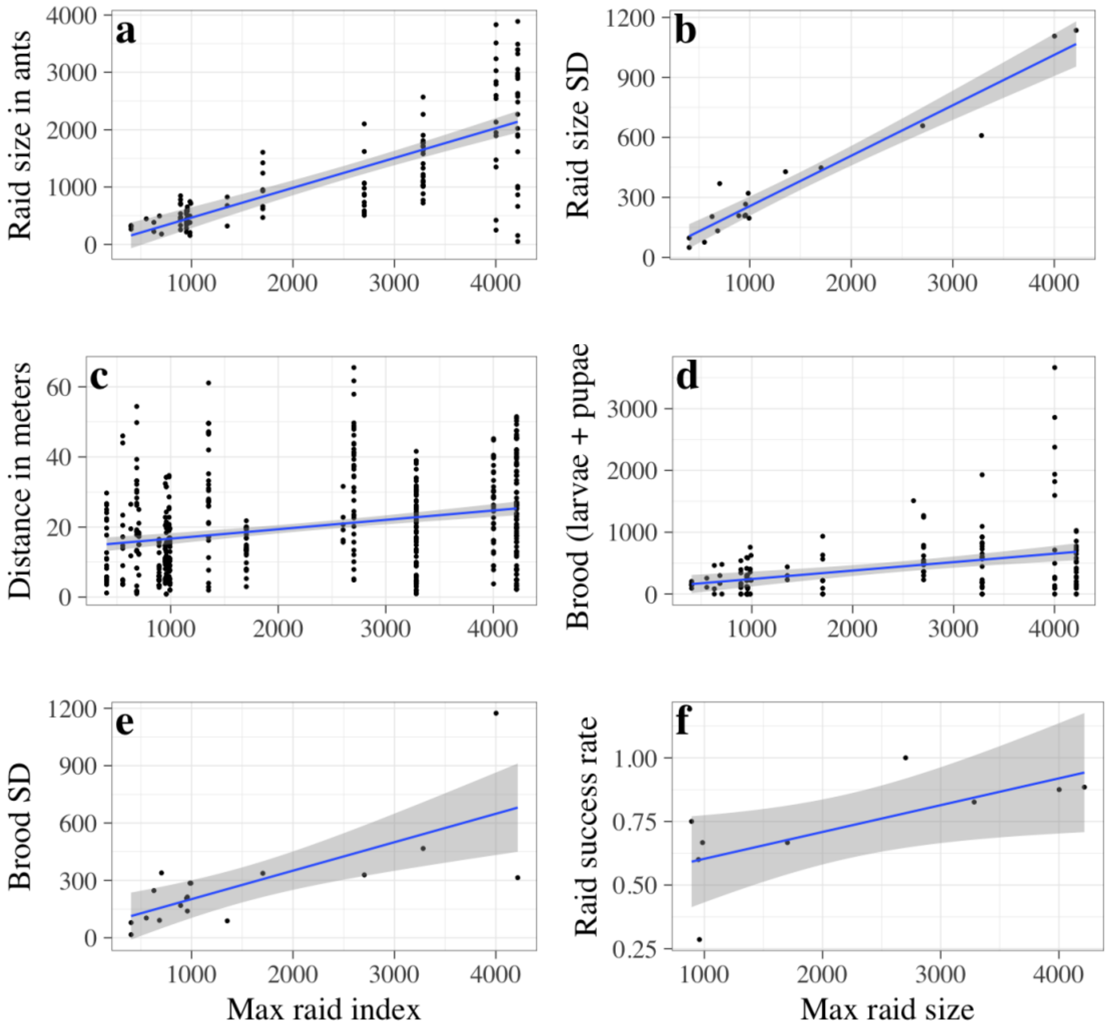

Figure 2.5: Linear relationships between max raid index and raid characteristics and outcomes. Points are individual raids, lines are best fit linear regression with 95% confidence interval shaded. For graphical purposes, lines displayed represent simple linear model where raids are considered statistically independent, but for reported statistics, nest identity was included as a random effect, and continuous variables were scaled (see methods). The largest raid from each nest was removed from dataset because it is the basis of the max raid index. (a) Size of raids as a function of max raid index. (b) Standard deviation of size of raids as a function of max raid index. (c) Distance of raids as a function of max raid index. (d) Brood captured per raid as a function of max raid index. (e) Standard deviation of brood captured per raid as a function of max raid index. (f) Nest-level success rate as a function of max raid index.

Raid Success

Raids were more likely to succeed when they started earlier in the day and went further distances, while the date of a raid had little effect on its eventual success or failure (GLMM with binomial logit link: success = 0.6274*[scaled raid distance] - 0.3875*[scaled time of day as fraction of day] -0.1022* [scaled date as days after spring equinox], distance $p < 0.001$, time of day $p < 0.001$, date $p = 0.361$; model run on 464 raids from 47 nests nested in 3 genetic subpopulations with nest identity nested within genetic subpopulation as random effects). Likelihood ratio tests that contrasted models missing each predictor against the full model confirmed the importance of distance and start time as predictors or raid success ($p < 0.001$, delta AIC = 26; time $p < 0.001$, delta AIC = 10) and the poor predictive power of date ($p = 0.36$, delta AIC = 1.16)

Raid Quality

In successful raids, those that traveled farther and had more raiders captured more brood. We found two models with delta AICc < 2.00. Both included raid distance and size as predictors, and one included subpopulation as well. However, our average model showed that genetic subpopulation did not have a strong effect on brood captured (GLM average of models with AICc < 2: log(brood) = 2.55 + 0.19(scaled log of number of raiders) + 0.12(scaled log of distance); subpop 2 effect: 0.05; subpop 3 effect: 0.04; Distance and Ant Count p < 0.001; all subpops p > 0.5).

DISCUSSION

Our data suggest that host exploitation by social parasites is sensitive to parasite condition, host quality, and environmental constraint. The traits of raids that varied most (distance and raid size) were linked to the outcomes of raids (brood and success). Though raids varied extensively within nests, we observed differences among nests in some of their raid characteristics and raid outcomes, often related to our metric of parasite condition: max raid index. By comparison, we found that raids were fairly uniform with respect to raid characteristics tied to the abiotic environment, as indicated by the start time and date of raids. The time and date of these *P. mexicanus* raids were similar to those observed in other studies of *Polyergus* populations(Czechowski 1975, 1977, Mori et al. 1991, 2001, Le Moli et al. 1994, Hasegawa and Yamaguchi 1995, Bono et al. 2006), suggesting a universal physiological constraint on foraging decisions for this genus of social parasite. Because we found no evidence of differences in raid characteristics or outcomes among subpopulations, though subpopulations are associated with distinct host *Formica*, we conclude that differences in host ecology do not affect raiding dynamics and may not play a large role in maintaining host specialization. Our method of subsampling filmed raids proved to be an effective way to capture detailed quantitative data on a large number of raids, the results of which were the basis of many of the other findings of this study.

The patterns in the distances and sizes of raids indicate that parasite condition and host quality are probably highly variable. Both distance and size vary enormously, and both were present in the two top LMMs describing brood captured per raid, which demonstrates their relevance to raid outcomes. Distance is also a significant predictor of the success or failure of raids, suggesting that *P. mexicanus* colonies will travel farther the more certain they are of a successful outcome. We did not consider raid size in the model for raid success in the interest of sample size, but think it likely that bigger raids are more likely to succeed based on personal observations in the field. *P. mexicanus* nests seem to have the capacity to tailor their raids to the quality of their target *Formica* nest, as has been shown for Italian populations of *Polyergus rufescens* (Le Moli et al. 1994). The wide range of brood captured per raid could mean that the resource quality of *Formica* nests varies widely, but also may indicate differences in a *P. mexicanus* colony's raiding abilities, as suggested by the strong relationship we find between max raid index and brood captured per raid.

Distance and size of *P. mexicanus* raids appear to be influenced and constrained by max raid index, with effects on raid outcomes. The increases in raid size, variation in raid sizes, distance, and brood per raid, associated with increasing max raid index suggests that larger *P. mexicanus* nests are capable of more versatile raids. Nests with large maximum raids still have raids that span the whole range of raid sizes, including sizes raided by nests with small maximum raids. In addition, only nests with large maximum raids obtained the highest amounts of brood per raids, but they still conduct raids that retrieve small amounts of brood. This suggests that big nests have access to a larger variety of potential *Formica* nests or they are able to more fully exploit *Formica* nests by getting more brood out of some of them. Finally, nests with larger maximum raids had a higher rate of successful raids, which further indicates that they are more proficient foragers than smaller nests.

We found no evidence that the max raid index alters the efficiency of raiding. Nests of all max raid index values had about 43% of raiders carrying brood in successful raids. This may indicate inherent tradeoffs incurred by raiders, i.e. that approximately 60% of raiding ants are needed to help with the attack but do not steal brood directly.

We found that the average size of raids was a constant percentage of max raid index; about 57%, regardless of max raid index, though the variation of individual raids within nests was very large. This constant proportion on average suggests that parasite condition plays a role to constrain the average raid size, while the high variation that remains suggests that within that constraint, raids size is calibrated to the quality of the host nest. Presumably, the amount of brood and the number of defending adult *Formica* would both increase the number of raiders sent by a *P. mexicanus* colony.

The greater distance traveled by nests with larger maximum raid sizes may indicate either an ability conferred only to those nests or a constraint only encountered by those nests. It is possible that nests that can mount larger raids incur fewer costs from long raids than nests whose raid sizes are constrained to be small because large-raiding nests can send more raiders, which may reduce the risks per raider. However, if this were true we would expect distance and raid size to be correlated, we did not find this. Alternatively, it is possible that nests with large raids are obligated to travel farther because they exhaust the host resources immediately around them. A study on *P. samurai* showed that dulotic ants can locally exhaust their host resource, creating a hole in the spatial distribution of host *Formica* nests that grows throughout the raiding season (Yasuno 1964). Our data support this idea: unlike variation in raid size and variation in brood obtained per raid, variation in raid distance does not increase with increasing max raid index. If we accept that a nest's maximum raid is related to nest size, then larger nests do not raid shorter distances, though they do have raids with the same size and outcome (i.e. brood per raid) as smaller nests.

The raid characteristics we measured were not highly correlated with each other (all < 0.5 Pearson's r), suggesting that they do not constrain each other and can be varied independently by an optimally foraging dulotic colony. Perhaps the most compelling evidence that the raid characteristics we measured reflect foraging decisions is that the strongest correlations were not between raid characteristics themselves, but between certain characteristics and raid outcome (brood per raid). Specifically, raid size and distance varied enormously within and among nests, and they had the two strongest (and most significant) correlations with brood per raid. This combination of correlation with raid outcome and extensive variation supports the role of host quality affecting parasite foraging decisions. Raiding nests assess the quality of their host nests and decide how far to raid and how many raiders to send. This idea was further supported by our LMM on successful filmed raids: The most probable model describing brood captured per raid included both raid size and raid distance.

The only significant correlation we found between measured raid characteristics was a negative relationship between raid date and raid size. Though the correlation was not strong enough to warrant conclusive inferences, it does encourage some biological hypotheses. The date of raids presumably correlates well with the phenology of host and parasite, so the relationship between date and raid size may reflect both parasite condition and host quality. *Polyergus mexicanus* raiders suffer mortality throughout the raiding season which would reduce the size of raids the colony is able to mount over the course of the raiding season. Another plausible reason raid sizes shrink somewhat over time is because host quality diminishes. *Formica* brood are disappearing over the course of the season both because they are being stolen by *P. mexicanus* raiders and they are pupating into adult workers, which are not viable objectives of dulotic raids.

A classic criticism of optimal foraging theory is that it presumes foragers have perfect knowledge of the patches they are foraging in. While most of our results are a testament to the usefulness of optimal foraging theory as a framework, the low rate of raid success highlights the criticism: many raids are not productive for the dulotic nest, especially for smaller nests. In our direct observations of failed raids raiders scramble around appearing confused, and eventually slowly trickle back to their nest empty-handed after expending much time and energy. The fact that 72% of raids failed, and the similar average success rate for individual nests, both show that there is a high level of uncertainty underpinning the optimal foraging decisions a parasitic nest makes when it raids. Small nests may often be forced to make the best of a bad job by choosing to conduct raids on well-defended *Formica* nests with sub-optimally sized raids instead of not raiding and having no chance at obtaining brood. Even for nests with large maximum raids, the variance of brood captured is high and only loosely correlated with raid size. Though nests with larger maximum raids had more successful raids, they suffered the same level of variability in brood captured per raider as smaller nests. Overall, these patterns indicate foragers do not meet the assumption of omniscience, but must have some mechanism for making optimal decisions and obtaining at least partial information on host quality. The overall predictive power of raid size and raid distance despite large variability reveals that raiders are foraging optimally.

Contrary to our expectations, the genetic subpopulation of raiding nests had little effect on raiding characteristics. Genetic isolation is probably the result of reproductive isolation of *P. mexicanus* nests due to specialization on different species of host *Formica* species (Chapter 1, D’Ettorre and Heinze 2001, Torres 2012). While host species might be expected to differ in many aspects of their life history relevant to their captors such as their foraging ability, brood care, and cuticular hydrocarbons; our data show that they at least do not differ appreciably in ways that affect *P. mexicanus* raids. Brood development for the three *Formica* host species likely have similar phenology because the dates of raids did not differ among different *P. mexicanus* subpopulations. The density of *Formica* nests is probably also similar across the three species because raid distances did not differ amongst *P. mexicanus* subpopulations.

The starting time of raids was also similar across subpopulations. We suggest that this indicates that environmental optima for raids are similar for all three subpopulations. Specifically, the starting time of raids is likely synchronized with the optimal temperature for ant activity, which would indicate that raids of the three subpopulations are occurring in similar microclimates with similar sun exposure. This seems plausible given the distribution of *P. mexicanus* nests from each genetic subpopulation: unlike some subpopulations (e.g., Cao et al. 2012), nests in our three subpopulations are intermixed across the landscape without geographic separation (see Chapter 1). Another reason *Polyergus* raiders from distinct subpopulations do not differ in their temporal onset of raiding is that they use the same environmental cue: *P. topoffi* (fomerly: *P. breviceps* Emery 1893, Trager 2013) raiders use the sun and polarized light to navigate (Topoff et al. 1984), and raids may be timed for when the sun's position maximizes its usefulness as a navigational cue.

The lack of differences among subpopulations in brood captured per raid, and brood captured per raider suggest that the three *Formica* host species do not differ in their fecundity (brood production) or defensive abilities against raiding *P. mexicanus* workers. Our data do not distinguish between these alternatives, but they do indicate that *P. mexicanus* subpopulations are capturing the same amount of brood per these two related measures of raiding effort.

Our failure to detect ecological differences in *P. mexicanus* genetic subpopulations suggests that ecology does not drive or maintain these genetic boundaries. Instead, we suggest that chemical communication and recognition differences among host species play a central role in the maintenance and generation of host races. It has long been recognized that ants have elaborate systems of chemical communication (Hölldobler and Wilson 1990) and work from taxonomically diverse dulotic species confirms the central role of chemical communication, recognition, and deception in interactions between parasitic ants and their hosts (D'Ettorre and Heinze 2001, Torres 2012).

The mating behavior of many ants may be preadapted to lead to the creation of host races prior to the evolution of socially parasitic behavior: if males and females chemically imprint on their maternal nests as a template for mate selection, nests that contain distinct hosts would have distinct signatures, and sexual ants dispersing from those nests would pick mates that match. Laboratory manipulations of host species and gas chromatography of hydrocarbons in the dulotic ant *Chalepoxenus muellerianus* confirm that both parasite mating preferences and cuticular hydrocarbons are influenced by host species (Beibl et al. 2007), and cuticular hydrocarbons of *P. mexicanus* workers are correspondingly similar to those of their resident host *Formica* species (Torres 2012).

The seasonality and start time of raids did not vary extensively in general and were similar to *Polyergus* populations studied elsewhere (Wheeler 1916, Topoff et al. 1984, Cool-Kwait and Topoff 1984, Le Moli et al. 1994, Hasegawa and Yamaguchi

1995, Bono et al. 2006). Most raids were constrained to a 70-day window in the summer, and most raids started within a 5-hour window and ended within a 4-hour window. This window of raid dates is conservative because it is limited by the arrival and departure dates of our field observers, which was always after raids had started, and before they had conclusively finished, respectively. Despite this, the rarity of raids at the tails of the distribution suggests that the observed range is close to the true limits of the raiding season. The date of raids is probably best considered an indirect abiotic factor. It is most likely driven by the parasitic colonies assessment of host quality, as measured by the amount of brood present in the host nest. Since the development of *Formica* brood is tied to time and temperature, date is a reasonable proxy for the brood condition of *Formica* nests. Future work should investigate to what extent parasite foraging decisions are based on indirect cues such as day length to decide to raid, or if they are driven by direct assessment of host colonies by scouts.

Raid start times generally occurred just after the peak heat of the day. This pattern has also been observed in other studies and likely reflects the optimal temperature for ant activity. We found that raids that started earlier were more likely to succeed than later raids. This result is likely a consequence of the abiotic environment and the physiology of ants. Raids that start later in the day are probably less likely to succeed because the air cools rapidly as the sun sets. Ant activity levels and metabolism are heavily influenced by ambient temperature. Since any raid which had *P. mexicanus* raiders return to their nest without any brood was counted as a failed raid, raids that turned around before reaching their goal because of low ambient temperature would count as a failure in our study. Also, the raiding ants are above ground and are likely more strongly affected by dropping air temperatures than the defending *Formica* workers of the nest they are raiding which are below ground. *Formica* workers often defend their nest by plugging entrances with pebbles and debris. In response to this, *P. mexicanus* raiders excavate pebbles and debris at the raiding site in attempts to find entrances. Cooler ambient temperatures should favor the defending *Formica* in this situation.

Our subsampling technique for filmed raids proved an efficient and useful way to get quantitative estimates on a large number of raids with minimal resources. Indeed, the estimates of brood per raid, raid size, and brood per raider that we obtained from this method are the basis for most of the key insights of this study. A particular advantage of this method is that it allows only a few researchers to capture detailed data from numerous neighboring and potentially interacting dulotic ant nests, even when they are concurrently raiding. Though we did not address conspecific interactions among parasites in this study, this technique should serve valuable to such studies in the future.

In this study, we use optimal foraging theory to characterize one aspect of a social parasite’s exploitation of its host: the raids of dulotic *P. mexicanus* ants. Dulotic ants in the genus *Polyergus* represent an especially interesting case for studying social parasitism because at least some species consume the majority of brood they capture (Cool-Kwait and Topoff 1984, Topoff 1985). Because of this, their raids represent both host exploitation as well as classical foraging, with another potential optimal foraging decision occurring after the raid: eat the captured brood or let it develop into a foraging host *Formica* worker?

In sum, this work illuminates several key aspects of the parasitic interaction and suggests patterns in host quality and parasite condition. Future work should assess those components directly by quantifying the location of host nests, and the sociometry of both host and parasite nests. This work indicates that parasites and hosts vary extensively, and this variation has consequences for the nature and outcome of host exploitation by parasites.

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CHAPTER 3

THE EFFECT OF HOST SPECIES ON SPATIAL RELATIONSHIPS OF NESTS AND RAIDS OF *POLYERGUS MEXICANUS*

INTRODUCTION

Roughly 40% of the organisms in any ecological community are parasites, and parasites play large if underappreciated roles in regulating these communities (Dobson et al. 2008). While there is an abundance of excellent research on the tiny fraction of parasites that are of medical importance, the majority of even this research has focused on the interactions among parasites and their hosts. Yet the interactions among parasites can be as relevant to the dynamics of parasitism as parasite-host interactions (Schmid-Hempel 2011).

Parasite-parasite interactions may have strong effects in social parasitism as well. Social parasitism is the behavioral analogue of traditional parasitism. Where classic parasites tap into host physiology for their own evolutionary benefit at the host’s expense, social parasites tap into the social arrangements of their host species by intercepting altruistic behaviors (Stuart 2002). For most social parasites, the society infiltrated is a family or group of close kin and the hijacked altruism is some form of parental care.

The commonality in mechanisms and interactions between social parasites and traditional parasites means studying one can yield insights in the other, and socially parasitic systems often have certain methodological advantages over other parasitic systems. For example, many social parasites are macroscopic as are their behaviors and the altruistic behaviors they receive. In contrast, most medically important parasites are endoparasites, which often makes observations and manipulative experiments invasive and ethically impossible.

When a population of parasite have access to multiple species of host, the parasite population can potentially form host-races: genetically isolated subpopulations of parasites that specialize on or prefer a particular host species (Goodloe et al. 1987, Gandon 2004, Balakrishnan and Sorenson 2007). Host races may form in response to past competition among parasites (Feder et al. 1995) and the existence of host races could influence present competition if parasites' host-preferences are strong: parasites that use distinct hosts should not engage in costly interference competition over host acquisition. To our knowledge, empirical support for this prediction is lacking, and the actual interactions between competition and host-races in parasitic systems are likely to be complex and contingent on numerous ecological factors, e.g.; the kinship relationships of competing parasites (Gandon 2004, Schmid-Hempel 2011). In this study, we look for spatial patterns consistent with competition, and the role of host-specificity in shaping those spatial patterns in a population of sympatric social parasitic ants.

Dulotic (often referred to as “slave-making”, see Herbers 2007) ants have a socially parasitic lifecycle defined by two key stages. The first is parasitic founding of nests by dulotic queens. Instead of excavating their own nest and laying and rearing their own eggs into sterile daughters (i.e. workers) like typical non-parasitic ants, dulotic queens search for free-living host nests to usurp. When a suitable host nest is found, the dulotic queen kills the resident queen and deceives the dead queen’s own workers into accepting her as their queen. Though many distantly related groups of ants are dulotic, acceptance by host workers is probably always accomplished via some form of chemical deception, and parasites are always closely related to their hosts (see D’Ettorre and Heinze 2001 for an excellent review). Once deceived, these host workers continue to perform all the typical tasks of worker ants including brood care of the dulotic queen’s offspring, foraging, nest excavation, and nest defense. In obligate dulotic ants, the dulotic queen and her descendants often rely on their host workers for all aspects of colony life and cannot even feed or drink without assistance from host workers(Wheeler 1905, Stuart 2002).

The second key feature of the dulotic ant life cycle is called raiding. This occurs when the dulotic queen’s own sterile daughters, having been reared to adulthood by host workers, steal larvae and pupae (henceforth “brood”) from neighboring free-living nests of their host species. This stolen brood is also reared to adulthood by existing adult host workers in the mixed-species parasitic nest that was usurped by the dulotic queen. Because host workers form chemical associations with nestmates and their home colony after pupating into adults, the stolen workers act as they would have in their natal nest; much like the workers of the assassinated host queen. These two steps – parasitic nest founding by dulotic queens and subsequent raids by her workers – ensure that dulotic queens continually maintain a large population of host workers.

Parasitic nest founding and raiding may be influenced by the conspecific interactions of queens and workers. Because of this, the spatial arrangement of raids and nests can reveal the nature of conspecific interactions, both among parasites and their hosts. The locations of parasitically founded nests are constrained to be a subset of the locations of their hosts' nests. Because of this, the spacing of active dulotic nests in an area in the present may be influenced first by host queens' decisions about nest site selection, and then by ecological factors affecting the continued survival of host colonies. Free-living host nests that survive are also subject to host-choice decisions by the parasitic queen, and finally by ecological factors that determine the survival of the parasitic mixed-species colony. The nests that survive in the present as active dulotic nests have a spatial arrangement that reflects their passage through all these ecological, evolutionary, and behavioral filters.

Competition among neighboring dulotic colonies is likely a strong ecological force shaping the spatial arrangement of nests. Indeed, competition has been described both as "one of the most important interactions within ant assemblages" (Lach et al. 2010) and "the hallmark of ant ecology" (Hölldobler and Wilson 1990). The most frequently cited evidence for intraspecific competition in ants is overdispersion; the uniform spacing of mature colonies (Parr and Gibb 2010). Though some form of interference competition is frequently the presumed or demonstrated mechanism driving overdispersion in ant nests, exploitation competition can lead to overdispersion as well (Gordon and Kulig 1996).

Dulotic raids also have spatial patterns that may reveal competition or other ecological forces. The paths of dulotic raids represent the current spatial relationships between dulotic nests, the resources they forage for (meaning free-living host nests that raiders forage for, as well as the food resources that their captured host workers forage for), and their neighboring competitors. Parasitic aids are analogous to foraging in that raiders search for a resource (hosts), and then exploit found resources. Because of this, we expect raiding ants to respond to competition as do foraging ants. Intra- and interspecific interference and exploitation competition is well known to affect foraging trails and territories of a diverse ant species. The interactions among foraging ants run the gamut from mutualistic or parasitic trail-sharing (Chomicki and Renner 2017) to classic non-overlapping territories (Adler and Gordon 2003).

Two aspects of dulotic ants' raids hint at the influence competition has on raiding: intraspecific raids, and raid overlap. Intraspecific raids are when a dulotic ant colony stages a raid on another dulotic ant colony. While this is a somewhat rare occurrence (approximately 1% of all raids we've observed at our site), it has been reported for a taxonomically broad group of facultative and obligate dulotic ant species (Topoff et al. 1984, Pollock and Rissing 1989, Le Moli et al. 1993, Pamilo and Seppä 1994). In the obligate dulotic genus *Polyergus*, to which our study species belongs, intraspecific raids are considered manifestations of interference competition both because they are considerably costlier to the raiding colony in terms of raider mortality (Topoff et al. 1984), and because conspecific territorially-motivated attacks are the most plausible explanation for the evolutionary origins of dulotic raiding itself (Topoff 1990).

There is some evidence that raids of neighboring dulotic ant nests do not overlap in both space and time to avoid costly intraspecific interactions like intraspecific raids (Bono et al. 2006b). In contrast to this finding, at our site raids intersect often, the raiding areas of nests can overlap extensively, and we have occasionally observed raids from neighboring dulotic nests cross each other in real time on their way to their respective host nest targets. The apparent lack of aggressive behavior we observed among raiders during these intersections differed markedly from the violence we've observed during intraspecific raids.

Host-specificity could explain the diversity of intraspecific interactions that occur between raiding nests. Parasites of the same host race may view each other as hostile competitors for the same host resource while those that do not share host species may exhibit tolerance towards each other. At our study site, dulotic *Polyergus mexicanus* nests form host-races for each of their three common host *Formica* species (Chapter 1, Torres 2012). Dulotic ants have high host specificity, even when multiple host species exist in sympatry, (Heinze et al. 1992, D'Ettorre and Heinze 2001, Bono et al. 2006a).

Here we examine the spatial arrangement of the nests and raids of a socially parasitic ant species that uses three sympatric hosts for patterns consistent with competition. We consider both the spatial relationships of all conspecific dulotic nests as well as only those within and across specific putative host-races. We first assess the level of clustering or overdispersion of dulotic nests. Then we measure how observed spatial overlap of raiding areas between neighboring nests compares to overlap in a spatial simulation that assumes dulotic raids have the same distances as observed raids, but no directional biases. We consider our results in the context of host-specificity, competition, and the longitudinal effects of competition at an early life-history stage (i.e. nest establishment and survival) may affect competitive interactions at a later stage (i.e. dulotic raids from mature nests).

METHODS

Study site

We studied a population of *Polyergus mexicanus* in the eastern Sierra Nevada Mountains near Truckee, CA at the Sagehen Creek Field Station (“SCFS", a University of California Natural Reserve; 39.432181, -120.241263, ~ 2000 m elevation). Note that *P. mexicanus* at this site were previously classified as *P. umbratus*, which are characterized by a long and often convex mesonotum compared to *P. mexicanus*. Though *P. umbratus* was recently synonymized with *P. mexicanus* (Trager 2013), recent genetic work indicates that *P. umbratus* is actually a distinct species, so the name may soon be resurrected (Trager, personal communication).

The site comprises a variety of habitats, but nests were typically found within 200 meters of dirt roads in disturbed (mechanically thinned for fire control) mixed-conifer forest on the south-facing slope of the Sagehen Creek drainage basin. Nests were often found associated with downed tree trunks, stumps of harvested trees, or the root structure of common understory plants such as *Ceanothus prostratus* and *Wyethia mollis*.

The elevation of study populations ranged from 1,931 to 2,124 meters over a contiguous area of approximately 9 km^2. We estimate a density of *P. mexicanus* nests at 8.4 per 100 m^2, which is greater than any we are aware of elsewhere in the literature for any species of *Polyergus.* While *P. mexicanus* nests at SCFS are only known to parasitize *F. accreta, F. argentea,* and *F. fusca*, there are approximately 20 species of *Formica* at our site, many of which are closely related quite similar to the known host species in habitat, behavior, and appearance.

Field sampling methods and design

From 2008 to 2011, we searched for raids and nests on two 10,000 m^2 study plots. In 2011, we conducted daily observations on four additional smaller (2,500 m^2) focal plots to provide more independent observations of unique pairs of interacting nests for behavioral studies. All plots were centered on a *P. mexicanus* nest and were chosen because of the high density of surrounding *P. mexicanus* nests in the area as revealed through preliminary pilot searches for nests. We created a grid of 10 x 10 meter quadrats within each plot using stakes and field flags and recorded the position of each *P. mexicanus* nest and raid point on field maps of each grid, in addition to taking GPS points of most *P. mexicanus* nests. This allowed us to make accurate (± 1m) maps of the relative positions of nests, and still anchor nests to real locations in the world (GPS accuracy ± 5m). Our observations and collection of specimens were not limited to plot boundaries: when we detected raids and nests near but outside plot boundaries, we included them in our study and referenced their position relative to the mapping grid. We searched for raids at these plots daily during the peak of the raiding season (typically during July) and used the conspicuous raids to locate both the mixed-species *P. mexicanus* nests and the nests of their host *Formica* species, which are inconspicuous.

We imported our field maps and GPS points into ARCGIS DESKTOP (ESRI 2015) using a Universal Transverse Mercator Zone 10N projection, with the 1983 North American Datum. We scanned the field maps and digitized them within the program, and converted GPS points to the correct projection and datum. We modeled raids as straight lines emanating from a *P. mexicanus* nest to its final observed point; typically a *Formica* nest, but sometimes simply the furthest point from the *P. mexicanus* nest that raiders were observed. Personal observations indicate that raids are almost always very close to straight lines, as observed for other species of Polyergus (e.g.; Hasegawa and Yamaguchi 1995).

To assess host species identity, we collected host *Formica* workers either from the *P. mexicanus* nest or from a *Formica* nest raided by the *P. mexicanus* nest. For each nest, we attempted to collect three host *Formica* workers, which we mounted according to museum standards for species identification. For two nests (Nest 19 at Site B and Nest 278 at Site 296), no Formica specimens were collected, thus slave-species IDs were not possible. We used the dichotomous keys developed by Francœur (1973) as well as several characters known to be diagnostic for the different local populations of host species (Phillip Ward and Candice Torres, personal communications) to determine the species of these *Formica* host workers. However, characters were sometimes ambiguous and *Formica* species within the sub-generic *fusca*-group (as are the three host species at SCFS) are notoriously difficult to identify (Mackay and Mackay 2002, Fisher and Cover 2007, Glasier et al. 2013).

Nest spatial analysis

We tested whether *P. mexicanus* nests were spatially clustered, overdispersed, or not different from randomly located on the landscape at each site by performing a geospatial statistical test called nearest neighbor analysis, which calculates the distance between each point and its nearest neighboring point (in this case, *P. mexicanus* nests) and compares it to the average distance that would be expected if all points were randomly arranged in the same space. Because we were also interested in the role of host *Formica* species preference in shaping competitive interactions among *P. mexicanus* nests, we also performed individual nearest neighbor analyses on *P. mexicanus* nests that used a particular host species at each site where we found at least two nests using that host species. In short, for each site we conducted up to four nearest neighbor analyses: One considering all *P. mexicanus* nests, and (if sample size permitted), one for only *P. mexicanus* nests using *F. accreta* hosts, one for only *P. mexicanus* nests using *F. argentea* hosts, and one for only *P. mexicanus* nests using *F. fusca* hosts.

Nearest neighbor calculations are very sensitive to the area size used in the analyses. Because of the nature of nearest neighbor tests and the opportunistic methods by which we searched for *Polyergus* nests, we developed a careful methodology for determining the area of study at each site that attempts to fairly estimate the actual area we searched for nests and raids at each site. We searched in either a 10,000 m^2 (for sites A and B) or 2,500 m^2 (all other sites) gridded plot, but included any nests whose raids we detected in or near these search areas. As such, the final analysis area included nests outside the bounds of the 100 m^2 or 50 m^2 study plots.

To accurately represent the real study area for the nearest neighbor analyses, we calculated the area of the bounding rectangle with the minimum area that included all known *Polyergus* nests at the site. We extended the boundary of this rectangle by half the average nearest neighbor distance of all nests at that site. If any part of the pre-designated gridded study area was not included in this shape, we added the area of that part to the total area we used for calculating expected nearest neighbor distance. In other words, we combined the designated search areas (i.e.; the pre-planned gridded study plots), and the search areas added due to raids and nests we discovered (i.e., any additional areas where raids or slave-making nests were detected) when we visited nests outside the gridded study plot. This method neither unfairly excludes nests that are right on the border of the observed area, nor area outside the study plots where we found no nests. Increasing the area analyzed is conservative when the hypothesis tested is that nests are overdispersed, not clustered.

The resulting areas used for the analysis for each site are shown as the shaded area in Figure 3.1.

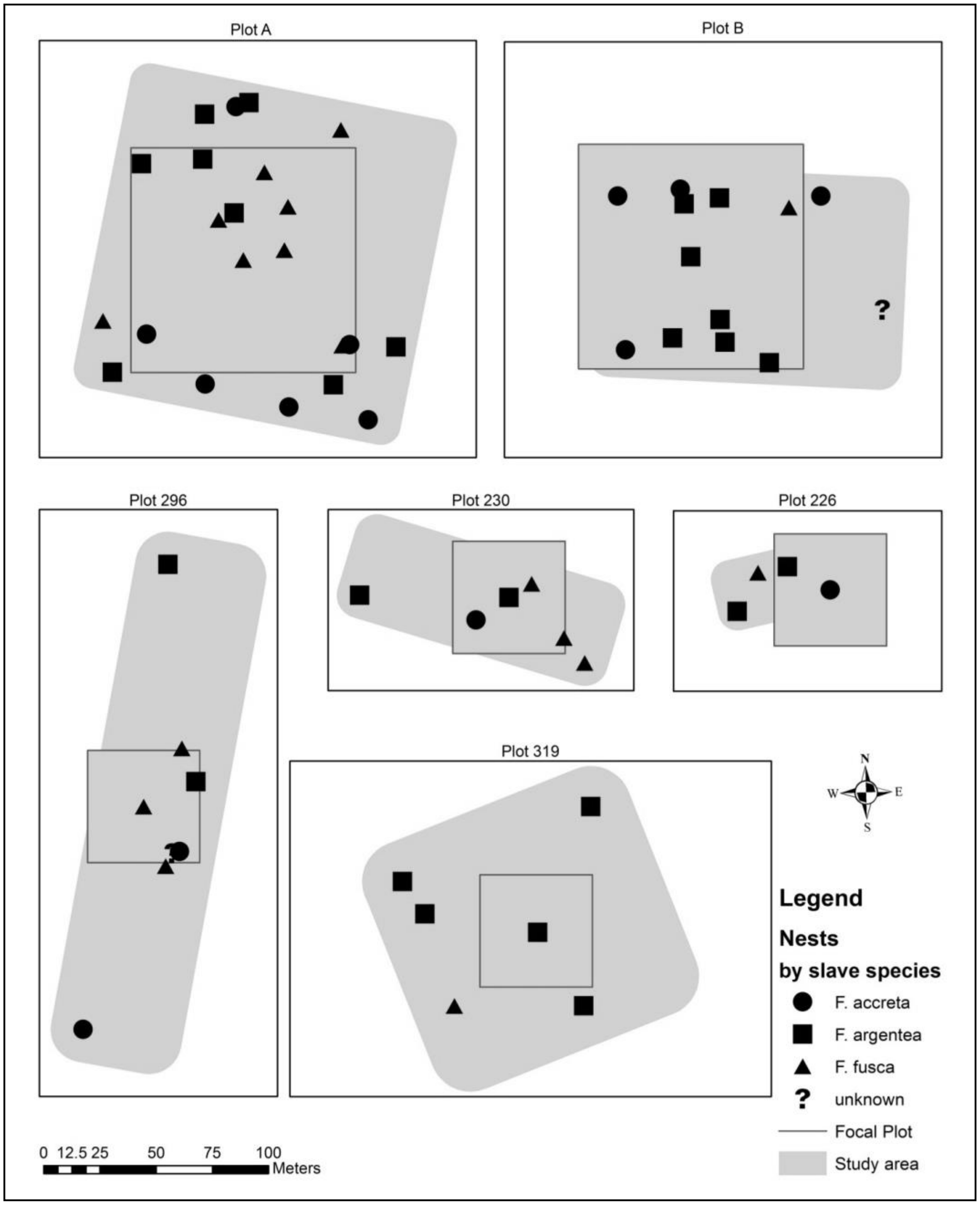

Figure 3.6: Maps of the six study plots. Orientation and scale are the same for all.

Raid spatial analysis

To measure the extent to which *P. mexicanus* raids responded to the presence of other nests, we calculated the raiding area for each nest, the amount that this raiding area overlapped with each neighboring nest's raiding area, and our null expectation of how much those areas should overlap if nests did not respond to each other's presence. To calculate the raiding area, we used ArcGIS to create a minimum convex polygon that encompassed a *P. mexicanus* nest and all the points it raided, for each *P. mexicanus* nest. We recorded the observed area of overlap between each nest's raiding area and each of its neighbors' raiding area in square meters.

We wanted to compare the pairwise overlap in raiding areas of neighboring *P. mexicanus* nests with a null expectation that preserved the innate differences nests have in the distances of their raids, but removed and bias in the directionality of raids. To do this, we wrote a Python script using the arcpy package to interact programmatically with ArcGIS. This script used the observed number of raids and distance of raids for each nest to randomly generate the same number of raids with identical distances but in random directions. It then recalculated the overlapping area of the minimum convex polygons of each nest's raiding area with each of its neighbors'. This simulated raiding area overlap was recreated 1,000 times for each pair of nests.

We determined which nest pairs had more or less raiding area overlap than the population of 1,000 randomly generated raiding area overlaps by performing a two-tailed t-test with $\alpha = 0.05$ for each nest pair. We used the t-test results to categorize each nest pair as “sharing space” if the observed area of overlap was greater than the null model, “avoiding” if the observed area of overlap was less than the null model, or “neutral” if the observed area of overlap was not distinct from the null model. Each nest pair was also assigned a “Host Species Status” of either the “same” or “different” host *Formica* species. Lastly, each nest pair was assigned to one of six possible specific “Host Species Pairs” based on the specific combination of two host species present in each nest. Host Species Pair names were abbreviated as illustrated in Table 3.1.

Table 2.1: Abbreviated names for the six possible *Formica* Host Species Pairs of *P. mexicanus* nests. By convention, the pair names are ordered alphabetically, i.e. "acc-arg" but not "arg-acc”.

First host species	Second host species	Abbreviated pair name
F. accreta	F. accreta	acc-acc
F. accreta	F. argentea	acc-arg
F. accreta	F. fusca	acc-arg
F. argentea	F. argentea	arg-arg
F. argentea	F. fusca	arg-fus
F. fusca	F. fusca	fus-fus

We performed two contingency analyses to determine the relationship between the host species and space-sharing status of pairs of neighboring *P. mexicanus* nests: One considering Host Species Status and another considering the actual identity of both host species as it affected space-sharing.

RESULTS

Polyergus mexicanus nests were overdispersed at two of the six study sites, and not different from randomly arranged at the other four. Nests were never clustered, whether we ignored their host species, or considered only nests of a particular host *Formica* species. Three sites had at least two *P. mexicanus* nests using *F. fusca* hosts, but nests at all three of these sites did not differ from random nest arrangement. All six sites had at least two *P. mexicanus* nests using *F. argentea* hosts, and these nests were overdispersed at two sites, but not different from random arrangement at the other four. Lastly, two sites had at least two *P. mexicanus* nests using *F. accreta* hosts, and these nests were overdispersed at both sites. The results of these nearest neighbor analyses are summarized in Table 3.2.

Most nests had raids that avoided neighbors' raiding space, with 73.33% of all nests sharing less raiding space than the null model predicted. In contrast to our predictions, this overall pattern was more pronounced for nests that did not share host species than for those that did ($\chi^2 = 6.159$, df = 2, p = 0.046; 79.8% of nest pairs with different host species, 61% of nest pairs with shared host species; Figure 3.2a).

Specific pairwise host species relationships also affected the amount of space shared by neighbors (Chi-squared test = 25.333, df = 10, p = 0.005). Nests that both used *F. accreta* hosts were the only group where raiding areas overlapped more often than they avoided each other (Figure 3.2b). For all other host species pairings, avoidance was the most common pairwise spatial arrangement between neighboring nests. Nest pairs that both used *F. fusca* hosts were the only host-sharing pair type

Table 3.2: Summary of nearest neighbor results for each site. Dashes indicate sites with too few nests to perform the test. Bold rows indicate $p < 0.05$.

Site	Host Species	N	Estimated Site Area (m^2)	Nearest Neighbor Ratio	z-score	p-value	Expected Mean Distance (m)	Actual Mean Distance (m)	Nest Density (nests / $1x10^4$ m^2)
226	*F. accreta*	1	3323	--	--	--	--	--	3.0
	F. argentea	2	3323	1.47	1.27	0.205	20.38	29.94	6.0
	F. fusca	1	3323	--	--	--	--	--	3.0
	all	4	3323	1.18	0.68	0.494	14.41	16.99	12.0
230	*F. accreta*	1	6121	--	--	--	--	--	1.6
	F. argentea	**2**	**6121**	**2.40**	**3.79**	**<0.01**	**27.66**	**66.39**	**3.3**
	F. fusca	3	6121	0.84	-0.53	0.597	22.58	18.98	4.9
	all	6	6121	1.28	1.33	0.182	15.97	20.52	9.8
296	***F. accreta***	**2**	**13974**	**2.16**	**3.13**	**<0.01**	**41.79**	**90.10**	**1.4**
	F. argentea	**2**	**13974**	**2.33**	**3.60**	**<0.01**	**41.79**	**97.46**	**1.4**
	F. fusca	3	13974	0.86	-0.48	0.63	34.12	29.18	2.1
	all	8	13974	1.40	2.14	0.032	20.90	29.17	5.7
319	*F. accreta*	0	14529	--	--	--	--	--	0.0
	F. argentea	5	14529	1.29	1.22	0.22	26.95	34.66	3.4
	F. fusca	1	14529	--	--	--	--	--	0.7
	all	6	14529	1.47	2.18	0.029	24.60	36.06	4.1
A	***F. accreta***	**6**	**21715**	**1.56**	**2.63**	**<0.01**	**30.08**	**46.99**	**2.8**
	F. argentea	8	21715	1.30	1.60	0.110	26.05	33.74	3.7
	F. fusca	8	21715	1.21	1.15	0.251	26.05	31.57	3.7
	all	22	21715	1.13	1.15	0.251	15.71	17.72	10.1
B	***F. accreta***	**4**	**14770**	**1.54**	**2.07**	**0.038**	**30.38**	**46.81**	**2.7**
	F. argentea	7	14770	0.75	-1.26	0.206	22.97	17.23	4.7
	F. fusca	1	14770	--	--	--	--	--	0.7
	all	13	14770	1.15	1.05	0.291	16.85	19.43	8.8

where avoidance was proportionally higher than some of the pair types that had different host species nest pairs (78.95% of fus-fus nest pairs avoiding, compared to 67.86% for acc-arg, 72.00% for acc-fus, and 94.44% for arg-fus, Figure 3.2b).

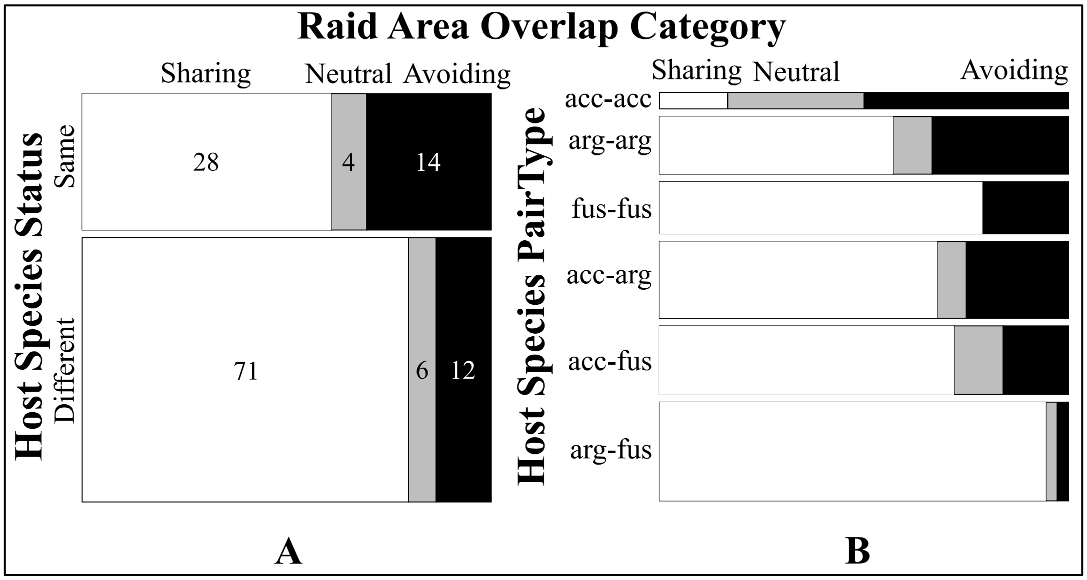

Figure 3.7: Mosaic plots of raid overlap and host species for pairs of neighboring *P. mexicanus* nests. Shading corresponds to raid area overlap category. Size of rectangles is proportional to number of observations in each category. (A) Numbers are the number of unique pairs of *P. mexicanus* nests in each category. (B) No numbers displayed, but categories are subsets of those shown in (A), so the sum of subcategories equals the numbers shown in (A).

CONCLUSION

While some of the spatial patterns we found are consistent with conspecific competitive interactions among parasites, overall, our data suggest a more complex interaction of several ecological forces. Our finding that parasitic nests are often overdispersed and never clustered indicates that competitive interactions probably influence the locations of present nest sites. However, overdispersion varied both by site and by host species. This could be driven by differences in host ecology including conspecific or heterospecific competition among hosts before usurpation by parasite queens. Many ant species are highly competitive, and nest spacing is often driven by territorial interactions with neighbors (Adams 2016).

Parasitic nests may also impose their own signature of competition on the current locations of their nests. This may happen at the nest founding stage; when parasitic queens search for host nests to usurp, or subsequently due to interference or exploitation competition. While the existence of interference competition via intraspecific raids is well documented at our site and in other dulotic ants (Topoff et al. 1984, Pollock and Rissing 1989, Le Moli et al. 1993, Beibl et al. 2005, Trager 2013), very little is known about exploitation competition in dulotic ants. There is ample evidence that exploitation competition for food and nest sites is common and a dominant force for many free-living ants (Lach 2005, Lach et al. 2010, Parr and Gibb 2010), but dulotic ants may also experience exploitation competition via their raids for more host workers. To what extent does such competition for hosts determine the fate and continued existence of parasite nests? It is likely that this question is central to understanding the ecology of socially parasitic ants, though it has been largely overlooked relative to host-parasite interactions.

The patterns we document in overlapping raided areas by neighboring nests indicate that competition is ongoing for the majority of neighboring *P. mexicanus* nests. As with patterns in nest location, our results suggest that the specific species of host may play a role in how neighboring nests interact via dulotic raids. While the evidence is in favor of mutual avoidance overall, the higher proportion of mutual avoidance between nest pairs that did not share host species relative to those that did share host species suggests that other ecological forces besides competition influence the spatial patterns of raids. One possibility is that while *P. mexicanus* nests avoid each other in general, nests that share host species must also share raiding grounds because the location of existing free-living host *Formica* nests are clustered. This possibility is intriguing in light of our conclusions about the locations of *P. mexicanus* nests, which are never themselves clustered but are a subset of the spatial distribution of all free-living host nests. If free-living host nests are clustered while parasitized host nests (i.e. *P. mexicanus* nests) are never clustered and often overdispersed, how do these two types of nests influence each other's spatial relationships? Parasitic queens' host nest selection biases and subsequent competition among parasites could create an overdispersed pattern of parasite nests, even from a clustered population of potential host nests.

Another possibility is that raiding may change the geography of host nests from overdispersed or random to clustered by reducing the numbers of nests in their immediate vicinity. There is evidence that raiding *Polyergus* nests can create holes in the distribution of host *Formica* nests that grow as the raiding season progresses (Yasuno 1964), and this phenomenon could ultimately generate a clustered distribution of remaining free-living host nests.

The specific host species of neighboring nests also had surprising effects on how parasitic neighboring nests shared raiding space. One specific pair type of nests that shared host species, acc-acc, was more likely to share space relative to our null spatial raiding model, in contrast to the general trend of avoidance. Another shared host pair type, fus-fus, was at the other extreme: it was the only shared host pair type whose proportion of avoiding nests rivaled that of types that didn't share host species.

While the sample size is too low to warrant strong inferences from these data, the trend they describe has intriguing connections to our data on nest location that suggest competition at one stage of a parasite's life cycle may mitigate competition at subsequent stages. *P. mexicanus* nests that shared *F. accreta* hosts were the rarest among our sites and always overdispersed when present, yet these nests were the only ones that shared raiding areas more often than not. *P. mexicanus* nests that shared *F. fusca* hosts are an interesting contrast: they were abundant yet never overdispersed, but had raids that appeared to avoid each other more often than the average nest pair. In other words, nests with *F. accreta* hosts appear competitive in their nest locations but not their raids, while nests with *F. fusca* hosts appear indifferent to neighbors in their nest locations, but competitive with neighbors in their raids. There are many forces that could give rise to such either pattern and the contrast between them. Future work should focus on the role of competition among hosts and parasites at several key life-history stages in shaping these patterns, and how the placement of both host and parasite nests affects the directions of raids. Additionally, we should focus on identifying key differences in host *Formica* that might generate these differences in parasite nests and their raiding behavior.

This study illustrates that spatial patterns can reveal the complexities of parasite-parasite interactions. These spatial patterns are likely driven by competition among hosts, among parasites, and host-parasite interactions. While host species specificity affects the dynamics of competition among parasites, the specific biological and ecological differences of host species may also affect the nature and strength of conspecific parasitic interactions. Data on the density, locations, and fates of host *Formica* nests; and the behavioral and physiological differences among host *Formica* nests will be invaluable in distinguishing among the many possibilities suggested by our findings, and should be the focus of future work. With these data and the present study, we will gain a much deeper understanding of conspecific parasitic interactions; a crucial and overlooked aspect of parasitology in general, and especially in the study of social parasitism.

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FINAL SYNTHESIS

Observations I made about the ecology and natural history of dulotic ants during my first visit to my would-be field site, Sagehen Creek Field Station (SCFS), compelled me to alter the focus of my intended thesis and generated a constellation of questions. From these questions, three themes have emerged: intraspecific interactions among parasites, host-specificity, and parasite diversity. These themes unite each chapter of this thesis and connect the specific results of this work to broader issues in behavioral ecology and parasitology.

The causes, consequences, and nature of intraspecific parasite interactions are the major theme of this work. Many results testify to the importance that parasite-parasite interactions can have in shaping the ecology of parasitism. By finding non-full sibling nestmates, I showed that the parasitic raids of these parasites might target each other as well as their interspecific hosts. This has important implications because it undermines the major selective advantage of being an obligate heterospecific parasite: the ability to evolve towards social parasitism without the constraint of having to evolve resistance against it. If dulotic ants have intraspecific raids and capture conspecific brood during them, then *P. mexicanus* may be both a parasite and a host as well.

Non-full sibling nestmates may be caused by several scenarios including polygynous nests (i.e. nests with multiple queens), multiply mating queens (i.e. polyandry), intraspecific raids, or even intraspecific parasitic nest founding by queens. Of these, intraspecific raids have the most empirical support. If intraspecific raids are the cause of non-full sibling nest mates, then many results here that indicate parasite-parasite interactions may be linked. For example, surprisingly high proportion of nests I detected with conclusively non-full-sibling nestmates may be connected to viscosity and kin-selected altruism among parasite nests. The presence of neighboring nests that are sometimes kin but mostly non-kin establishes the existence of population viscosity, which could influence which neighboring conspecific nests are raided by an aggressive nest. Inclusive fitness would suggest that non-kin are more likely to be the target of intraspecific raids than kin, all else being equal. Alternatively, if all things are not equal, local competition and constraints on dispersal may drive the opposite pattern in which kin are more likely to conflict than non-kin. Since kin share more genes than non-kin, it is harder to detect if nestmates have different parents from each other when the parents are kin. This means that my genetic technique of counting alleles among nestmates to determine the minimum number of parents they could have is less likely to find evidence of intraspecific raids on kin than non-kin. While I think it more likely that intraspecific raids target non-kin, it is important to recognize this potential source of bias.

Intraspecific raids may also be connected to the spatial patterns I documented. The existence and threat of intraspecific raids, which typically result in the death of the raided colony and extensive worker mortality for both the raiding and raided nests, could drive the patterns of overdispersion in *P. mexicanus* nest spacing at many sites. Intraspecific raids could also explain why the majority of nest pairs have raids that share less space than expected.

The second central theme of this work is host specificity. Host specificity connects to the first theme of intraspecific interactions in many ways. It may influence gene flow (Chapter 1) if there is positive assortative mating among parasites based on host species. Gene flow can then influence interactions between nestmates as well as interactions between neighboring nests which may be close kin. In addition to the genetic implications, host specificity can directly affect intraspecific interactions among parasites, as demonstrated with the host-species specific effects on nest spacing and raiding area I documented (Chapter 3).

Interestingly, Chapter 2 shows that host species identity has little effect on the characteristics and outcomes of raids. This seems to indicate that the ecology of the host species is similar among the three hosts, at least in ways that would affect raiding characteristics and outcomes. This poses and interesting question: If hosts are so similar to each other, why do parasites specialize? It is possible hosts may differ in some way we have not quantified. Another intriguing possibility is that the important difference is primarily the chemical signaling environment itself: even if all hosts are ecological nearly identical, they do differ in the chemical signature of their cuticular hydrocarbons, and *P. mexicanus* ants appear to mimic the signature of their specific host species (Torres and Tsutsui 2016). Perhaps differences in the chemical communication pathways of different host species drive parasites towards specialization. Parasites that switch hosts would have diminished ability to exploit their hosts due to chemical miscommunication. This scenario has precedent in avian brood parasites, where the common cuckoo seems to be constrained to particular host species more because of the limitations of egg mimicry than by ecological differences of potential hosts (Krüger 2007).

The last central theme of this thesis is parasite diversity. One example of this is the finding that the *P. mexicanus* population at SCFS comprises three distinct genetic subpopulations (Chapter 1 and Torres 2012) . Another is the striking diversity I record in basic characteristics of colonies (estimated size) as well as individual raids. I was able to understand the sources and interactions of some of this diversity. I found that nests with a higher size index had better raid outcomes and had more diverse raids. I also found that some of the variable characteristics of raids could be connected to some of the variability in raid outcomes (i.e., brood captured). Even after taking into account these relationships, much diversity in raid outcomes still remains unexplained even for raids with similar characteristics. For example, though nest size index explained some patterns, both raid characteristics and outcomes had high variability within single nests. I think some of this unexplained variation in raids can be attributed to environmental stochasticity that requires dulotic nests to conduct raids with imperfect information about their target host nest. Another likely factor is the as yet unmeasured diversity in the quality of the host target of each raid. I discuss this in more detail below when I consider future research directions.

The themes that emerge from this thesis come with attendant questions that could be explored with this specific system, yet inform bigger questions in behavioral ecology and parasitology. First, better resolving the link between genes and host use patterns would be a key contribution to understanding both host-parasite and parasite-

parasite interactions and setting the stage to ask bigger questions about social parasitism and host-use. To address this problem, we need to collect data on the diversity of hosts raided and parasitized by *P. mexicanus* nests. My research relied on the general conclusion of past work with this population and other *Polyergus* spp. populations that *P. mexicanus* nests have high host species fidelity. Thus, we used as few as three *Formica* host worker specimens from either a raided *Formica* nest or the raiding *P. mexicanus* nest to assume the species identity of all the enslaved *Formica* workers and raided *Formica* nests for each *P. mexicanus* nest. Because host fidelity has important consequences for host-parasite interactions, host population ecology, and intraspecific competition among parasites, it is worth testing this assumption in future work by identifying the species of more enslaved *Formica* in each *P. mexicanus* nest and also by identifying the *Formica* species at each raided nest to understand how strong host species specificity is. To understand host-switching, we should collect data the species of host nest chosen by new queens during parasitic nest founding. Do queens ever attempt to usurp a nest of host species that differs from their maternal nest? Do raiders ever raid more than one host species? What are the fates of such trans-host-species nest-founding and raid events?

A related issue that future work should address is the reliability of host morphology for determining species identity. In the genus *Formica*, to which all *Polyergus* hosts belong, it is notoriously difficult to identify species based on morphology (Mackay and Mackay 2002, Fisher and Cover 2007, Glasier et al. 2013). We relied on several keys, expert advice about locally diagnostic morphological features, and expert verification of some of our determinations. Nevertheless, even experts are prone to disagree about the species status of specimens from this site. One reason for this may be that closely related *Formica* species can hybridize. Applying modern genetic approaches to known host species across *P. mexicanus's* range would clarify the connection between morphological characters and species, reveal any hybridization, and potentially provide genetic tools as an alternative to morphological species identification. A fast, cheap, accurate, and high throughput method for identifying host *Formica* species, such as is done in recent work with Ultra Conserved DNA Elements (UCEs) (Smith et al. 2014), would render many of our questions about host species use tractable. Work like this could do more than estimate the frequency and importance of host switching, it could also reveal the within-species diversity of hosts inside a single parasite nest, from which we could infer how many distinct host nests are raided by different parasite nests.

Another important future direction is understanding the link between queen and male dispersal and genetic structure. I found a weak correlation between distance between nests and relatedness of nestmates, yet queens parasitize new host nests on foot, so why aren't more nearby nests close relatives? Future studies could follow the fate of new queens and new *P. mexicanus* nests to see how far away queens are actually traveling, how many survive to make nests, and how the fate of those nests relates to interactions with their maternal nest.

Sex-biased dispersal has numerous implications for population structure, mating systems, competition, and social dynamics. Since males fly to mate, while females walk, the relationship between distance and relatedness of neighboring nests may be complex. Future work should quantify the differences between male and female *P. mexicanus* dispersal and the ecological consequences of those differences. The mandibular gland sex pheromone that queens use to attract males has been successfully isolated and synthesized (Greenberg et al. 2007), making it possible to set up regularly spaced baited traps across the landscape to collect male *P. mexicanus* specimens for genetic sampling. By assessing the relatedness of males to nests across the landscape, we could estimate male dispersal distance. Queens can be followed directly from their maternal nests to their final destination.

A final suggestion for future research directions is to focus on host ecology and its effect on the diversity of parasite behaviors and raid outcomes I observed. Most of my insights into the behavioral ecology and population genetics of this social parasitism were made with almost no information about the host ecology other than the species identity of captured and raided adult hosts and the locations of raided host nests. Identifying the locations of all host nests, not just those that were raided, would be very useful for understanding how patterns of host use are related to host characteristics. If both the quality of host nests and the location of raided host nests as a subset of all host nests were known, we could add that to our existing data on parasite nest locations to determine the relative effect of intraspecific parasitic interactions versus host-parasite interactions in shaping the spacing of nests and the shape of raiding areas.

A better understanding of host *Formica* ecology and biology would also inform the larger question about the degree and nature of host specificity. Obtaining sociometric details about host colonies like the number of queens, the amount of brood, and the ratio of brood to workers could all affect key aspects of parasitic behavior like raid frequency, size, and nest-founding by queens (Tschinkel 2010). Understanding the kinds and magnitudes of the biological differences among hosts is directly related to understanding the costs of host switching and the host-specific adaptations that parasites might co-evolve to better exploit their hosts.

Many of results of this work are interconnected. Perhaps the most interesting and promising future work should take an integrative approach to unite these separate themes. I already integrated my finding on parasite genetic structure and its relationship to host species to understand differences in raid characteristics and outcomes. Future work could extend this integration of genetic data by applying the genetic patterns (including relatedness) I described to the spatial patterns of nests and raids I documented, and contrast the patterns that emerge with my existing findings on the effects of host species on parasite spatial relations.

My work on dulotic raids would also be much improved by an integrative approach. I considered the characteristics and outcomes of raids from an optimal foraging perspective, and separately considered the effects of intraspecific interactions on the spatial patterns of raids and parasitic nests. How do the ecological forces that influence optimal foraging decisions interact with the intraspecific forces like competition that influence the geography of nests and raids? Raid distance is a key feature of raids that units both topics: it is associated with successful raids and raids that obtained more brood; and it is an implicit component of the raid areas of neighboring nests and how they overlap. I assumed that distance was influenced only by optimal foraging decisions (and not by intraspecific interactions) in the spatial simulation I used to test for raiding area overlap: the model tested the idea that raids were biased in their direction, but not their distance. While that is a reasonable assumption that revealed much about intraspecific interactions among parasitic nests, it is plausible that neighboring conspecific nests affect the distances of raids as well as the direction. Again, better knowledge on the spatial distribution and ecology of host *Formica* nests would be useful here. If we knew the locations and quality of free-living host nests, we could compare the effect of suitable host nest locations to the effect of locations of putative conspecific competitors on raid distance as well as direction.

Successful experiments require a clear understanding of the study system to be relevant, but many details of this study system had not been documented well for any dulotic ant system before this work. My findings, though based on observational methods alone, lay the groundwork for future experimental manipulations. For example, the selective removal of nests of both the parasite and host could be combined with the techniques employed in this thesis to understand both optimal foraging in raids and intraspecific territoriality. Though labor intensive, the removal of nests could also be a rich source of more observational data regarding the sociometery (the quantitative measurement of all parts of an ant society) of removed colonies (Tschinkel 2010). Other manipulations could provide a detailed picture of optimal foraging decisions by manipulating the size of raiding colonies (via removal of raiding workers), or the quality of host (by manipulating the amounts of brood and workers in surrounding host nests) and measuring corresponding changes in raids characteristics and outcomes.

Parasitism is a ubiquitous feature of the biological world and social parasitism is bound to occur any time animals form societies. Much of the insight we have gained about both of these phenomena comes from a host-centric approach: How do hosts resist parasites? How do parasites affect their hosts? For dulotic ants, the conspicuousness and diversity of raids, one of two main manifestations of the parasitism, demanded a different focus. My close attention to the lives of these social parasites has confirmed that parasites are not monolithic, that parasites resemble foragers in the diverse ways they seek and exploit their hosts, and that parasite-parasite interactions should not be ignored in favor of host-parasite interactions. The social parasites studied here are diverse and complex in many regards, from their genetic structure to the strategies they employ in exploiting their host. They have abundant opportunity to interact and affect each other and all the evidence suggests that they do.

In part, the insights I have been able to make in this work are a result of the unique properties of the local ecosystem I studied. The high density of dulotic nests and diversity of co-occurring hosts were instrumental in both steering me towards and answering somewhat questions about social parasite diversity, strategies, host preferences, and interactions. The site's extraordinary features should now be used in combination with the results of this work, and better data on the natural history and ecology of host species to develop this system into a powerful natural laboratory for continued exploration of how social parasites function and what it means for the ecosystems they inhabit.

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