Mating Biology, Social Structure,

And the Evolution of Reproductive Conflict in Ants

by

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ABSTRACT

In many social groups, reproduction is shared between group members, who
compete for position in the social hierarchy for reproductive dominance. This
reproductive conflict can lead to different means of enforcing reproductive differences,
such as dominance displays or limited control of social hierarchy through antagonistic
encounters. In eusocial insects, archetypal colonies contain a single, singly-mated fertile
queen, such that no reproductive conflict exists within a colony. However, many eusocial
insects deviate from this archetype and have multiply-mated queens (polyandry), multiple
queens in a single colony (polygyny), or both. In these cases, reproductive conflict exists
between the matrilines and patrilines represented in a colony, specifically over the
production of sexual offspring. A possible outcome of reproductive conflict may be the
emergence of cheating lineages, which favor the production of sexual offspring, taking
advantage of the worker force produced by nestmate queens and/or patrilines. In extreme
examples, inquiline social parasites may be an evolutionary consequence of reproductive
conflict between nestmate queens. Inquiline social parasitism is a type of social
parasitism that is usually defined by a partial or total loss of the worker caste, and the
"infiltration" of host colonies to take advantage of the host worker force for reproduction.
It has been hypothesized that these inquiline social parasites evolve through the
speciation of cheating queen lineages from within their incipient host species. This "intra-
specific" origin model involves a foundational hypothesis that the common ancestor of
host and parasite (and thus, putatively, the host at the time of speciation) should be
functionally polygynous, and that parasitism evolves as a "resolution" of reproductive
conflict in colonies. In this dissertation, I investigate the hypothesized role of polygyny in

the evolution of inquiline social parasites. I use molecular ecology and statistical approaches to validate the role of polygyny in the evolution of some inquiline social parasites. I further discuss potential mechanisms for the evolution and speciation of social parasites, and discuss future directions to elucidate these mechanisms.

I got into grad school to make others proud.
I am graduating out of it to make myself proud.
My goal today is to be better than yesterday, and not as good as tomorrow.

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To say that a PhD is the product of work supported by many people would be tautological. "Breaking down an open door", as the French say. This dissertation is no different.

First, my heartfelt gratitude goes to my advisor Christian Rabeling. I truly cannot overstate his support, his patience, and his understanding, and how much it meant to me. He has always preached a comprehensive and rigorous academic approach to projects, and has instilled a weariness of any path of least resistance. Perhaps the defining example of this is chapter 2 of this dissertation, the first project I worked on as a rotation student in the lab, and which has lasted for over six years, as discoveries in other projects warranted more data be added to mine. Rather than publish an incomplete study, the decision was made to ensure that this project be as complete as possible, turning a relatively small natural history study into the largest dataset I have ever worked on. It is my hope that the work presented here reflects this standard of scientific rigor and comprehensiveness.

My committee must also be recognized for their roles in my success, for always being available to provide help and guidance not only for my projects, but also for helping me grow and become a better scientist. Jennifer Fewell's uncanny ability to find the weakest points of my scientific arguments and poking at them, ensuring continuous reflection for improvements. Gro Amdam instilled a drive for creativity and reasonable ambition. Olav Rüppell kept me honest when my enthusiasm carried me away from healthy skepticism. Finally, I am particularly grateful for Stephen Pratt's support since our arrival to Arizona State University, providing me with space and resources for my

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CHAPTER 1

INTRODUCTION

### 1.1 Speciation

One of the most notable goals in evolutionary biology is investigating what conditions favor the evolution of reproductive isolation in sympatry (sympatric speciation). How lineages can diverge in the presence of gene flow has been highly scrutinized, particularly as a deviation from the traditionally accepted models of speciation in allopatry (Bourke and Franks 1991; Filchak et al. 2000; Savolainen and Vepsäläinen 2003; Savolainen et al. 2006; Turner and Hahn 2010; Hadid et al. 2013; Cruickshank and Hahn 2014; Rabeling et al. 2014b; Egan et al. 2015; Toews et al. 2016; Morales et al. 2017; Ragland et al. 2017; Sachdeva and Barton 2017). Perhaps unsurprisingly, models of ecological speciation have been developed with less reliance on geography, focusing more on the forces of evolution involved in the process, which may happen in either sympatry or allopatry (Nosil 2012). Nonetheless, these models must include two elements: quantifiable variation is one or more phenotypic traits, and a reduction of gene flow associated with this variation (Nosil 2012). This limitation of gene flow between morphs may be a result of changes in life history (e.g. phenology), natural history (e.g. changes in host or substrate), behavior (e.g. assortative mating), or, more likely, a combination of these factors (Filchak et al. 2000; Savolainen et al. 2006; Ragland et al. 2017; Sachdeva and Barton 2017). Thus, in systems in which speciation with gene flow is suspected, trait-based approaches are especially powerful to elucidate the mechanism(s) of divergence. For example, palm trees on Lord Howe Island, in the

Tasman Sea, have been proposed as a model of ecological speciation in sympatry. Two closely related species have been identified as having diverged after the formation of the volcanic island, and display strict differences in soil substrate preference associated with a shift in flowering time. These shifts has resulted in a spatiotemporal barrier to gene flow leading to speciation (Savolainen et al. 2006; Babik et al. 2009; Papadopulos et al. 2011). Similarly, host plant variation in the apple maggot fly *Rhagoletis pomonella*, associated with differences in mating phenology as well as variations in sensory mechanisms, have contributed to the formation of distinct host races in the species, which may constitute the early stages of speciation in sympatry (Prokopy et al. 1988; Filchak et al. 2000; Tait et al. 2021). In ants, obligate inquiline social parasites have also been proposed as potential candidates to exemplify speciation with gene flow, based on phylogenetic and natural history observations (Buschinger 1990; Bourke and Franks 1991).

**1.2 Obligate social parasitism**

*Types of social parasitism in ants*

In ants, more than 400 species are social parasites – species that rely on their hosts' social structure to benefit their own individual fitness (Rabeling 2020). These species are divided in three distinct life history strategies: (1) The queens of temporary social parasites invade host nests, killing the queen and using the remaining workers to raise their first brood. Past this first generation, the parasitic brood develops into functional workers and the parasite colony is self-sufficient and free-living. (2) The

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queens of dulotic ants similarly invade a host colony and use its worker force for brood

rearing and colony maintenance, but their workers cannot rear brood. Thus, they must

rely on frequent raids on neighboring host colonies to obtain new workers. (3) Obligate

inquiline social parasites are workerless (or nearly workerless) species that rely entirely

on the workers present in their host nest for food and reproduction throughout their

lifetime. They are often host-queen-tolerant, and coexist in nests with their hosts (Table

1) (Buschinger 2009; Rabeling 2020).

Table 1: The three major types of social parasitism in ants, with the traits most often used to define each type.

|  | Worker caste | Host queen tolerant |
|---|---|---|
| **Temporary social parasitism** | Present | Never |
| **Dulosis** | Mostly present | Almost never |
| **Inquiline social parasitism** | Mostly absent | Almost always |

In a context of speciation research, particular interest has been given to obligate

inquiline social parasites, because they may provide good examples of sympatric

speciation. These social parasites are a highly diverse group (Hölldobler and Wilson

1990; Buschinger 2009), who "infiltrate" colonies of their host species and rely on the

present host workers to reproduce. They are notable for their drastic reduction (and

sometimes loss) of the worker caste. In some cases, host queens of colonies parasitized

by inquilines are not able to produce fertile offspring, such that the parasite offspring

benefits from all of the colony's reproductive effort, although the possible mechanisms

by which this sterilization happen are not known (Schultz et al. 1998; Rabeling and Bacci

2010). This type of social parasitism has evolved independently at least 40 times in ants, and nearly 100 inquiline social parasite species are known. In addition, many social parasites have converged on derived natural history traits compared to their hosts, such as a drastic body size reduction (see Fig. 1), asynchronous reproduction, and/or changes in the location of mating (i.e. in or on the nest as opposed to mating flights). In contrast, the origin of dulotic and temporary social parasites likely emerged as a result of either predation, or territoriality contests between competing species (Alloway 1980), and are unlikely to have arisen as a result of sympatric speciation.
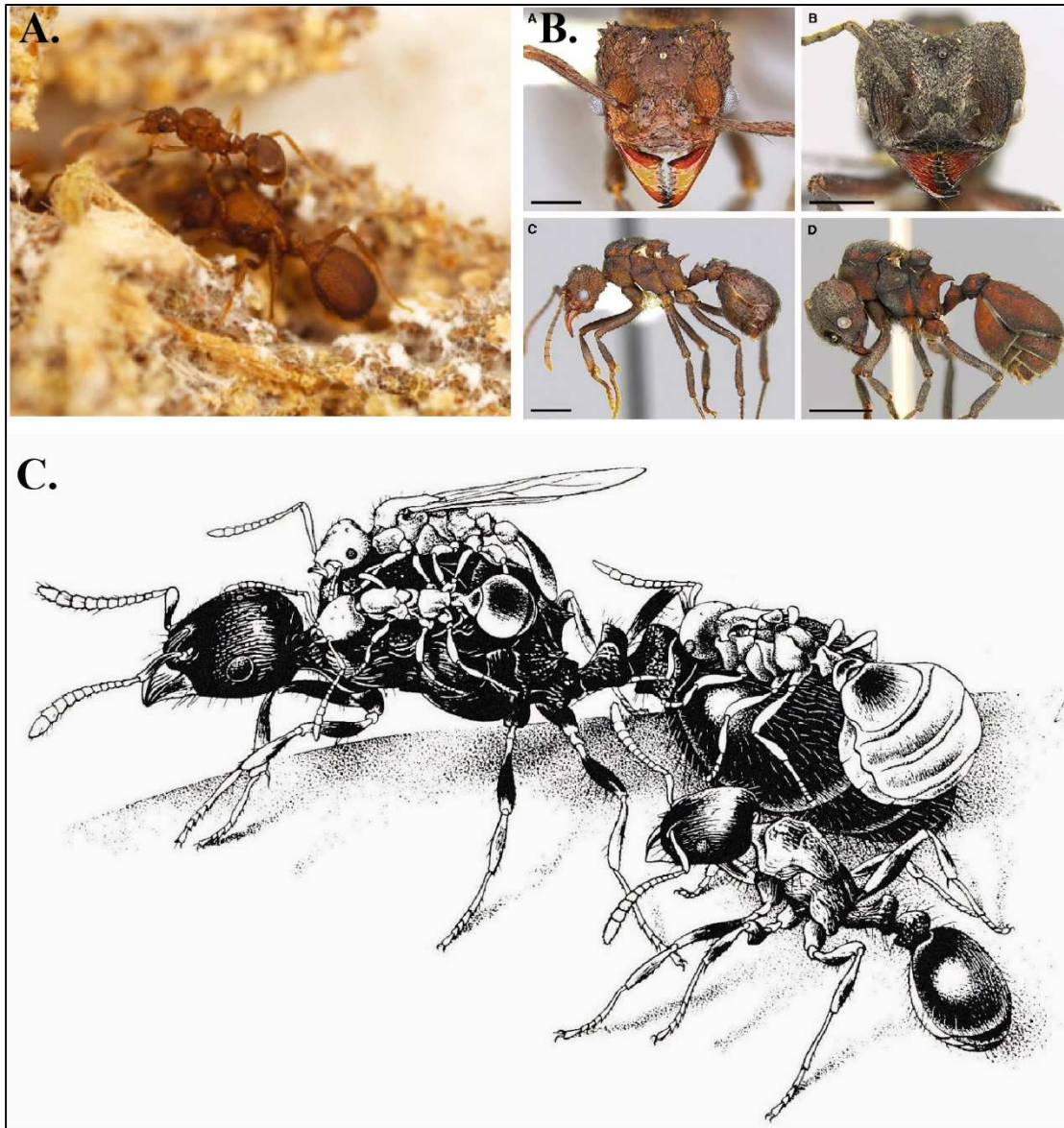
Figure 1: Examples of inquiline social parasites in ants . A. *Mycocepurus castrator* (top) rides on the back of a queen of the fungus-growing ant *M. goeldii* (photo by Scott Solomon). B. Frontal and side view of gynes of *Acromyrmex charruanus* (left) and its host *A. heyeri* (right)(Rabeling et al. 2015). C. Gynes of the "ultimate ant social parasite", *Tetramorium inquilinum*, riding on the back of a *T. impurum* queen, so-called because it features most morphological, social, and behavioral characteristics associated with inquiline social parasites (Wilson 1971).

*Emery's rule*

Emery's rule originated from an observation made by the entomologist Carlo Emery that socially parasitic ants tend to parasitize closely related species, and his inference that parasites have thus "originated from closely related forms, which [then] serve them as slave or host species" (Emery 1909). There has been a lot of debate about the validity and veracity of this generalization, and regarding its utilization as a source of evidence for investigating the mechanisms of evolution of social parasitism (Ward 1985, 1996; Maschwitz et al. 2000; Sumner et al. 2004; Huang and Dornhaus 2008). On a few occasions, Emery's rule has been formally tested cladistically and phylogenetically (Ward 1985, 1996; Agosti 1994; Parker and Rissing 2002; Savolainen and Vepsäläinen 2003; Sumner et al. 2004; Pitts et al. 2005; Rabeling et al. 2014b), using allozymes (Heinze 1991), or taxonomic data (Huang and Dornhaus 2008). The results of tests of Emery's rule have been conflicting between different species. While most inquilines have been found to agree with Emery's rule in a 'loose' form – that parasites are closely related to their hosts – not all species have been found to be their host's closest relative. Parasitic species of *Cataglyphis*, *Solenopsis* or *Leptothorax* have been found to be more closely related to other species within their genera than to their respective hosts (Heinze 1991; Agosti 1994; Pitts et al. 2005), while some host-parasite pairs are demonstrably sister species, such as *Myrmica hirsuta* and its host *M. sabuleti*, the leaf-cutting ant parasite *Acromyrmex insinuator* and its host *A. echinatior*, the lower attine *Mycocepurus castrator* and its host *Myc. goeldii*, or two of the three parasites of *Leptothorax acervorum, L. goesswaldi* and *L. kutteri* (Elmes 1978; Heinze 1991; Savolainen and

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Vepsäläinen 2003; Sumner et al. 2004; Rabeling et al. 2014b). All these tests display a wide discrepancy between evidence used, be it morphometric data (Elmes 1978), allozymes (Heinze 1991), morphology-based cladistics (Agosti 1994; Pitts et al. 2005), phylogenies based on a single CO-I fragment (Sumner et al. 2004), or advanced multiple-marker phylogenies (Savolainen and Vepsäläinen 2003; Rabeling et al. 2014b). The strict interpretation of Emery's rule – that social parasites should be their host's closest relatives – does not consider secondary evolutionary events occurring after the divergence of the host and parasite clades, such as secondary speciation, host shifts, and extinctions. Thus, the strict interpretation of Emery's rule may be difficult to detect in older host-parasite relationships. It remains clear, however, that the vast majority of inquiline ant species are closely related to their hosts (Le Masne 1956; Huang and Dornhaus 2008; Rabeling et al. 2014b).

*Evolution of inquiline social parasites*

Hypotheses for the evolution of inquiline social parasites need to account for both the close relatedness of host and parasite species and provide a mechanism of host nest invasion by parasites. Thus, it has been hypothesized that some inquiline social parasites have evolved via the speciation of reproductive cheaters in a polygynous common ancestor of the host and parasite clades in sympatry (Buschinger 1986, 1990, 2009; Bourke and Franks 1991). In this "intra-specific origin" model, obligate inquiline social parasites originate as intra-specific parasites resulting from selfish lineages in a common ancestor. Cheating involves reproductive skew in the allocation of different queens

towards reproduction in a colony, where a cheating queen may shift her reproductive

allocation towards the production of sexual offspring (as opposed to sterile workers), thus

maximizing direct fitness returns. This may result in the speciation of obligate inquiline

social parasites under two conditions: (1) reproductive cheating is heritable; and (2)

cheating is associated with a reduction in gene flow between the cheating and non-

cheating morphs (e.g. resulting from assortative mating; Fig. 2). The origin of

reproductive cheating, and in this framework of inquiline social parasitism, involves a

shift in the caste determination developmental trajectory. This apparent shift, leading to a

reduction/loss of the worker caste, is associated with numerous morphological,

behavioral, and life history traits, such as a reduction in body size, changes in mating

behavior, and in dispersal strategies (Wilson 1971; Rüppell et al. 2001a, 2002; Howard

2006). Thus, the evolution of inquiline social parasites involves the concerted evolution

of multiple traits forming complex phenotypes, and potentially leading to the speciation

of obligate parasite species.
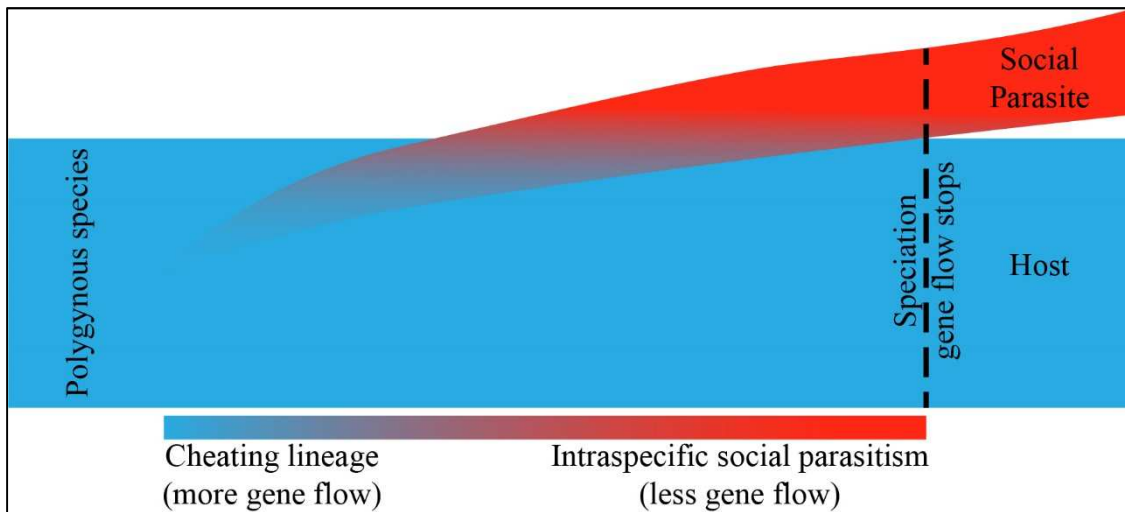


Figure 2: Conceptual schematic of the intra-specific model for
the evolution of inquiline social parasites in ants

Alternatively, inquiline social parasites may have evolved following different routes not involving intra-specific cheating, and not necessarily involving polygyny in an incipient host. The occurrence of strictly monogynous hosts, as well as host-parasite clades which seem to violate Emery's Rule, for example in *Nylanderia* (Messer et al. 2016, 2020), point to alternative models of evolution of inquiline social parasitism in different systems. Traditionally, the "intra-specific" model has been posited against an "inter-specific" model relying on the independent transition to parasitism from a closely related free-living species, thus not evolving reproductive isolation in the face of gene flow (Hölldobler and Wilson 1990; Rabeling 2020). Other models have been better supported which point to the evolution of inquiline (workerless) social parasitism from either dulotic or temporary social parasites (Buschinger 2009). Evidence for such transitions have originated primarily from parasites in *Temnothorax*, where two inquiline social parasites are nested in different clades of dulotic species (Buschinger et al. 1988; Douwes et al. 1988; Suefuji and Heinze 2015) and share some natural history traits with their dulotic relatives (such as queen intolerance). Thus, they are believed to be "degenerate" dulotic species. Similarly, *Formica talbotae*, an inquiline social parasite of *F. obscuripes,* was recently found to be nested in a clade of temporary social parasites in the *F. difficilis* species group, suggesting that the species evolved workerlessness from a temporary social parasite ancestor (Borowiec et al. 2020). It appears likely that the evolution of inquiline social parasitism follows a diversity of modes, and different models are supported in different systems (Buschinger 2009; Rabeling 2020).

Importantly, it should be noted that inferring a mechanism for the evolution of inquiline social parasitism should not usually rely solely on phylogenetic evidence. Indeed, the relative position of host and parasite species rarely provide enough evidence to determine whether an inquiline species evolved intra- or inter-specifically. In some cases, this origin can be inferred by recovering the host species as paraphyletic due to the parasite (Rabeling et al. 2014b; Nettel-Hernanz et al. 2015), but these cases are rare and fortuitous, as they rely on an extremely recent divergence of host and parasites. More often, secondary evolutionary events, such as speciation and extinctions in host or parasite clades, or host shifts, will result in a dilution of any phylogenetic pattern present at the time of speciation. Any investigation of the mechanisms of evolution of social parasites should also rely on detailed natural history and population genetic observations in hosts and parasites. In this dissertation, I investigate some of the central hypotheses of the evolution of inquiline social parasitism, specifically as they relate to an intra-specific, sympatric origin of inquiline species.

**1.3 Social structure, levels of selection, and speciation of inquiline social parasites**

*Social structure and inquiline social parasitism*

A model of intra-specific origin of inquiline social parasitism, arising through the speciation of intra-specific cheating lineages, is best explained by the occurrence of functional polygyny, i.e. the presence of multiple reproductively active queens in a single colony. Although strict monogamy is thought to have been a necessary ancestral state for

the evolution of eusociality in ants (Hughes et al. 2008a), polygyny has been commonly observed in many species, such that variation in colony social structure is widespread (Hölldobler and Wilson 1977; Rissing and Pollock 1988; Frumhoff and Ward 1992; Keller and Reeve 1994; Bourke and Franks 1995; Boomsma et al. 2014), either as an obligate feature of colonies, or as a social polymorphism in species.

While polygyny has been shown to be associated with multiple beneficial effects for ant colonies, such as increased disease resistance and/or improved productivity (Oldroyd and Fewell 2007), the presence of multiple queens in colonies also introduces conflict for reproduction, specifically over the production of sexual offspring vs. sterile workers. Additionally, polygyny can reduce the average intra-colonial relatedness, weakening the benefits of kin selection over colony reproduction. Reproductive cheating may arise if lineages evolve to exploit a polygynous social structure, shifting reproductive allocation toward alate production, and taking advantage of workers produced by nestmate queens to that end (Buschinger 1990; Bourke and Franks 1991). Cheating lineages may also be expressed as nepotistic workers, which would favor sexual brood directly produced by their mothers over brood produced by other queens (Snyder 1993; Hannonen and Sundström 2003). Thus, the emergence of parasitic traits may arise as a consequence of polygyny, such that the social structure has been hypothesized as an important pre-condition for the evolution of inquiline social parasitism in sympatry (Buschinger 1990; Bourke and Franks 1991).

Polygyny in social insects may be either primary, resulting from the co-operative founding of colonies by multiple queens, or secondary, in which new queens are adopted

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by mature colonies. While co-founding is documented to some extent in ants, only a few

cases are known to result in functional primary polygyny (e.g. the leaf-cutting ants *Atta*

*texana* and *Acromyrmex versicolor*, or the seed harvester ant *Pogonomyrmex*

*californicus*; Rissing et al. 1986; Mintzer 1987; Haney and Fewell 2018). Conversely,

secondary polygyny is thought to be more widespread, and accordingly has been

observed more often across ants (Bourke and Franks 1995; Boomsma et al. 2014; see also

chapter 3). While both primary and secondary polygyny may result in potential conflicts,

secondary polygyny has been singled out as a precursor state to inquiline social

parasitism because it also provides a route for colony infiltration that may be exploited by

parasites, as colonies may be tolerant to supernumerary queens (Bourke and Franks

1991). Additionally, while primary polygyny involves colony founding and the

cooperative production of the initial worker force of the colony by all queens, adopted

queens in secondary polygynous colonies arrive to an already established worker force,

and thus may start the production of sexual offspring immediately. Thus, secondary

polygyny introduces not only reproductive conflict between nestmate queens, but also an

opportunity for cheating lineages to evolve and thrive.

*Cheating, parasitism, and levels of selection*

Reproductive cheating in polygynous colonies is defined as a shift in reproductive

allocation toward sexual offspring in some queens, and arises from a mismatch between

selection pressures at different levels of selection. Typically, colonies of eusocial insects

are the primary subject of selection, where colony members benefit from kin selection

through the inclusive fitness from queens. In polygynous colonies, however, the success of a specific lineage is `desynchronized` from the colony success because the colony's reproductive output must now be shared between the different queens, particularly if nestmate queens are non kin. It is important to note that reproductive cheating in colonies need not be detrimental to the colony fitness or the fitness of nestmate queens, if the relatedness between cheating and non-cheating lineages in the colony is high enough to provide inclusive fitness to non-cheaters through the success of cheaters. If, on the other hand, reproductive cheating results in a decrease of the fitness of non-cheating queens at the expense of the individual fitness of cheating queens, this may be viewed as a parasitic relationship. Further, if such cheating is heritable, and results in some form of lineage sorting either via assortative mating or any other barrier to gene flow, this might result in the eventual speciation of intra-specific parasites into inter-specific obligate inquiline social parasites. Individual selection might even favor such reproductive isolation, as males issued from cheating lineages would benefit from selectively mating with cheating females, reinforcing the reproductive isolation between parasites and non-parasites. Indeed, many obligate inquiline social parasites display strikingly distinct mating behaviors from their hosts, with many species mating in nests rather than in flight, mating at different times compared to their hosts, or having distinct mating systems (Gallardo 1916; Buschinger 2009; Rabeling and Bacci 2010). These differences may point to potential sources of reproductive isolation during the emergence of these parasitic species.

*Alternative adaptations and inquiline social parasitism*

One of the most challenging aspects of elucidating the origin of inquiline social parasitism lies in explaining a shift in reproductive allocation toward sexual offspring, decreasing, and often stopping the production of sterile workers. Bourke and Franks (1991) hypothesized that smaller queens, specialized on polygyny, would promote the emergence of inquiline social parasitism by favoring conditions promoting selfishness. In short, smaller queens, for which independent founding is unfavorable, might evolve to specialize on joining established colonies, and follow an evolutionary trajectory toward social parasitism.

Of course, such a scenario necessitates mechanistic links between polygyny, reproductive cheating, and mating isolation. While it can be difficult to distinguish lineages that specialize on a specific social structure if no other phenotypic differences exist, there are many examples in ants where social structure is associated with other traits, and where a basis for these correlated polymorphisms has been investigated. For example, both the invasive fire ant *Solenopsis invicta* and the alpine ant *Formica selysi* have been found to have independently evolved social chromosomes – supergenes which form a genetic basis for social polymorphism, as well as differences in behavioral and morphological traits (Wang et al. 2013; Purcell et al. 2014). In many species, alternative queen morphs known as microgynes have been described (Fig. 3). These small queens (in comparison to the `normal` macrogynes of the same species) are typically associated with secondary polygynous social structures, and often are characterized by additional trait

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polymorphisms in behavior and physiology (Rüppell and Heinze 1999; Wolf and Seppä 2015).
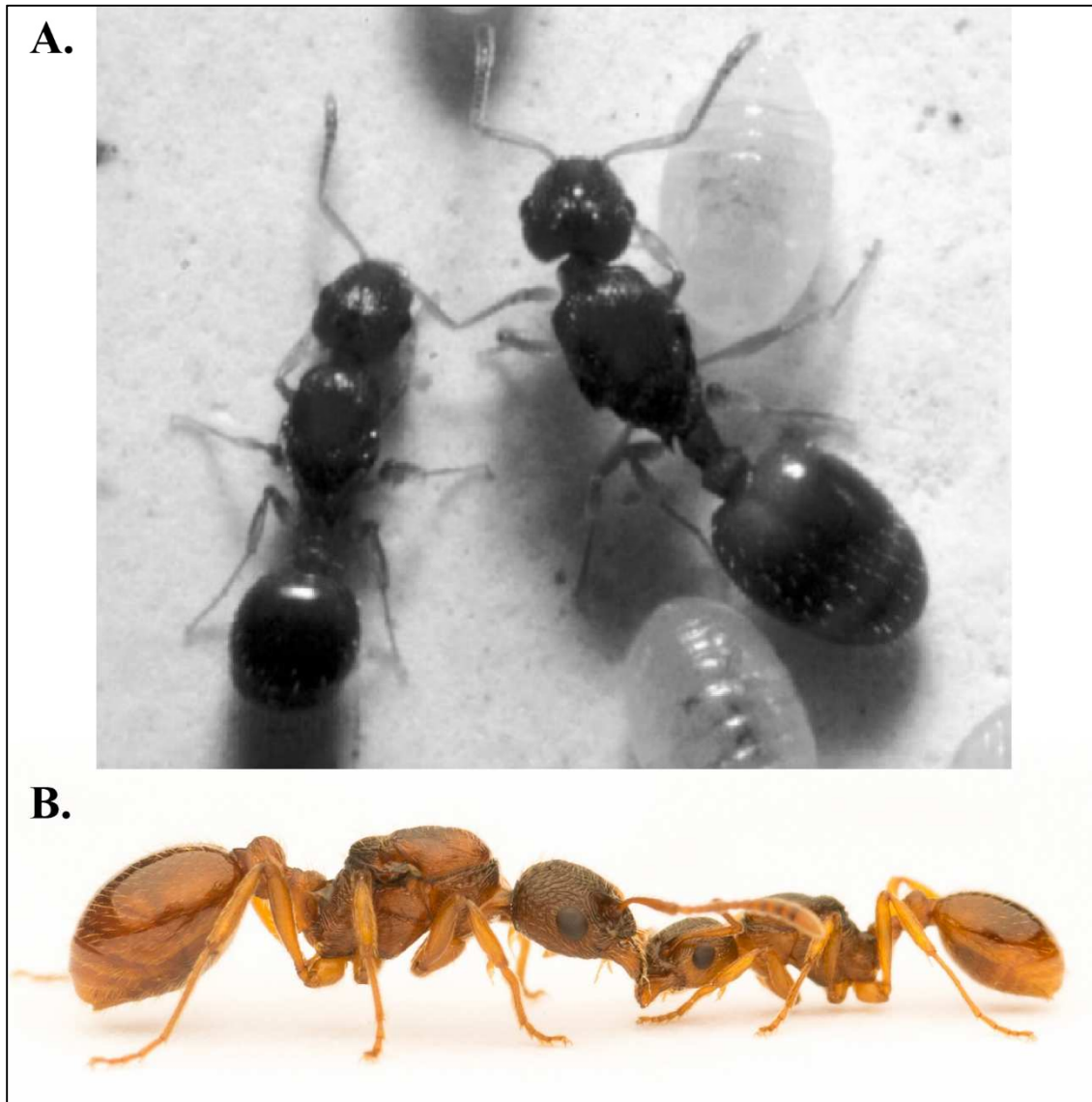


Figure 3: Two examples of microgyny in ants: A. Macrogyne (right) and microgyne of the western rock ant *Temnothorax rugatulus*. Reproduced under license from Springer Nature (Rüppell and Heinze 1999). B. Macrogyne (left) and microgyne of the European fire ant *Myrmica rubra* (photo by Michal Kukla).

In many cases, microgyne morphs have been described as alternate dispersal morphs associated with habitat saturation and high population densities (DeHeer and Tschinkel 1998; Rüppell et al. 2001a; Howard 2006). Alternatively, microgynes in other species have been proposed as intra-specific inquiline social parasites (Elmes 1973; Hora et al. 2005a), with some having been raised to the species level as hetero-specific social parasites (Seifert 1993; Steiner et al. 2006; Feitosa et al. 2008). In cases where the mechanistic basis for queen polymorphism have been investigated, results have varied greatly between species and sometimes within species, from fully penetrant genetic control (e.g. *S. invicta*, *F. selysi,* or *Myrmica rubra*; Wang et al. 2013; Purcell et al. 2014; Leppänen et al. 2015), to highly plastic and environment-specific (e.g. *Temnothorax longispinosus*; Howard and Jeanne 2013), to complex mosaics of genetic and environmental factors (e.g. *T. rugatulus*; Rüppell et al. 2001b). While both proposed explanations (alternative reproductive morph or cheating morph) have been kept distinct in the literature, it appears unlikely that they would be strictly mutually exclusive.

Body size is a well-known determinant of caste fate in ants, where smaller brood tend to develop into workers and larger brood develop into queens (Trible and Kronauer 2017). Accordingly, a shift in the size threshold of developmental trajectory where brood develops into sexual morphs at smaller sizes and given less resources might explain the occurrence of small queens as a distinct alternative morph (Nonacs and Tobin 1992; Wolf and Seppä 2015). Such developmental shift may be favored by selection at the individual level, as brood would develop into a sexual caste while being fed as a future worker would be under normal conditions. For this reason, this hypothesis is referred to as the

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selfish brood hypothesis (Nonacs and Tobin 1992). Under such a mechanism, microgynes, which would lay smaller brood on average compared to macrogynes, would consequently produce a higher proportion of sexual offspring compared to macrogynes (Nonacs and Tobin 1992; Wolf and Seppä 2015). Thus, it appears that the evolution of queen polymorphism, stemming from shifts in developmental switches, may covary with the evolution of worker caste reduction (West-Eberhard 2003). Indeed, in some species, microgynes have been shown to produce significantly higher proportion of sexual offspring compared to macrogynes (Elmes 1976; Rüppell et al. 2002; Bengston, Dahan, & Rabeling, *unpublished*), supporting a potential correlation, but not necessarily a causation, between body size polymorphism and reproductive cheating in these species. Thus, the covariance of queen size polymorphisms with differential reproductive allocation to different castes appears to be a well-suited candidate mechanism for the origin of reproductive cheating. Behavioral, life history, and morphological differences may then participate in the reinforcement of mating isolation between morphs, leading to lineage sorting and eventually speciation of inquiline social parasitism.

Anecdotally, this hypothesis appears promising as observations in inquiline social parasites suggest that species with more marked body size differences compared with their hosts also tend to have more dramatic worker caste reduction (or altogether worker caste elimination), while parasite species which are closer in body size to their host queens (e.g. *Acromyrmex insinuator*) tend to produce workers in the wild. While these observations are anecdotal, and as such do not demonstrate any causal link, such an association may be worth investigating in future studies.

**1.4 Future Directions in research**

The evolution of inquiline social parasitism in sympatry involves a series of behavioral, natural history, and morphological changes. While investigations focusing on trait differences between hosts and parasites can provide good evidence supporting such hypotheses (e.g. see chapter 2), the best way to test hypotheses regarding the emergence of reproductive isolation between host and incipient parasites involves investigating reproductive cheating and parasitism within species, or in very recently diverged host-parasite pairs.

Because of their potential for reproductive cheating, microgynes can represent strong candidates for investigating the potential emergence of inquiline social parasitism in sympatry. The genetic and environmental factors that influence queen morph determination have major impacts on the evolutionary trajectory and eventual fate of microgynes. If morph determination is governed at least in part by genetic factors, individual selection can favor cheating and eventually lead to social parasitism. Conversely, if queen morph is determined through environmental factors, it appears unlikely that microgynes would evolve into socially parasitic lineages (Wolf and Seppä 2015). Finally, genetic-by-environment factors may contribute to microgyny, resulting in a complex pattern of morph determination (Rüppell et al. 2001b; Wolf and Seppä 2015). Genetic accommodation, the genetic change in the response threshold of an environmental developmental switch (West-Eberhard 2003, 2005), can explain the emergence of microgynes via the selfish brood hypothesis, and can fully explain an association between queen morph and reproductive cheating. Thus, investigating genetic

and gene-expression differences between queen morphs during development has the potential to provide useful information regarding the evolutionary and developmental mechanisms underpinning queen polymorphism and reproductive cheating, and can also lead to testing hypotheses regarding the role of microgynes in the evolution of inquiline social parasitism.

Such studies might include next-generation sequencing in macro- and microgynes, and in the brood laid by either morphs, including (but not limited to) massively-parallel sequencing to detect any consistent genetic differentiation or transcriptome sequencing to detect differences in gene expression during development (Bengston et al. 2018). These studies have the potential to reveal key mechanisms in the emergence and evolution of inquiline social parasitism, and would provide invaluable insights regarding the evolution of reproductive isolation in sympatry.

CHAPTER 2

DECOUPLED EVOLUTION OF MATING SYSTEM AND SOCIAL STRUCTURE IN *ACROMYRMEX* LEAF-CUTTING ANTS

**2.1 Introduction**

The evolution of mating behavior and reproductive biology is central to the evolution of complex animal societies (Clutton-Brock 1985; Bourke 2014). Extensive research has been conducted linking mating biology evolution and social evolution in mammals, birds, and arthropods (Rubenstein and Wrangham 1986), notably identifying trait associations involving life history, social organization, parental care, and mating systems (Maynard Smith 1977; Clutton-Brock 1985; Choe and Crespi 1997; Stanford 1998; Lukas and Clutton-Brock 2012b). Mating systems in particular have been associated with cooperative breeding and social evolution in insects, birds, and mammals, and monogamy was identified as one important trait associated with the evolutionary origin of cooperative breeding in animal societies (Hughes et al. 2008a; Cornwallis et al. 2010; Lukas and Clutton-Brock 2012a).

The eusocial insects have been used as a model for studying the evolution of complex societies and cooperative breeding because of their complex social organization. Eusocial insects are characterized by overlapping generations, cooperative brood care, and an irreversible reproductive division of labor (West-Eberhard 1975; Wilson 1975). Lifetime monogamy is considered a precondition for the evolution of eusociality, as a strictly monogamous mating biology allows for maximum relatedness between individuals of the same colony (Hughes et al. 2008a; Boomsma 2009; Cornwallis et al.

2010; West and Gardner 2010; Lukas and Clutton-Brock 2012a). However, secondary deviations from lifetime monogamy have been observed in many eusocial insects. Interestingly, the evolution of mating biology appears asymmetric in different eusocial insect lineages. Polygyny, the presence of multiple egg-laying queens in a colony, has primarily been observed in ants, but is only observed in a small proportion of social bees, wasps, and termites (Hartke and Baer 2011; Boomsma et al. 2014). In contrast, polyandry - multiple mating by females - has frequently been observed in honeybees, but is only known from few examples in ants and vespine wasps, and is virtually unknown from bumblebees, stingless bees, polistine wasps, and termites (Strassmann 2001; Hartke and Baer 2011). Only very few taxa, all of which are ants, are known to display both polygyny and polyandry simultaneously (Keller and Reeve 1994; Pedersen and Boomsma 1999c; Schmid-Hempel and Crozier 1999; Kellner et al. 2007).

The factors underlying the evolution of polygyny and polyandry in eusocial insects have been extensively debated. Multiple hypotheses have been proposed to explain both phenomena, particularly in eusocial Hymenoptera. Two main hypotheses for the evolution of polyandry in eusocial insects are widely accepted (Ratnieks and Boomsma 1995; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Strassmann 2001; Boomsma et al. 2009): (1) The sperm-limitation hypothesis posits that multiple mating events may be required for queens to acquire enough sperm to reproduce throughout their lifetimes (Fjerdingstad and Boomsma 1998; Kraus et al. 2004; Kronauer and Boomsma 2007). (2) The genetic-diversity hypothesis proposes that increased genetic variation within colonies is beneficial and adaptive due to an increased resistance

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of genetically variable colonies to pathogens and parasites (Kraus and Page Jr. 1988; Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999; Schmid-Hempel and Crozier 1999; Tarpy 2003; Seeley and Tarpy 2007), and/or higher colony productivity due to a more efficient partitioning of task specializations among workers, through genetic polyethism (Page Jr et al. 1989; Crozier and Fjerdingstad 2001; Hughes et al. 2003; Julian and Fewell 2004; Oldroyd and Fewell 2007; Waddington et al. 2010).

On the other hand, the evolution of polygyny is addressed by two principal explanatory frameworks: (1) Ecological factors, which associate high potential cost to dispersal and/or independent nest founding due to unfavorable environmental conditions, both biotic and abiotic (Bourke and Franks 1995). Newly mated females may be selected to attempt rejoining their natal colonies (or a nearby conspecific colony), resulting in secondary polygyny from queen adoption. Queen adoption is beneficial if the likelihood of successful dispersal and colony founding is low, under pressures such as competition for resources, low nest site availability (in the case of high population density), and high predation (Bourke and Franks 1995; Seppä et al. 1995; Banschbach and Herbers 1996; Pedersen and Boomsma 1999c; Ingram 2002; Purcell et al. 2015). Similarly, primary polygyny, resulting from the association of colony-founding queens, may result from ecological pressures where solitary colony foundation is unfavorable, and co-founding results in a higher probability of a successful colony being established (Cahan and Julian 1999; Haney and Fewell 2018). (2) Social/genetic factors may promote the evolution of polygyny (both primary and secondary) by allowing for, and sometimes promoting, the co-existence of queens in colonies. The proposed benefits are aligned with the genetic

diversity benefits for the evolution of polyandry, including more efficient division of labor in a diverse workforce, greater resistance to disease and parasites, and overall more productive colonies (Bourke and Franks 1995; Schmid-Hempel and Crozier 1999; Oldroyd and Fewell 2007). In such cases, however, the lower cost of multiple mating is expected to favor the emergence of polyandry in most cases (Keller and Reeve 1994).

The main difference of polygyny versus polyandry for intracolonial relatedness is that polygyny introduces more genetic variation into a colony compared to polyandry because females produced by a single, multiply mated queen are half siblings, with an minimum relatedness of 0.25, while females produced by different unrelated mothers should not be more related than any two individuals from that population (i.e. they are only as related as the inbreeding coefficient in their population) (Pedersen and Boomsma 1999c). In some cases the increased genetic diversity introduced by polyandry is alleviated or cancelled out by inbreeding (Trontti et al. 2007), and by the fact that males mating with a single female are often related (Pedersen and Boomsma 1999b; Kellner et al. 2007). Empirical evidence has shown that experimentally combining workers from different colonies (which should then be as related as two workers produced by unrelated nestmate queens) increased disease resistance in socially polymorphic species across eusocial insects (Baer and Schmid-Hempel 1999; Tarpy 2003; Hughes and Boomsma 2004; Reber et al. 2008).

In contrast to these benefits of polygyny and polyandry, reduced colony relatedness also increases the potential for conflict in eusocial insect colonies. Nepotism (the preferential rearing of related brood by workers) is an expected consequence of

multiple reproductive lineages coexisting in a colony. While it has not been demonstrated clearly in cases of polyandry (Page Jr et al. 1989; Breed et al. 1994), empirical evidence has demonstrated occurrences of nepotism in polygynous species (Hannonen and Sundström 2003). While this phenomenon increases the inclusive fitness of nepotistic lineages, it may result in an overall reduction of colony productivity (Page Jr et al. 1989).

Polygyny itself introduces conflict over reproduction between nestmate queens, particularly for the production of reproductive females (Reeve and Ratnieks 1993; Bourke and Franks 1995). In extreme cases, this conflict can result in the evolution of obligate inquiline social parasites, as has been documented for inquiline social parasites that speciated directly from their hosts (Savolainen and Vepsäläinen 2003; Sumner et al. 2004; Rabeling et al. 2014b; Leppänen et al. 2015). These obligately parasitic species are often workerless or nearly-workerless, and "infiltrate" colonies of their hosts, taking advantage of host workers to rear their offspring (Buschinger 2009; Rabeling 2020). Secondary polygyny has been associated with the evolution of inquiline social parasitism, as it would not only provide an opportunity for socially parasitic nest foundation, but also provide the opportunity for reproductive cheating to appear, and empirical evidence supporting this association has recently come to light (Bourke & Franks, 1991; Buschinger, 2009; Dahan & Rabeling *In Review*).

Fungus-growing ants (Myrmicinae, Attini, Attina) have been studied in great detail for most of the 20th century to unravel the evolution of cooperation and conflict (Hölldobler and Wilson 1990, 2011). The leaf-cutting ant genera *Atta* Fabricius, *Acromyrmex* Mayr, and *Amoimyrmex* Cristiano, Cardoso & Sandoval contain a total of 53

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species (17, 33, and 3 described species, respectively) (Rabeling et al. 2019; Cristiano et al. 2020; Bolton 2021), which are distributed throughout the New World's tropics and subtropics. The colony and social structures of *Acromyrmex* leaf-cutting ants are known to be variable, and therefore these species are of particular interest for studying the evolution of mating behavior (Boomsma et al. 1999; Villesen et al. 1999, 2002; Murakami et al. 2000). All free-living (i.e., non-socially parasitic) leaf-cutting ant species investigated so far, which includes at least seven *Acromyrmex* species, are obligately polyandrous. In contrast, the queens of fungus-growing ants in distantly related genera are predominantly singly mated (Villesen et al. 1999, 2002). Additionally, some species of *Acromyrmex* (*A. heyeri, A. echinatior, A. subterraneus bruneus*, and *A. subterraneus molestans*), as well as *Amoimyrmex striatus,* are reported to be facultatively polygynous (Delabie 1989; Bekkevold *et al.* 1999; Diehl *et al.* 2001; Souza *et al.* 2004; Boomsma *et al.* 2014), whereas others, such as *A. landolti* and *A. octospinosus*, as well as most species of *Atta* are monogynous (with the exception of the primarily polygynous *Atta texana*, see Mintzer 1987). Therefore, *Acromyrmex* is one of the rare ant genera in which both polygyny and polyandry co-occur in multiple species (Keller and Reeve 1994; Pedersen and Boomsma 1999c; Schmid-Hempel and Crozier 1999; Kellner et al. 2007), representing a series of natural replicates that allows for comparative investigations of the evolutionary causes and consequences of complex mating behaviors in eusocial Hymenoptera, especially with respect to the co-evolution between multiple mating and polygyny. Here, I investigate the mating biology and population genetic colony structure of five species in the genus, and conduct a comparative evolutionary analysis of mating

biology and colony structure of *Acromyrmex* leaf-cutting ants. I use this comparative framework to provide an analysis of the co-evolutionary dynamics between polyandry and polygyny. Finally, I discuss our results in the context of the evolutionary consequences of polygyny and polyandry.

**2.2 Methods**

*Species collection and sampling*

Five leaf-cutting ant species were included in the study: *Acromyrmex ambiguus, A. crassispinus, A. heyeri, A. lobicornis,* and *A. lundii.* Notably, all of these species except *A. lobicornis* are known hosts of two obligate inquiline social parasites in the genus, *Pseudoatta argentina* and *A. charruanus. A. lobicornis* is the closest relative of the monophyletic clade including these social parasites (Rabeling et al. 2015). Workers, and when present, sexual reproductives (alates), of each species were collected from nests in their native ranges in Uruguay and Southern Brazil, between March 2012 and November 2013 (Fig. 4; Appendix 1). All samples were preserved in 100% ethanol and identified visually with a Leica M205 C stereomicroscope, using published taxonomic keys (Gonçalves 1961; Fowler 1985, 1988). Voucher specimens were deposited in the Social Insect Biodiversity Repository (SIBR) at Arizona State University in Tempe, Arizona.

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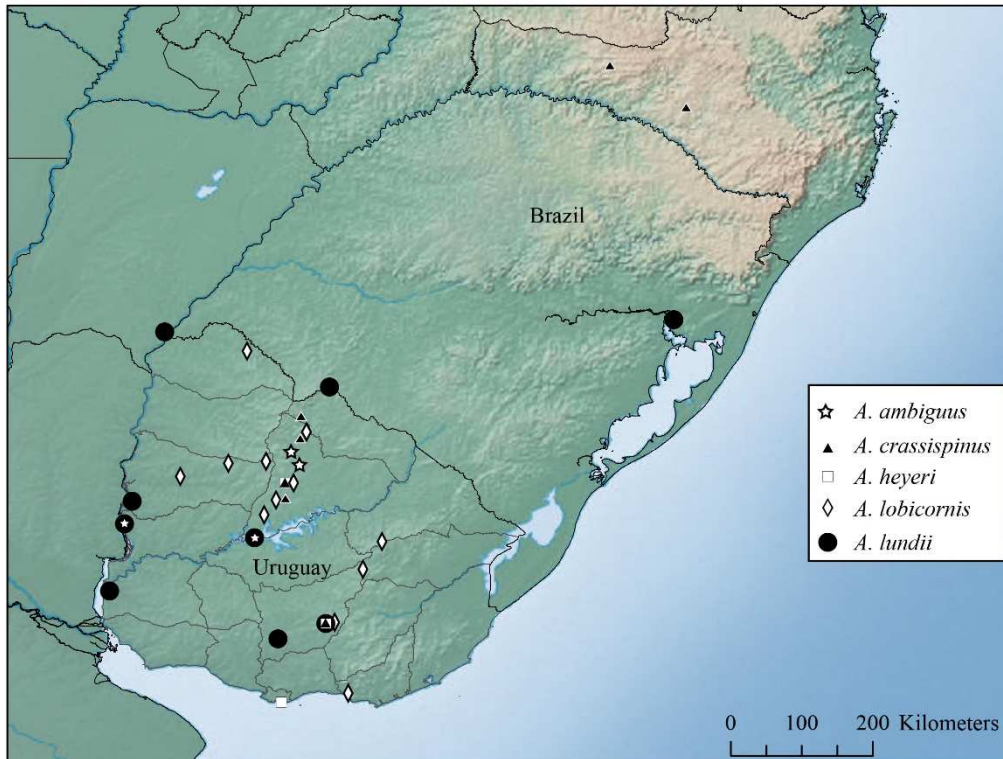
Figure 4: Sampling map depicting field study sites where *Acromyrmex* colonies were
collected

*Microsatellite selection and colony genotyping*

  To infer individual genotypes, six microsatellite loci were selected out of 22 loci

recently characterized for *A. lundii* (Rabeling et al. 2014a), based on allele diversity and

range overlap in each locus, to obtain the most informative sampling (Table 2). The

selected markers were used in three duplex PCR reactions. For each screened colony, I

sampled between 16 and 48 individuals, with a minimum of 16 workers and a maximum

of 16 males. Colony samples were maximized based on field sampling, and a total of 89

colonies were analyzed for *A. crassispinus* (n = 18), *A. heyeri* (n = 25), *A. lobicornis* (n =

18), and *A. lundii* (n = 28). Additionally, I genotyped four colonies of the related species

*A. ambiguus*, but could not complete more sampling (see SI). If sampling 48 individuals per colony was impossible, I reduced sampling by increments of 8 individuals, to a minimum of 16 workers. In all but two species, all genotypes available were from workers. For *A. crassispinus*, female alates were available in six colonies, in which I sampled 5–24 alates; in *A. heyeri*, both female and male alates were sampled from five colonies (8-12 female alates, 10-16 males). In total, I genotyped 4,614 individuals, generating 27,684 genotyped loci. DNA was purified using the Qiagen PureGene blood & tissue kit. Markers were amplified in duplex PCR reactions, each containing 1–10 ng of template DNA. Genotyping was conducted at each locus by fragment analysis, and genotypes were visually scored against a Genescan LIZ-500 standard using 6-Fam and NED tagged primers (Applied Biosystems). All scoring of genotypes was conducted in Geneious 8.1.4 (Kearse et al. 2012).

Table 2: PCR reaction designs, using six microsatellite markers developed for *A. lundii*.

| Locus | Primers | 5' Tag | Duplex |
|---|---|---|---|
| **Alun_44** | F: TATACACACCAATCGCGTCG<br>R: TGTATGTGCATTGATAGTACACGC | 6FAM | B |
| **Alun_9** | F: TGAATTCCTTGCCGAACTCC<br>R:AGGACGGATCGACAGTGAGC | NED | B |
| **Alun_32** | F: GCTTACTCATTCGCATTCGC<br>R: GGTAATACCATCGGACTTTGCG | 6FAM | C |
| **Alun_21** | F: CCTCCTCGCACATAATTCGC<br>R: GATACTTCGAACGACCTTGATCC | NED | C |
| **Alun_29** | F: CAAGTCCCGATGATTTGCG<br>R: GGAAGGGAGAGAACGCGG | 6FAM | G |
| **Alun_36** | F: AGCAGAAATCTCATATTAGGCACC<br>R: TCAGTGTATCTCTGGTTGAACGC | NED | G |

*Inference of parental genotypes*

Parental genotypes were inferred for each colony, first by using the COLONY software package (Jones and Wang 2010), followed by manual resolution using the COLONY output as a starting point. The number of queens in a colony was inferred from the results of the parental inference. Offspring with three missing loci or more in the final dataset (i.e. loci that failed to amplify after at least two PCR attempts) were discarded, as were whole colonies where more than 25% of the offspring had two missing loci or more. Whole colonies with more than 25% of discarded workers were also excluded. COLONY was run using entire species-specific datasets, updating the allele frequencies between each run. The software is equipped to handle such missing loci: the input allelic dropout rate, representing the frequency with which alleles change in offspring due to mutations, was set as $10^{-4}$, as this is the fast limit of microsatellite mutation rate observed in arthropods (Bhargava and Fuentes 2010). The genotyping error rate, accounting for extrinsic factors resulting in genotyping errors (null alleles, human error, etc.), was also set at $10^{-4}$ for each locus, as the default for the software. The results for each colony were then visually inspected to confirm the validity of the inferred maternal genotypes, and manually rechecked, correcting the inference to produce the most conservative estimate, and correcting any genotyping error. In many cases, several resolutions could account for the observed genotypes within a single colony. In these cases, resolutions were selected based on several criteria, in order of importance:

1. Parental inferences with the least number of queens (maternal genotypes) were favored;

2. If genotypes of multiple queens differed at a single locus within a colony, a single queen was inferred as mother of all the offspring of these queens, and the discordant offspring would be accounted for as genotyping error;

3. Inferences approaching a 1:1 Mendelian ratio for maternal alleles were favored (sensu Kronauer et al. 2004);

4. Inferences with the least number of sires (paternal genotypes) were favored;

5. Inferences minimizing the number of discarded individuals were favored;

6. Inferences minimizing the genotyping error rate were favored.

These criteria of selection ensured that I always chose the most conservative resolution (i.e. resulting in the lowest possible number of queens and sires) for the genotypes obtained from a single colony. Due to our sampling and inference approach, it was possible to underestimate the number of queens and sires, but never to overestimate these values. After manual resolution, maternal genotypes contributing less than four offspring (for n = 48), or two offspring (for n ≤ 24) were ignored and their offspring discarded. This threshold was selected not only to be able to calculate the segregation ratios of queens, but also to minimize false positives (offspring clustering to an additional matriline due to mutation, migration from a neighboring colony, or genotyping error); while maintaining a high probability of sampling offspring of all queens in a colony, even with moderately high reproductive skew between queens (see Appendix 1).

*Inference of mating frequencies and intra-colonial relatedness*

The effective number of mates per queen was inferred using the formula

$$m_e = \frac{(N-1)^2}{\sum p_i^2 (N+1)(N-2) + 3 - N}$$

where N represents the total number of successfully assigned offspring, and $p_i$ represents the proportion of offspring produced by the $i^{th}$ father (Nielsen et al. 2003). I then calculated inter-individual relatedness ($r$) within all colonies following the Queller & Goodnight method (Queller and Goodnight 1989).

*Reproductive skew*

We investigated reproductive skew (describing inter-individual differences in offspring production) between the different patrilines mated to each single queen, using the Pamilo-Crozier skew index (Pamilo and Crozier 1996). In all cases, only workers were used to determine reproductive skew. The index represents the deviation between the number of patrilines observed mated to a queen and the effective mating frequency of a queen, standardized to the maximum variance in offspring contribution:

$$S = \frac{M - m_e}{M - 1}$$

Here, M is the inferred number of males mated to a queen, and $m_e$ is the effective mating frequency of that queen (see above). This index varies between 0, representing no skew, with equal contribution between all sires mated to a queen, and 1, representing a case where the reproductive skew is absolute and a single sire is producing all offspring. Note that the inference method presented here means that the value of S can never be exactly 1, since I am relying on patriline detection in the offspring generation to infer the males represented in the colony.

*Statistical analyses*

We investigated variation in empirical mating frequency (number of males mated to each queen), effective mating frequency, reproductive skew, and average intra-colonial relatedness for all five species. Observed mating frequency, effective mating frequency, and skew index were compared between species using Kruskal-Wallis tests, performing post-hoc pairwise Mann-Whitney U tests where appropriate, applying a false discovery rate correction in all cases (FDR = 0.05). The response variables (number of mates, effective mating frequency, and reproductive skew) can be expected to violate assumptions of normality, therefore, non-parametric tests were favored. In order to assess the roles of effective mating frequency and social structure on average intra-colonial relatedness between species, a linear regression was fit across all species with relatedness as a response variable, and social structure, effective mating frequency, and their interaction, as well as specie, as predictor variables. Effective mating frequency was log-transformed for this analysis. Assumptions of residual normality and homoscedasticity were met for the regression (Fig. S1). I excluded *A. ambiguus* from all interspecific analyses to avoid statistical issues involving low power and uneven sampling, as I were only able to analyze four colonies. For the intra-specific estimates of mating behavior, I chose to report the data for the four *A. ambiguus* colonies, however, I would like to caution that these results should be regarded as preliminary. Within species, I compared both the average observed relatedness (r) and the effective mating frequency between polygynous and monogynous colonies. Because of our sampling, these comparisons were only possible for *A. heyeri* and *A. lobicornis*. In these cases, I used student's t-tests (with

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equal variance) to compare the groups, except to compare effective mating frequency in *A. heyeri*, where a Welch two sample t-test was more appropriate to account for unequal variance. In all cases, I used the average value for each colony as a replicate within a group to avoid pseudoreplication (averaging the effective mating frequency of all queens in a colony for polygynous colonies). In all cases, I set the significance threshold at $\alpha =$ 0.05. All analyses were performed in R 4.1.0, using the packages 'related' and 'ggplot2' (Wickham 2009; Pew et al. 2015; R Core Team 2021).

### 2.3 Results

*Social structure of* Acromyrmex *leaf-cutting ants*

We investigated the social structure of five South American species of *Acromyrmex* leaf-cutting ants based on 27,684 genotypes among six loci generated for 4,614 individuals. I detected different social structures and mating behaviors among these species. All 28 colonies of *Acromyrmex lundii* were inferred to be monogynous. In contrast, *A. crassispinus* (2/18 polygynous; 11%), *A. heyeri* (5/25 polygynous, 20%), and *A. lobicornis* (5/18 polygynous; 28%) were socially polymorphic, consisting of both monogynous and polygynous colonies (Table 3). Among the facultatively polygynous species, most polygynous colonies comprised two matrilines, with the exception of one colony of *A. crassispinus* in which three queens were inferred, and one colony of *A. lobicornis* in which four queens were inferred (Table 3). One out of four *A. ambiguus* colonies was polygynous with two matrilines, whereas the other three colonies were monogynous, suggesting that *A. ambiguus* is also facultatively polygynous.

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Table 3: Summary of social structure in colonies of five *Acromyrmex* leaf-cutting ants. The numbers in parentheses in the top row represent the number of polygynous colonies over the total sampling for the corresponding species.

| | *A. ambiguus* | *A. crassispinus* | *A. heyeri* | *A. lobicornis* | *A. lundii* |
|---|---|---|---|---|---|
| *Number of colonies sampled* | 4 | 18 | 25 | 18 | 28 |
| *Frequency of polygyny* | 25.00% (1/4) | 11.00% (2/18) | 16.00% (4/25) | 22.22% (4/18) | 0.0% (0/28) |
| *Maximum number of queens* | 2 | 3 | 2 | 4 | 1 |
| *Minimum number of mates* | 4 | 1 | 2 | 1 | 1 |
| *Maximum number of mates* | 10 | 12 | 12 | 4 | 6 |
| *Average number of queens per colony (std. deviation)* | 1.25 (0.50) | 1.17 (0.51) | 1.15 (0.37) | 1.39 (0.78) | 1 (N/A) |
| *Observed mating frequency M (std. deviation)* | 6.00 (2.35) | 6.476 (3.73) | 5.50 (2.57) | 2.88 (1.08) | 4.18 (1.63) |
| *Effective mating frequency $m_e$ (std. deviation)* | 5.92 (2.41) | 4.51 (2.85) | 4.43 (2.20) | 2.36 (0.80) | 3.33 (1.40) |
| *Reproductive skew S (std. deviation)* | 0.09 (0.14) | 0.41 (0.23) | 0.26 (0.15) | 0.26 (0.21) | 0.25 (0.21) |
| *Intra-colony relatedness r (std. error)* | 0.42 (0.10) | 0.46 (0.03) | 0.36 (0.03) | 0.46 (0.03) | 0.46 (0.03) |

*Multiple mating and effective mating frequency of* Acromyrmex *queens*

In all five species, queens used sperm from multiple males to produce offspring, although there were marked differences between species (Table 3; Kruskal-Wallis test:

$\chi^2_3 = 21.66$, $p = 7.67 \times 10^{-5}$). Specifically, the observed mating frequency of *A. crassispinus* queens (average observed mating frequency M = 6.48) is higher than that of the other three species, although it is indistinguishable from that of *A. heyeri* queens (M = 5.50; Table 3; Fig. 5a). Conversely, queens of *A. lobicornis* are mated to significantly fewer males compared to the other species (M = 2.88). It is worth noting that queens of *A. crassispinus* and *A. heyeri* also mate with a higher maximum number of mates (max. 12 mates) compared to *A. lobicornis* (max. 4 mates) and *A. lundii* (max. 6 mates) (Table 3). Preliminary analysis suggests that queens of *A. ambiguus* mate with a high number of males (M = 6.00, max 10 mates), appearing comparable to queens of *A. crassispinus* and *A. heyeri*.

Interestingly, in spite of the higher empirical mating frequency in *A. crassispinus*, this species had a similar effective mating frequency ($m_e$ = 4.51) compared to *A. heyeri* ($m_e$ = 4.43) and to *A. lundii* ($m_e$ = 3.33) (Table 3). Similar to the pattern observed for the empirical mating frequency, the effective mating frequency of queens of *A. lobicornis* ($m_e$ = 2.36) was significantly lower than in the other species (Kruskal-Wallis test: $\chi^2_3 = 17.81$, p = 4.81 x $10^{-4}$; Fig. 5b). In all cases, the observed number of mates was much lower than the number of sampled offspring, ensuring unbiased estimation of $m_e$ following the assumptions of the estimator (Nielsen et al. 2003). In *A. ambiguus*, the effective mating frequency of queens was comparatively high ($m_e$ = 5.92).
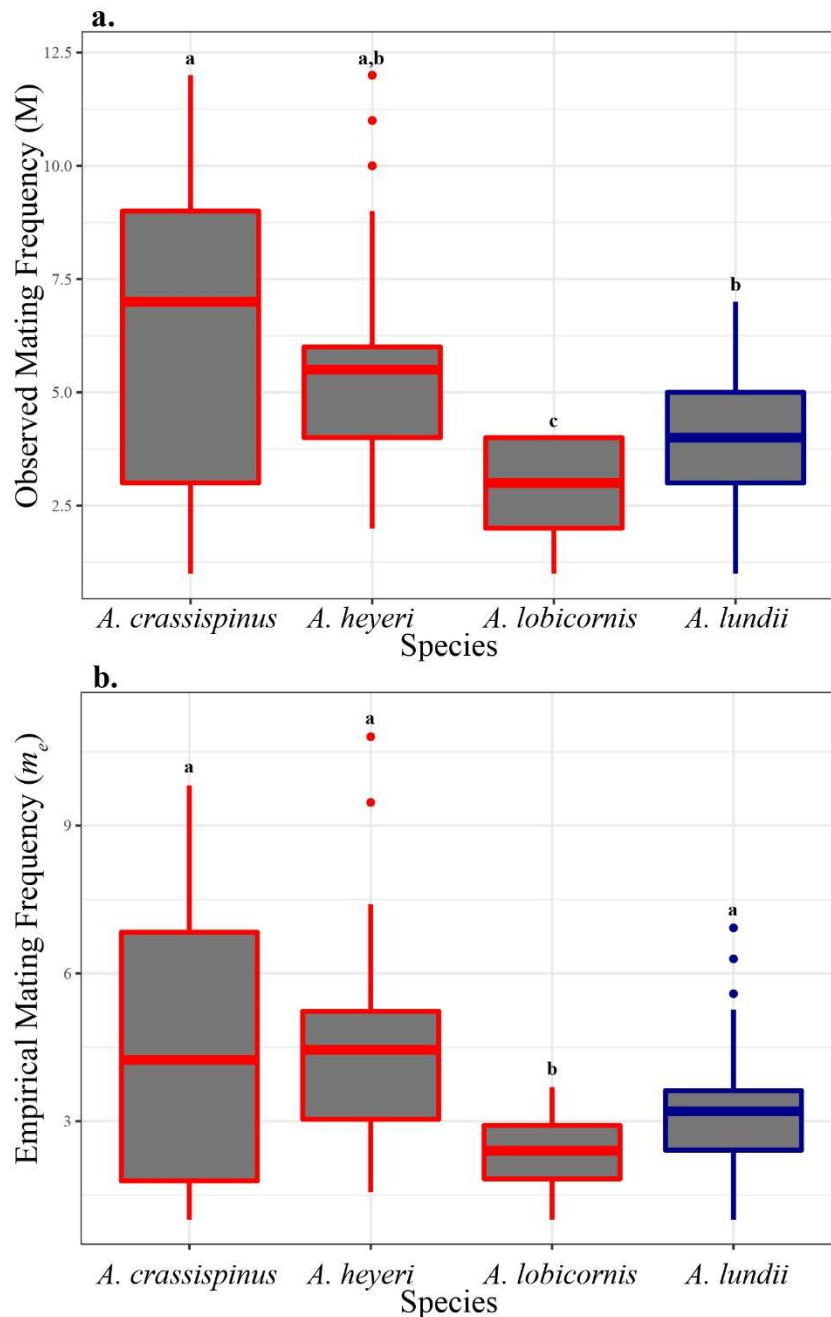
Figure 5: Mating frequency in four species of *Acromyrmex* leaf-cutting ants. A) Empirical mating frequency, the total number of males mated to a single queen. B) Effective mating frequency, the effective number of males contributing equally to a queen's offspring production (see text). Letters above the boxes represent the significance groups recovered from pairwise Mann-Whitney test with FDR correction ($\alpha = 0.05$). Red (grey) boxes represent facultatively polygynous species, blue (black) boxes represent monogynous species. (Parenthesis colors refer to grayscale equivalences).

*Reproductive skew*

We detected reproductive skew between patrilines in all species (Fig. 6). While the values recovered varied between ~0.1 and ~0.45 (a value of 0 indicates an equal reproductive share between males, whereas a value of 1 indicates that all offspring are sired by a single male), no significant differences were found between species (*A. crassispinus*: S = 0.41, *A. heyeri*: S = 0.26, *A. lobicornis*: S = 0.26, *A. lundii* = 0.26; Kruskal-Wallis test: $\chi^2_3$ = 6.82, *p* = 0.08; Table 3, Fig. 6). These statistical results suggest that variation in skew within and between species thus appears to be governed by random variation between colonies potentially reflecting that genotyped individuals represent a snapshot in time during the colonies lifetimes.
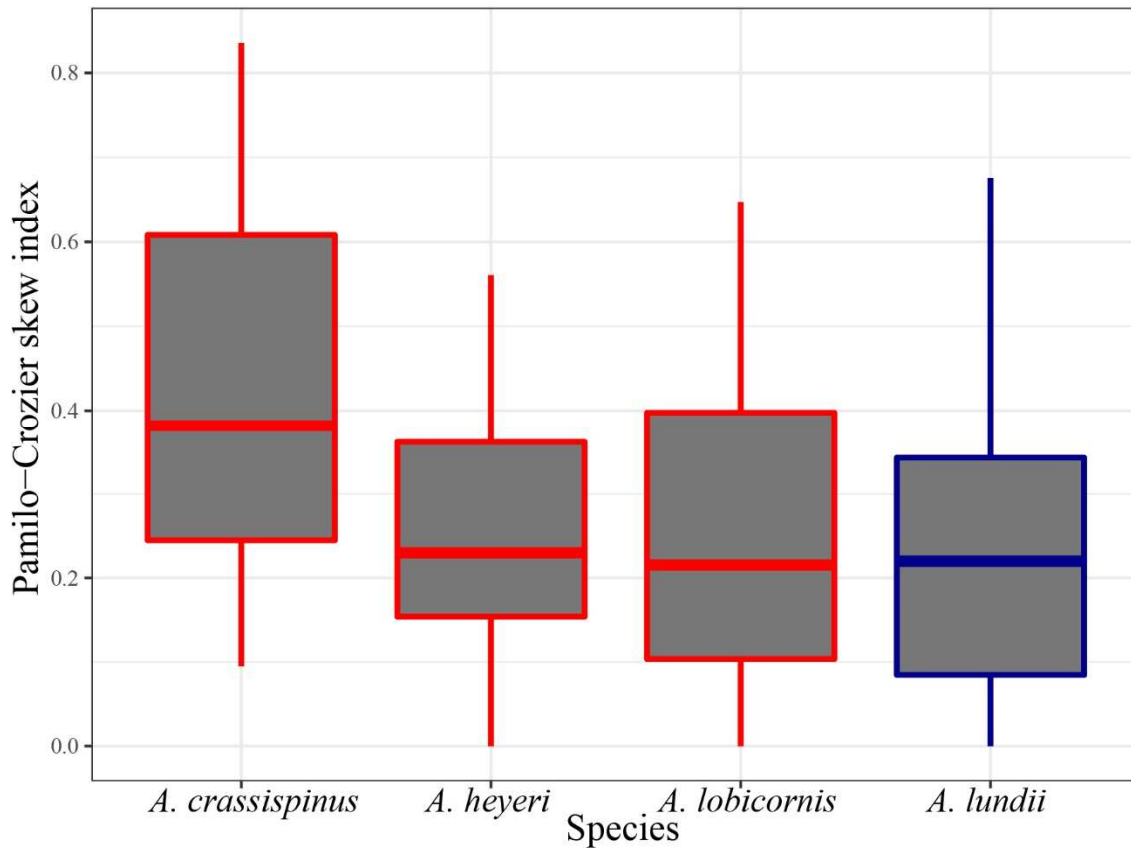
Figure 6: Pamilo-Crozier skew index in workers of four species of *Acromyrmex* leaf-cutting ants. Red (grey) boxes represent facultatively polygynous species, blue (black) boxes represent monogynous species. (Parenthesis colors refer to grayscale equivalences).

*Intra-colonial relatedness*

Based on the multiple regression, I found no evidence for a significant difference in average intra-colonial relatedness between species (ANOVA: $F_{2,72} = 3.10$, $p = 0.0513$; Table 3, Fig. 7). When scrutinizing the regression parameters, it appears that *A. heyeri* colonies show a slightly lower intra-colonial relatedness (r ± std. error = $0.36 \pm 2.8 \times 10^{-2}$) compared to *A. lundii* (r = $0.46 \pm 2.6 \times 10^{-2}$), *A. crassispinus* (r = $0.46 \pm 3.2 \times 10^{-2}$) and *A. lobicornis* (r = $0.46 \pm 3.2 \times 10^{-2}$). Effective mating frequency had a highly significant effect on intra-colonial relatedness (estimated regression parameter $b_{mating} = -0.234$, $F_{1,72}$

= 44.61, p = 4.86 x 10$^{-9}$; Fig. 7). We also found a significant difference in how effective mating frequency affected relatedness in monogynous vs polygynous colonies ($F_{1,72}$ = 4.054, p = 0.0478; Fig. 7). On the other hand, social structure alone had no detectable effect on relatedness ($F_{1,72}$ = 0.0193, p = 0.890, Fig. 7). Relatedness in colonies of *A. ambiguus* appeared indistinguishable from that inferred in colonies of the other species (r = 0.42 ± 0.10).

*Relatedness and effective mating frequency variation within species*

Comparing the effective mating frequency and intra-colonial relatedness between polygynous and monogynous colonies yielded contrasting results in *A. heyeri* and *A. lobicornis*. In *A. heyeri*, there was a significant difference in average effective mating frequency of queens in polygynous species (average $\overline{m_e}$ ± std. error = 3.17 ± 0.41) compared to the effective mating frequency of queens in monogynous colonies ($m_e$ = 4.92 ± 0.51; Welch t-test: $t_{14.35}$ = 2.65, p = 0.0187; Fig. 8a). On the other hand, I detected no significant difference between the intra-colonial relatedness of monogynous (r ± std. error = 0.38 ± 0.02) and polygynous (r = 0.26 ± 0.07) colonies of *A. heyeri* (t-test: $t_{23}$ = 2.00, *p* = 0.057; Fig. 9a).

Conversely, in *A. lobicornis*, these results were reversed. There was no significant difference in the effective mating frequency of polygynous colonies ($\overline{m_e}$ = 2.24 ± 0.39) compared to that of monogynous colonies ($m_e$ = 2.53 ± 0.21, t-test: $t_{16}$ = 0.65, p = 0.525; Fig. 8b). I found that average relatedness was significantly lower in polygynous colonies of *A. lobicornis* (r = 0.33 ± 5.90 x 10$^{-2}$) compared to monogynous colonies (r = 0.51 ±

3.66; t-test: $t_{16} = 2.67$, p = 0.0167; Fig. 9b). These results suggest relatedness may be affected in different ways by mating frequency and social structure in different species of leaf-cutting ants.
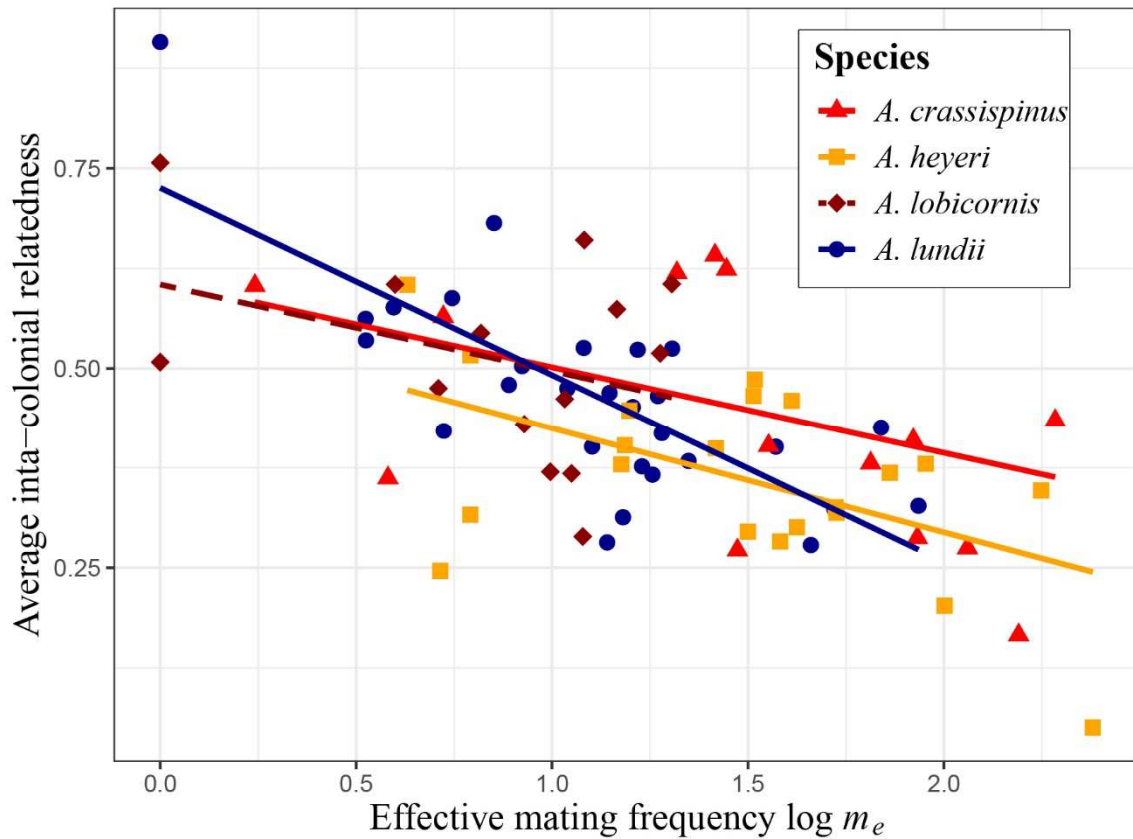
Figure 7: Scatterplot of average intra-colonial relatedness vs. effective mating frequency (natural log) in four species of *Acromyrmex* leaf-cutting ants. Warm (grey)colors represent facultatively polygynous species, the blue (black) line and circles represent monogynous species. *A. lobicornis* (a polygynous species, diamonds) is represented with a dashed line for convenience in grayscale. (Parenthesis colors refer to grayscale equivalences).
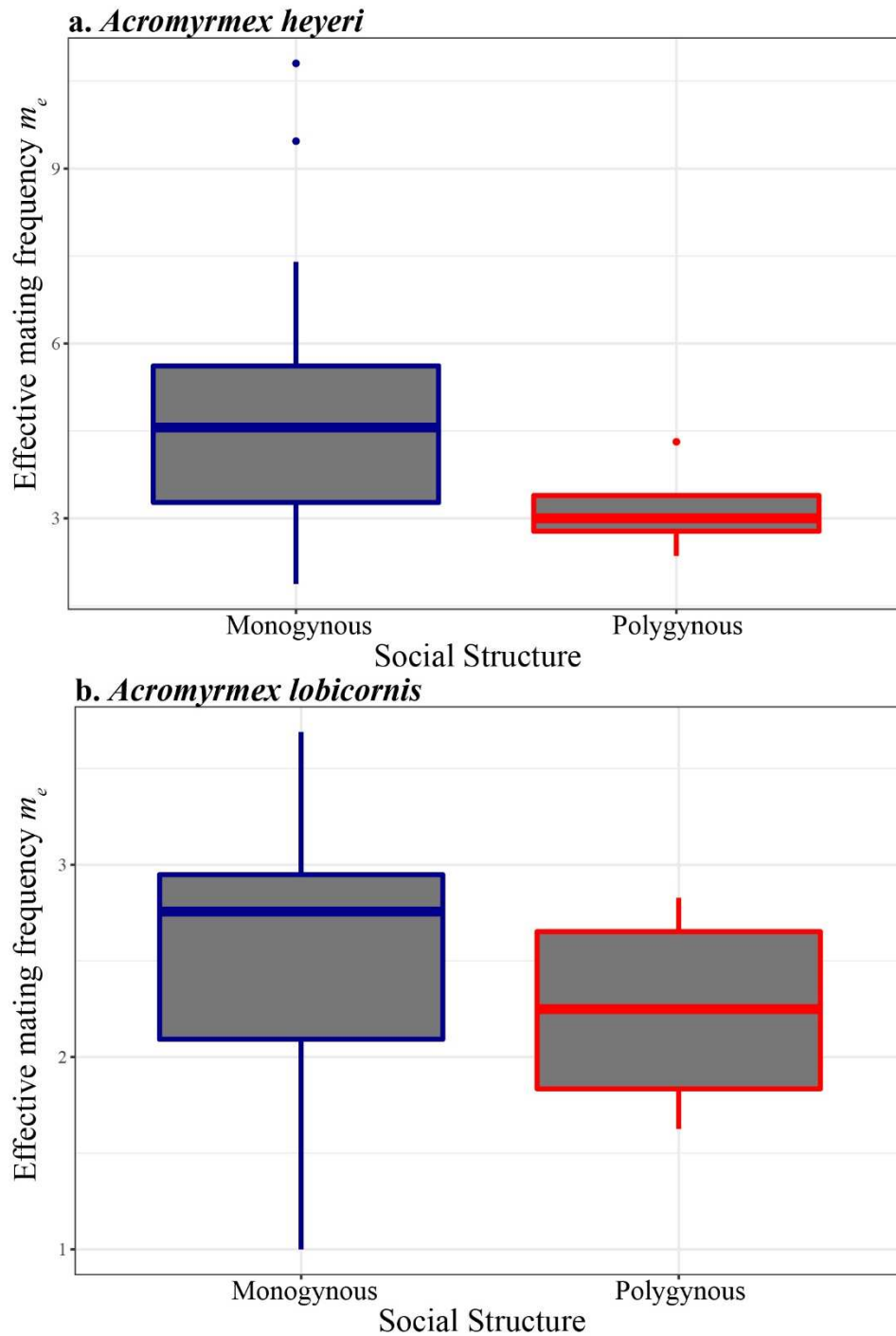
Figure 8: Effective mating frequency in monogynous and polygynous colonies of (a) *Acromyrmex heyeri* and (b) *A. lobicornis*. Red (grey) boxes represent facultatively polygynous colonies, blue (black) boxes represent monogynous colonies. (Parenthesis colors refer to grayscale equivalences).
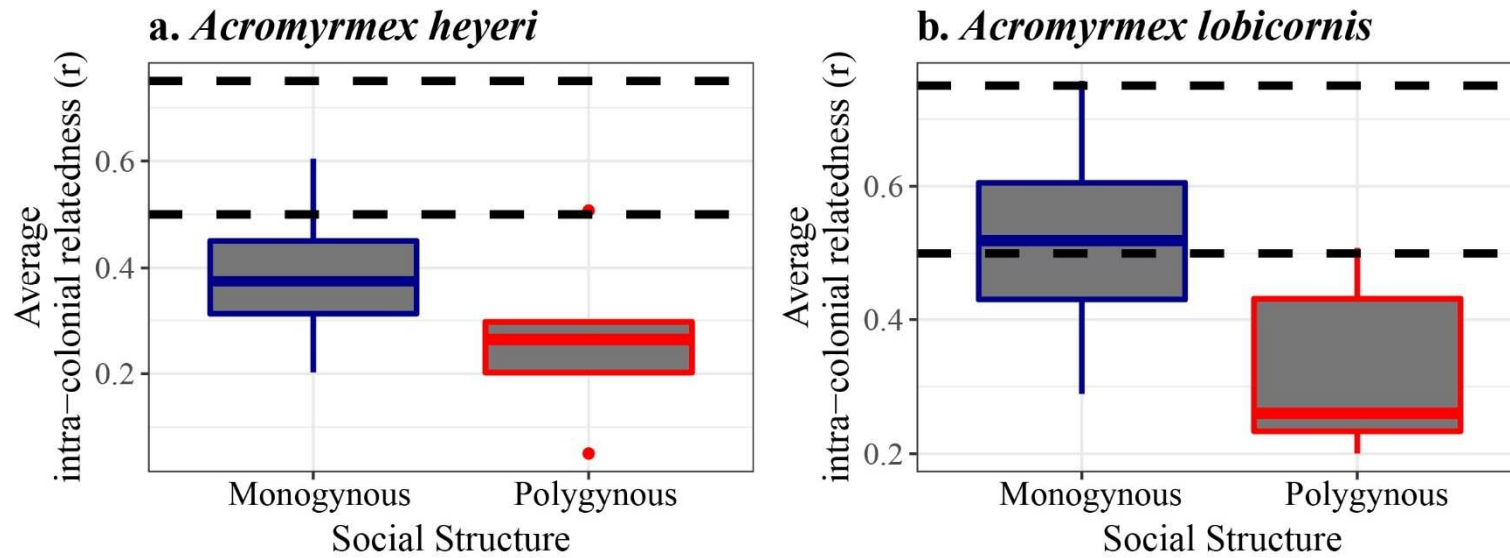
Figure 9: Average intra-colonial relatedness in monogynous and polygynous colonies of (a) *Acromyrmex heyeri* and (b) *A. lobicornis*. Red (grey) boxes represent facultatively polygynous colonies, blue (black) boxes represent monogynous colonies. (Parenthesis colors refer to grayscale equivalences).

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**2.4 Discussion**

In this study, I characterize the social structure and mating biology of five species of South American *Acromyrmex* leaf-cutting ants. Our analysis infers one species, *A. lundii*, as monogynous, while the other four species, *A. ambiguus, A. crassispinus*, *A. heyeri*, and *A. lobicornis* are facultatively polygynous. All five species are polyandrous. Our findings suggest that variation in relatedness is independent from variation in mating biology, and that species have no appreciable differences in reproductive skew among males mated to the same queens. Finally, I find that variation in mating biology and social structure have little effect on the intra-colonial relatedness.

*Patterns of association between mating biology and social structure in ants*

Our results reveal that mating biology is variable among South American species of *Acromyrmex* leaf-cutting ants and that mating behavior and social structure occur in different combination across leaf-cutting ants. Polygyny and a relatively high mating frequency ($m_e \geq 3.50$) were repeatedly observed, with variation in both traits occurring independently of one another between species. One of the studied species, *A. lundii*, appears to have reverted to obligate monogyny, while another, *A. lobicornis*, showed a significantly lower effective mating frequency compared to the others. It is theoretically possible that the inferred monogyny of *A. lundii* could stem from the non-sampling of polygynous colonies. However, the frequency of polygynous colonies in the other sampled species ranged from 11-28%, and the number of sampled colonies was larger for *A. lundii* than for any other species in our study. Calculating the probability of not

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sampling a polygynous colony out of 28 total colonies based on a binomial distribution, and assuming a conservative rate of polygyny of 10%, the binomial probability of sampling no polygynous colonies is 0.05, suggesting that our results are unlikely to stem from sampling error. Interestingly, these inter-specific variations in mating biology appear to have little impact on average intra-colonial relatedness in species (Fig. 7). The only noteworthy variation seems to be seen in colonies of *A. heyeri*, which have slightly lower relatedness on average (Fig. 7). Variation in intra-colonial relatedness across species is not influenced by social structure, but was significantly affected by effective mating frequency (Figs. 7, Table 4). Interestingly, the effect of effective mating frequency on intra-colonial relatedness was itself dependent on the species social structure, with a larger effect in the strictly monogynous *A. lundii* compared to the other, socially polymorphic species (Fig. 7). The overall general pattern of trait association between social structure, mating biology, and relatedness appears to be unrelated to the phylogenetic relationship between the investigated species (Fig. 9).
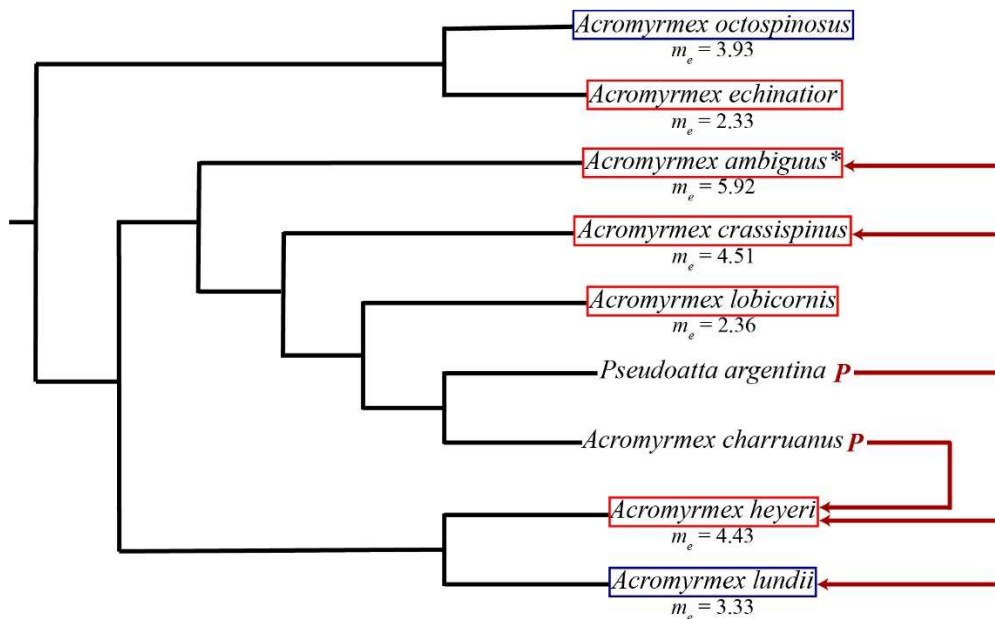
Figure 10: Phylogenetic distribution of mating biology and social structure in *Acromyrmex* leaf-cutting ants. Species boxed in red (grey) are socially polymorphic (i.e., facultatively polygynous), species boxed in blue (black) are obligately monogynous. A dark red (black) P denotes inquiline social parasites, and arrows show host-parasite relationships. Data for *A. ambiguus*, denoted by an asterisk, is based on low sample size. The *Acromyrmex* phylogeny is modified from Rabeling, Bollazzi, Delabie, Schultz, & Bacci, 2018. Information about the social structure and mating frequency of the Central American leaf-cutting ant species *A. echinatior* and *A. octospinosus* were generated by Boomsma et al. (1999) and Bekkevold et al. (1999). (Parenthesis colors refer to grayscale equivalences).

Table 4: Summary of patterns of trait association between mating biology and social structure among six species of *Acromyrmex* leaf-cutting ants. High effective mating frequency was defined as a value of $m_e$ = 3.50 or above. High mean intracolonial relatedness was defined as a value of r = 0.40 or above.

| | $m_e$ | Social structure | Mean intracolonial relatedness | Source |
|---|---|---|---|---|
| *A. crassispinus* | High | Polygyny | High | This study |
| *A. echinatior* | Low | Polygyny | High | Bekkevold et al. 1999 |
| *A. heyeri* | High | Polygyny | Low | This study |
| *A. lobicornis* | Low | Polygyny | High | This study |
| *A. lundii* | Low | Monogyny | High | This study |
| *A. octospinosus* | High | Monogyny | Low | Boomsma et al. 1999 |

Previous studies investigated the mating biology of four Central and South American *Acromyrmex* leaf-cutting ant species. Diehl and colleagues (2001) conducted a qualitative investigation of mating biology and colony structure in the South American species *A. heyeri* and *Amoimyrmex striatus*, finding facultative polygyny and prevalent polyandry in both species, which is consistent with our results. Boomsma and colleagues (1999) investigated the mating biology of the monogynous Central American species *A. octospinosus*, while Bekkevold and colleagues (1999) investigated both mating biology and social structure in its close relative *A. echinatior*. The monogynous *A. octospinosus* was found to have a high effective queen mating frequency ($m_e$ = 3.93; n = 22), and a mean pedigree relatedness (calculated from effective mating frequency rather than from genotypic data) of 0.38. *Acromyrmex echinatior* was found to be facultatively polygynous, but with a relatively lower effective queen mating frequency ($m_e$ = 2.33; n =

20), although that estimate was based on monogynous colonies only (n = 13). Average intra-colonial relatedness in these *A. echinatior* colonies was estimated from the effective mating frequency to be 0.41. Based on these results, *A. echinatior* appears similar to *A. lobicornis* with a low effective mating frequency, facultative polygyny, and high intra-colonial relatedness, while *A. octospinosus* presents a unique pattern of trait association with high effective mating frequency, monogyny, and low intra-colonial relatedness.

Overall, among the six *Acromyrmex* species with detailed sampling for mating biology and social structure, I cannot identify any consistent pattern of trait association between social structure and mating biology. In fact, only *A. echinatior* and *A. lobicornis* appear to have converged on a common association phenotype (with polygyny, low effective mating frequency, and relatively high mean intracolonial relatedness). All other species were found to display unique patterns of trait associations (Table 4). Evolutionary changes in social structure and mating frequency also appear to be phylogenetically unrelated (Fig. 9). Importantly, polygyny and polyandry are not consistently negatively correlated in these *Acromyrmex* leaf-cutting ants.

*Independent evolution of polygyny and polyandry*

When comparing *Acromyrmex* species to other fungus-growing ant species, and to other species across the ant tree of life, the co-occurrence of polygyny and polyandry in the same species is relatively rare, or at least a rarely documented phenomenon. If polygyny and polyandry evolve chiefly to increase the genetic variability within a colony, the prediction follows that polygyny and polyandry should be negatively correlated in

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eusocial insects, that is, polygynous species should be primarily monandrous, and monogynous lineages polyandrous (Keller and Reeve 1994). This association has been identified across ants (Schmid-Hempel and Crozier 1999; Hughes et al. 2008b). In fungus-growing ants, this pattern is weak, and for *Acromyrmex* leaf-cutting ants, I do not observe this negative association. Outside of *Acromyrmex* only few fungus-growing ant species in the genera *Mycocepurus* and *Sericomyrmex* are known to display facultative polygyny (Villesen et al. 2002; Rabeling et al. 2007, 2009, 2014b). The evolutionary transition from monandry to multiple mating has been well documented as a single evolutionary transition at the origin of the so-called "higher attines" (Villesen et al. 1999, 2002; Nygaard et al. 2016), comprising the leaf-cutting ants, *Acromyrmex*, *Amoimyrmex*, and *Atta*, as well as the genera *Mycetomoellerius*, *Paratrachymyrmex*, *Sericomyrmex*, *Trachymyrmex*, and *Xerolitor* (Sosa-Calvo et al. 2018; Solomon et al. 2019). Thus, it appears that facultative polygyny evolves independently of polyandry in fungus-growing ants. Notably, functional secondary polygyny is absent in the closely related leaf-cutting ant genus *Atta*, despite the fact that *Atta* species are known to be polyandrous (Fjerdingstad et al. 1998; Fjerdingstad and Boomsma 2000; Evison and Hughes 2011). When both traits co-occur in a clade, selection may act on both independently, modulating each trait individually to reach an optimum based on the individualized ecological and social niche occupied by a species (e.g. pathogen resistance, worker caste polymorphism, etc. See Introduction). Alternatively, these traits may not be under strong enough selection to overcome drift, and trait associations could be the result of random variation in these traits in these species. However, based on the strong evolutionary

implications of both polygyny and multiple mating, it seems unlikely that drift only would drive the evolution of these traits. Our results show remarkable consistency in average intra-colonial relatedness in Central and South American species of *Acromyrmex* (Table 4, Figs. 7, 9). Considering the observed effect of mating frequency and social structure on relatedness in these species (see Fig. 7), and the dynamic variation of both of these traits in the investigated species, the apparent consistency of species-wide average relatedness suggests that species may be converging on an optimum intra-colonial relatedness, mediated via a complex combination of multiple mating and multi-queen breeding, evolving independently of one another. An alternative explanation may be that relatedness is not under selection in these colonies, and the observed similarities is simply the result of chance convergence due to the trait associations in different species and other, unaccounted factors.

Outside of the fungus-growing ants, studies investigating the mating biology of *Formica*, *Myrmecia*, and *Myrmica* species, in which polyandry and polygyny co-occur, have produced different and often contradicting results (Pamilo 1993; Pedersen and Boomsma 1999c; Qian et al. 2011). These findings further support the hypothesis that, while polyandry and polygyny are negatively correlated in general, when both traits co-occur in groups of closely related species, they can evolve independently at shallow evolutionary timescales, dynamically filling species-specific ecological and social niches.

*Ecological factors promoting polygyny and polyandry*

Two major hypotheses have been proposed to explain the evolution of polyandry in eusocial insects. The sperm limitation hypothesis suggests that multiple males are needed to provide enough sperm to the queen of large colonies. The hypothesis has been widely tested in the literature, and has received support in honeybees (Kraus et al. 2004), as well as in the leaf-cutting ant *Atta colombica* (Fjerdingstad and Boomsma 1998) and army ants (Kronauer and Boomsma 2007). In this case, polyandry is expected to evolve more readily in association with the evolution of large colony sizes. In *Acromyrmex*, investigated species form large colonies (Burchill and Moreau 2016 and references therein; personal observations). Interestingly, in the fungus-growing ants, the transition to large colony size is observed in the ancestor of the leaf-cutting ants, after the inferred origin of polyandry in "higher" attines (Villesen et al. 2002; Burchill and Moreau 2016). Most notably, the hypothesis predicts that polyandrous species with larger colonies should exhibit significant reproductive skew among patrilines following natural variation in sperm contribution from males, because the queen has to use the entirety of the sperm stored in her spermatheca (Kraus and Moritz 2010; Barth et al. 2014). This pattern was observed in the Panamanian species *A. echinatior* (Stürup et al. 2014), and was inferred for South American *Acromyrmex* species in this study. Paternal skew was observed for all multiply mated queens, thus providing circumstantial support for this prediction of the sperm limitation hypothesis.

The genetic diversity hypothesis posits that increased genetic variation in a colony is beneficial, as it would lead to (a) increased parasite resistance and (b) increased colony

efficiency, and this idea has been more widely supported across bees and ants (Kraus and Page Jr. 1988; Sherman et al. 1988; Keller 1995; Liersch and Schmid-Hempel 1998; Schmid-Hempel and Crozier 1999; Crozier and Fjerdingstad 2001; Hughes et al. 2003; Palmer and Oldroyd 2003; Fjerdingstad and Crozier 2006; Trontti et al. 2007; Oldroyd and Fewell 2007; Waddington et al. 2010). The workers of leaf-cutting ants are highly polymorphic with large workers accomplishing foraging and exploration tasks outside the nest, while small workers remain inside the nest tending to the brood and the fungus garden. In *A. echinatior*, worker morph determination is complex and has been inferred to be in part genetically determined (Hughes et al. 2003; Hughes and Boomsma 2007). In this system, high mating frequency by the queen was interpreted to be favorable because it increases the genetic diversity of workers and allows for a better optimization of colony efficiency through the ratio of large vs. small workers (Hughes and Boomsma 2007). More generally in leaf-cutting ants, worker task specialization is associated with variability in size, contrasting with the rest of the fungus-growing ants, in which task specialization typically depends on age-dependent factors (Murakami et al. 2000). This could represent a shift in the determination of division of labor in colonies, in which genetic factors affecting worker size determination are linked with factors determining worker task specialization.

Functional polygyny in ants may be either primary, resulting from multiple queens co-founding colonies (pleometrosis), or secondary, resulting from the adoption of new queens in established mature colonies. While many examples of pleometrotic species are known, only a few have been documented to actually result in functional primary

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polygyny (Rissing et al. 1986; Mintzer 1987; Bourke and Franks 1995; Trunzer et al. 1998; Johnson 2004). In contrast, secondary polygyny appears to be more common in ants (Hölldobler and Wilson 1977; Bourke and Franks 1995; Boomsma et al. 2014). The mode (primary or secondary) of polygyny in the *Acromyrmex* species sampled here is unknown. Pleometrosis has been observed in *A. crassispinus*, but in all cases all but one queen died before colony maturation (Diehl-Fleig and de Araújo 1996). In contrast, primary polygyny has been well characterized in the North American desert species *A. versicolor* (Rissing et al. 1986, 1989), and has been observed under laboratory conditions in the South American species *Amoimyrmex striatus* (Diehl-Fleig and de Araújo 1996). On the other hand, queen adoption has been observed in the laboratory in a single species, the South American *A. subterraneus molestans* (Souza et al. 2005). Taken together, our results are consistent with the hypothesis that relatedness is the ultimate subject of selection, resulting from the trade-off between kin selection favoring high relatedness and the ecological benefits of genetic diversity outlined above. Multiple mating and polygyny appear to evolve along independent trajectories, and their respective effect on relatedness is dynamic and inconsistent between strictly monogynous or socially polymorphic species.

In our sampling, polygyny and mating frequency did not appear to correlate with known variation in nest structure. *Acromyrmex* ants are an interesting group among fungus-growing ants because the nest architecture of different species varies considerably. Some species excavate subterranean nests, while others assemble superficial mounds using thatch (Bollazzi et al. 2008). Some species show plasticity in

their nest architecture preference, in direct relation to soil temperature variation at different latitudes (Bollazzi et al. 2008). The *Acromyrmex* species I investigated either build superficial thatch mound nests only (*A. lobicornis*, *A. heyeri*), or exhibit plasticity in their nest-building behavior (*A. ambiguus, A. lundii, A. crassispinus*) depending on climatic factors (Bollazzi *et al.* 2008). Notably, I did not detect a pattern between monogynous and polygynous species and by extension between nest architecture and mating frequency.

*Polygyny, polyandry, and reproductive conflict in eusocial insects*

Polygyny and polyandry (and the reduced relatedness in colonies where they occur) can introduce the potential for reproductive cheating, and it is well known that the presence of multiple reproductive queens and/or males in a single colony can introduce direct conflicts over reproduction (Bourke and Franks 1995). When multiple queens are present or multiple males mated to a queen, rare cheaters may be favored if they evolve a mechanism for their offspring to be more likely to develop into sexual offspring, taking advantage of the worker production from other queens and/or males in the colony, to maximize their fitness returns out of their reproductive investment. For example, in polyandrous lineages, rare "royal" patrilines, that produce more sexual offspring than sterile workers have been detected in bees and ants (Moritz et al. 2005; Hughes and Boomsma 2008). Similarly, cheating queens in polygynous ant colonies have been detected in multiple species (Elmes 1976; Rüppell et al. 2002), and obligate workerless or nearly workerless inquiline social parasites in ants exploit this potential for reproductive

cheating. Inquiline social parasite species infiltrate their hosts' colonies and take advantage of the existing worker force to produce sexual offspring (Hölldobler and Wilson 1990). Several researchers have proposed the hypothesis that secondary polygyny is a critical trait involved in the evolution of social parasites in ants (Buschinger 1990; Bourke and Franks 1991). The transition to social parasitism is particularly relevant for *Acromyrmex* leaf-cutting ants, because five inquiline social parasites are known in the genus, including one species in the parasitic satellite genus *Pseudoatta*. Our sampling includes four *Acromyrmex* species that are known hosts to two inquiline social parasite species (Rabeling and Bacci 2010; Rabeling et al. 2015). *Acromyrmex lundii*, *A. heyeri*, and *A. crassispinus* are hosts of *Pseudoatta argentina* (Gallardo 1916; Bruch 1928; Rabeling and Bacci 2010; Rabeling et al. 2015)*, whereas *A. heyeri* is the only known host of *A. charruanus* (Rabeling et al. 2015). One host species, *A. lundii*, was inferred to be monogynous, while the other host species are facultative polygynous. In particular, the most closely related species to the parasitic clade, *A. lobicornis*, was found to be polygynous, strengthening the association between polygyny in a host species and the evolution of social parasitism.

*Conclusions*

In summary, I found a complex and dynamic association between colony structure and mating biology in leaf-cutting ants of the genus *Acromyrmex*. Our findings suggest that *Acromyrmex* leaf-cutting ants are socially polymorphic, and the association between polygyny and polyandry seems to be governed either by species-specific fitness optima

that may be related to ecological conditions (or independent evolution resulting from random Brownian motion), rather than a general pattern of anti-correlation between multiple mating and multi-queen breeding. Specifically, the relationship between mating biology, social structure, and relatedness both within and between species appears complex.

CHAPTER 3

MULTI-QUEEN BREEDING IS ASSOCIATED WITH THE EVOLUTION OF

INQUILINE SOCIAL PARASITISM IN ANTS

### 3.1 Introduction

Both vertebrate and invertebrate species have been the subject of many studies

investigating the role of ecology in the evolution of social structure, and how variation in

social systems can lead to major evolutionary transitions (Maynard Smith and Szathmáry

1995). Among eusocial insects, ancestral lifetime monogamy provided the key conditions

favoring the origin of eusociality across Hymenoptera (Hughes et al. 2008a; Boomsma

2009), and the ants share a single common ancestor suggesting that eusociality evolved

once during the Cretaceous (Borowiec et al. 2019). However, many extant ant species

display social structures deviating from a single, monandrous queen in a colony (Hughes

et al. 2008b). While polyandry (multiple mating by females) and polygyny (multi-queen

breeding) are expected to have negative impacts on intra-colonial relatedness, weakening

the effects of kin selection and preventing the evolution of eusociality, these social

structures may be beneficial to colonies once eusociality has been established, providing

fitness benefits to the colony as a whole (Bourke and Franks 1995; Schmid-Hempel and

Crozier 1999; Oldroyd and Fewell 2007; Hölldobler and Wilson 2009). On the other

hand, divergences in social structure in ant colonies may also result in the evolution of

alternative life-history strategies, such as alternative dispersal morphs, co-operative

colony foundation, or reproductive cheating, and nepotism (Trunzer et al. 1998; Rüppell

and Heinze 1999; Hannonen and Sundström 2003; Hughes and Boomsma 2008).

In particular, multi-queen nesting (polygyny) can evolve as a response to rapidly changing ecological conditions, and can have profound impacts on the social evolution of ant species (Pedersen and Boomsma 1999a; Rüppell et al. 2001a, 2003). In social insects, polygyny may be primary, resulting from the cooperative foundation of colonies by multiple queens (i.e., pleometrosis), or secondary, resulting from the adoption of new queens in established colonies (Hölldobler and Wilson 1990). While primary polygyny is generally associated with pleometrotic colony founding under ecological conditions that promote crowding of foundress queens (Bartz and Hölldobler 1982; Rissing et al. 1989), secondary polygyny has been a particular focus of research studying the evolutionary consequences of variation in social structure, because it provides avenues for the evolution of alternative life-history strategies (Hölldobler and Wilson 1977; Rüppell et al. 2002; Boomsma et al. 2014), and it is thought to be the prevalent mechanism for polygyny in ants (Hölldobler and Wilson 1990; Bourke and Franks 1995). Secondary polygyny has also repeatedly been associated with the evolution of socially parasitic life-histories in ants (Buschinger 1990, 2009; Bourke and Franks 1991).

Social parasites are species that take advantage of the social structure of their eusocial hosts to benefit their direct fitness. Three main life-histories of social parasitism occur in ants (Hölldobler and Wilson 1990; Buschinger 2009; Rabeling 2020). Temporary social parasites found colonies by invading host nests, killing the host queen(s), and taking advantage of the remaining host workers to raise their first brood. Dulotic species similarly found colonies as temporary social parasites by invading host nests, but subsequently rely on frequent raids on neighboring colonies for new workers.

Finally, inquiline social parasites are predominantly queen-tolerant workerless or nearly-workerless ant species which infiltrate established host colonies and take advantage of the present worker force to rear their own sexual brood (Hölldobler and Wilson 1990).

Inquiline social parasites are of particular interest to evolutionary biology because they are phylogenetically highly diverse and the currently known 96 inquiline species evolved at least 40 times independently across 25 genera across the formicoid clade of the ant tree of life (Rabeling 2020). Empirical studies revealed that many inquiline social parasites are closely related to their hosts, a pattern known as 'Emery's rule' (Bourke and Franks 1991; Huang and Dornhaus 2008). Thus, models describing the origin of social parasitism must account for the rule and explain why this pattern exists. In this vein, two models have been proposed to explain the origin of inquiline social parasitism in ants. The inter-specific origin model proposes that social parasites originated through the allopatric speciation of facultatively parasitic lineages of species closely related to the incipient host(s). In contrast, the intra-specific origin model proposes that inquiline social parasites originated through the sympatric speciation of cheating lineages from the incipient host species (Buschinger 1990, 2009; Hölldobler and Wilson 1990; Bourke and Franks 1991; Ward 1996). In all cases, parasites rely on similar resources, social cues, and environmental conditions as their hosts, requiring them to be fairly closely related to their hosts (Bourke and Franks 1991). In the intra-specific model, hosts and parasites must necessarily form a monophyletic clade **at the time of speciation**, providing a strict explanation for why parasites and hosts tend to be more closely related than any two species within a genus (Ward 1996; Huang and Dornhaus 2008). On the other hand, the

inter-specific model must include a free-living non-host species as sister to the parasite species, and cannot account for a strict interpretation of Emery's rule. So far, empirical phylogenetic studies supported the intra-specific route of inquiline social parasite evolution in *Acromyrmex*, *Ectatomma*, *Mycocepurus*, and *Myrmica* ants (Savolainen and Vepsäläinen 2003; Sumner et al. 2003; Jansen et al. 2010; Rabeling et al. 2014b; Leppänen et al. 2015; Nettel-Hernanz et al. 2015). The inter-specific model has so far garnered more support in *Pseudomyrmex* and *Temnothorax* ants (Ward 1996; Prebus 2017). Secondary speciation events of host and/or parasite species and host shifts can obscure the original transition to inquiline social parasitism given enough time, and lead to ambiguous resolutions between models, for example in *Pogonomyrmex*, *Solenopsis*, and a clade of Malagasy *Pheidole* (Parker and Rissing 2002; Shoemaker et al. 2006; Fischer et al. 2020).

To explain the evolutionary transition from a cooperative eusocial lifestyle to a socially parasitic life history, secondary polygyny has repeatedly been suggested as one of the key factors (Buschinger 1990, 2009; Hölldobler and Wilson 1990; Bourke and Franks 1991) because: (1) less efficient nestmate and brood recognition in polygynous colonies may yield a greater non-nestmate tolerance (Beye et al. 1998; Starks et al. 1998; Fournier et al. 2016); (2) the presence of multiple queens in colonies may provide an opportunity for supernumerary queens to cheat, focusing on the production of sexual offspring without contributing to the colony's sterile worker force (Rüppell et al. 2002); (3) workers and queens of polygynous colonies tolerate supernumerary egg-laying individuals (Hora et al. 2005b); and (4) the queen adoption behavior of established,

secondary polygynous colonies may provide a nest-invasion 'channel' for social parasites to exploit (Buschinger 2009). These predictions have different, yet not mutually exclusive implications regarding the evolutionary dynamics of inquiline social parasitism. The former two predictions are at least partially required for intra-specific cheating to evolve, and support a sympatric origin model of inquiline social parasitism. On the other hand, the latter two predictions are required for the maintenance of current inquiline social parasitism, targeting specific host behaviors associated with secondary polygyny, and do not necessarily favor either speciation model for the origin of inquiline social parasitism. While a strong association between polygyny and inquiline social parasitism would lend support to any one of these predictions equally, a weak correlation would fail to support the latter two, as it would show that polygyny is not necessarily required for the maintenance of inquiline social parasitism. Indeed, secondary evolutionary events, after speciation of a parasite species, such as diversification or extinction events, or host shifts, are expected to weaken an association between polygyny and inquiline social parasitism.

The predicted association between polygyny and inquiline social parasitism has been addressed in numerous empirical studies and literature reviews (Buschinger 1986; Bourke and Franks 1991; Boomsma et al. 2014), however, it was never tested in a statistically rigorous framework. Here, I aim to provide a formal statistical test of the prediction that polygyny and inquiline social parasitism are associated, by conducting a meta-analysis of the social structure of ants, and performing a phylogenetically corrected test of independence between social structure and parasitism. The role of both facultative

polygyny and obligate polygyny have been discussed in previous reviews, with

contradicting predictions (Bourke and Franks 1991; Boomsma et al. 2014). Thus, I

further test whether obligate or facultative secondary polygyny are associated with social

parasitism, in order to test whether either social structure may favor social parasitism

more than the other (Bourke and Franks 1991; Boomsma et al. 2014). Finally, I discuss

our results to assess the plausibility of the four hypotheses linking polygyny and social

parasitism (as outlined above).

## 3.2 Methods

*Social structure in ants*

We compiled a dataset of ants with known social structures using previously

published reviews as a starting point, and complemented the sampling with a thorough

literature search adding species and updating information on taxonomy and social

structure of species (Appendix 2) (Hölldobler and Wilson 1977, 1990; Rissing and

Pollock 1988; Frumhoff and Ward 1992; Keller and Reeve 1994; Schmid-Hempel and

Crozier 1999; Hughes et al. 2008b; Boomsma et al. 2014). For each species in the

dataset, I confirmed its social structure (monogyny/facultative polygyny/obligate

polygyny), by researching the primary literature reference to its social structure in the

field. As in Rissing and Pollock (1988), I validated a species as monogynous if at least 5

field colonies were collected. Records based on lab-reared colonies only were discarded,

as well as records only reported in reviews. Every species was checked individually to

ensure that the most up-to-date information was recorded. Species with known primary

polygyny were excluded, as the intra-specific model predicts that secondary polygyny is associated with inquiline social parasitism. I also excluded all social parasites, because I specifically tested predictions about the social organization of the free-living hosts. In total, our final dataset comprised 331 species, including 51 hosts of inquiline social parasites.

There is a possibility that lineages reported as monogynous in the literature might be "false negatives", i.e., lineages where monogyny was inferred because polygynous colonies have not been observed. It is impossible to prove that a species is obligately monogynous, so a certain probability of false negative species can be expected. In contrast, falsely claiming a species as polygynous appears less likely, as a polygyny inference relies on positive observations of colonies with multiple queens; and therefore, false positives are much less probable in our dataset. False negatives might bias an analysis by adding erroneous data points in the "monogynous/non-host" group, simply because of the much larger sampling in the "non-host" category. I attempted to curb this effect by stringently curating the dataset obtained from previous studies. Thus, I rejected any species where the mating biology was not directly referenced and addressed in the primary literature, as well as species where the colony structure was only inferred from lab colonies.

*Phylogenetic inference and correction*

In order to correct for phylogenetic non-independence, I assembled a cladogram of as many ant species as possible from available published phylogenies (Appendix 2). I

did so by "grafting" genus-level phylogenies together within subfamilies, and then "transplanting" these subfamily cladograms to the appropriate tip of a subfamily-level phylogeny of Formicidae. If a genus lacked an appropriate species-level phylogeny in the literature, the genus was treated as representing a hard polytomy. I discarded any species present in the phylogeny but not in the social structure dataset, and *vice versa*, resulting in a final dataset mapped to a cladogram of 294 species, including 48 host species of inquiline social parasites. In some cases, I had to discard phylogenies which included social parasites, because they did not include information about the social structure of host or non-host species. I then inferred ancestral states of social structure using a Maximum Likelihood optimization method, inferring the social structure at a node as the one with the highest posterior probability. This procedure allowed me to track the number of independent evolutionary transitions between social structures along our cladogram. In contrast, I inferred transitions to becoming a host for inquiline social parasites only if all of the descendants of a node were hosts, unless a case of host shift could reasonably be inferred, e.g. based on the paraphyly of a host clade relative to the parasite (see supplementary methods). As a result, I recovered a contingency table of independent evolutionary transitions inferred for both social structure and susceptibility to inquiline social parasites, following methods in (Ridley 1983; Harvey and Pagel 1991). For example, if a transition to becoming a host happened on a branch which started from a node inferred as polygynous, such even was counted in the Host x Polygynous cell of the contingency table. Inversely, if a reversal to monogyny was inferred on a branch whose parent node was an inferred host, or if a branch that included a transition to host had a

monogynous parent node, I counted these in the Host x Monogyny cell. This method is the same to that described in Schmid-Hempel and Crozier (1999).

*Statistical methods*

We analyzed the results to test two hypotheses: (i) that hosts of social parasites are more likely to be polygynous compared to non-hosts; and (ii) that either facultative or obligate polygyny is overrepresented among hosts of social parasites. I tested the former by testing for the independence of social structure (monogyny vs. polygyny) from hosting (or not hosting) an inquiline social parasite. I used a one-tailed Fisher's exact test, as our hypothesis had an *a priori* directional prediction (i.e. that host and polygyny should be associated). In contrast, I tested the second hypothesis that either obligate or facultative polygyny are involved in the evolution of inquiline social parasitism by testing for overrepresentation of either facultative or obligate polygynous species among hosts and non-hosts. Because this hypothesis lacks an *a priori* directional prediction, and the sample size was sufficiently large, I used a $\chi^2$ test of independence. For all tests, a significance level of 0.05 was used. All analyses were conducted in R 3.4.3, using the packages 'ape' 'phytools' and 'base' (Paradis et al. 2004; Revell 2012; R Core Team 2021).

Table 5: Contingency table of social structure (polygyny or monogyny) in species hosting (or not hosting) obligate inquiline social parasites, before phylogenetic correction.

|  | Host species | Non-host species |
|---|---|---|
| **Monogyny** | 9 | 127 |
| **Polygyny** | 42 | 153 |

Table 6: Contingency table of polygyny type (facultative or obligate) in species hosting (or not hosting) obligate inquiline social parasites, before phylogenetic correction. Polygynous species for which information regarding obligate or facultative nature of the polygyny are excluded from this table.

|  | Host species | Non-host species |
|---|---|---|
| **Facultative polygyny** | 29 | 90 |
| **Obligate polygyny** | 2 | 26 |

Table 7: Contingency table of social structure (polygyny or monogyny) in species hosting (or not hosting) obligate inquiline social parasites. Numbers represent independent evolutionary events. Expected values are given in parentheses.

|  | Host species | Non-host species |
|---|---|---|
| **Monogyny background** | 8 (12.45) | 26 (21.55) |
| **Polygyny background** | 33 (28.55) | 45 (49.45) |

Table 8: Contingency table of polygyny type (facultative or obligate) in species hosting (or not hosting) obligate inquiline social parasites. Numbers represent independent evolutionary events. Expected values are given in parentheses.

|  | Host species | Non-host species |
|---|---|---|
| **Facultative polygyny** | 24 (21.52) | 24 (26.48) |
| **Obligate polygyny** | 2 (4.48) | 8 (5.52) |

### 3.3 Results

*Social structure in ants*

In total, our final dataset comprised 331 species, including 51 hosts of inquiline social parasites (15.41%). Of the total 331 species, 136 (41.09%) were inferred as monogynous, while 119 (35.95%) were facultative polygynous, 28 (8.46%) were obligate polygynous, and 48 (14.50%) were polygynous without, although the whether that polygyny was obligate or facultative was not specified (Tables 5 and 6; Appendix 2). Among the 51 hosts, 42 (82.35%) were polygynous, compared to 153 among the 280 non-host species (54.64%). Twenty-nine host species (56.86%) were facultatively polygynous, 2 (3.92%) were obligate monogynes, and 11 (21.57%) polygynous species had no reference to any social polymorphism (Table 6). After matching the dataset with our assembled cladogram, the sampling was reduced to 272 species spanning 11 subfamilies, consisting of 111 monogynous species and 161 polygynous species. Of these 272 species that were represented in the cladogram and for which reliable information on social organization was available, 47 (17.27%) were hosts of inquiline social parasites. After estimating the evolutionary history of social structure in ants, I recovered 78 transitions from monogyny to polygyny, as well as 34 reversals to monogyny (Table 7).

*Association between polygyny and social parasitism*

We found a significant association between polygyny and hosting inquiline social parasites (Fisher's exact test: Odds ratio = 0.423, $p = 0.0443$, Table 7), indicating that polygynous species were significantly over-represented among hosts of inquiline social

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parasites. Specifically, hosts of social parasites were more than twice as likely to be polygynous compared to non-hosts. In contrast, I did not find a significant association between either obligate or facultative secondary polygyny and social parasitism ($\chi^2$ test of independence: $\chi^2_1 = 3.0115$, $p = 0.0827$, Table 8).

### 3.4 Discussion

We conducted a meta-analysis across the ant tree of life to statistically test for an association between social structure and being a host of inquiline social parasites, with a phylogenetic correction for independent evolutionary events. I found that polygynous species are over-represented among hosts of inquilines, confirming a long-standing but hitherto untested observation in the social parasitism literature (Buschinger 1990, 2009; Bourke and Franks 1991; Boomsma et al. 2014). In contrast to previous conflicting predictions (Bourke and Franks 1991; Boomsma et al. 2014), I did not find a significant association between either obligate or facultative polygyny and inquilinism.

An association between polygyny and inquiline social parasitism could stem from distinct but related behavioral traits observed in polygynous ant colonies. Bourke & Franks (1991) outlined four key scenarios how polygyny is important for the origin and maintenance of social parasitism: Polygyny is (1) reducing the intra-specific non-nestmate recognition ability in workers of ancestral colonies; (2) providing an avenue for cheating by adopted queens to take advantage of the existing worker force; (3) increasing tolerance of host colonies for supernumerary egg-laying females; and (4) providing a nest-invasion mechanism easily hijacked by the parasites.

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*Polygyny and inquiline social parasitism*

The association of polygyny with social parasitism is important for both the intra-specific origin and the inter-specific origin model of social parasite speciation, especially relating to the tolerance of non-nestmate individuals and the queen adoption mechanism of polygynous hosts. However, while the role of multi-queen nesting is circumstantial in the inter-specific origin model (as parasites may target monogynous species as well, see below), it is critical in an intra-specific model, in which parasites evolve from intra-specific lineages. A sympatric origin requires polygyny in the incipient stages of the speciation process, as it would begin as reproductive cheating between nestmate queens. Here, I found a significant association between polygyny and inquiline social parasitism. While not definitive evidence of the intra-specific origin model, our results add to the mounting phylogenetic evidence favoring the sympatric, intra-specific speciation model for the origin of inquiline social parasites in some species (Savolainen and Vepsäläinen 2003; Rabeling et al. 2014b; Leppänen et al. 2015; Nettel-Hernanz et al. 2015). Furthermore, these observations are consistent with predictions of mechanistic models for sympatric speciation (see below) (Bourke and Franks 1991; Wolf and Seppä 2015; Leppänen et al. 2016) Overall, our results emphasize the importance of polygyny across ant species in the initial emergence of inquiline social parasitism, facilitating the emergence of cheating and the adoption of additional queens either intra- or inter-specifically.

*Monogyny and inquiline social parasitism*

The occurrence of monogynous hosts of inquiline social parasites is at first glance inconsistent with the previously outlined arguments that polygyny promotes the evolution of inquiline social parasitism (Buschinger 1986; Bourke and Franks 1991). Nonetheless, empirical studies revealed that several social parasites have monogynous host species including, for example, inquiline species in the genera *Nylanderia* and *Acromyrmex* (Rabeling et al. 2019; Messer et al. 2020). To distinguish between the different evolutionary dynamics of social parasite speciation, i.e., the origin and the maintenance of social parasitism in a host population, it is important to understand whether hosts of social parasites were monogynous at the time when the social parasite originated, or whether host monogyny could be a consequence of a co-evolutionary arms-race between host and parasite (Davies et al. 1989; Grüter et al. 2018). Arms race dynamics are known to affect the social organization of the host species, and population studies of dulotic *Temnothorax* ants revealed that the frequency of monogynous colonies increased in highly parasitized host populations, presumably as a co-evolutionary response to parasitism which allowed for improved parasite detection and rejection by the host (Foitzik and Herbers 2001; Herbers and Foitzik 2002). In general, secondary evolutionary events in either host or parasite, including host shifts, speciation and extinction events, as well as changes in social structure of either host or parasite colony, may obscure the original conditions under which social parasitism originated. If our results are correct, at least eight independent origins of inquiline social parasitism occurred in a monogynous background (Table 7), suggesting that this specialized life history can evolve in

monogyny, even if rarely. Further studies are necessary to evaluate whether these eight

monogynous events truly represent social parasites that evolved in monogynous hosts and

whether host monogyny reflects the social colony organization at the time of parasite

speciation. Once primarily monygynous hosts can be validated, it will be insightful to

study the interactions and evolutionary dynamics between host and parasite species.

Investigating potential natural history traits or common ecological niches in these

systems may provide insights towards elucidating the mechanisms of evolution of

inquiline social parasitism in monogynous species, and the convergence of a socially

parasitic life history.

Social structure and reproductive ecology are at the core of the evolution of

inquiline social parasitism (Buschinger 2009). Increasingly, however, other traits in the

inquiline syndrome (*sensu* Wilson (Wilson 1984)) are believed to play important roles in

the emergence of reproductive cheating. Queen polymorphisms involving differential

dispersal strategies, for example, have provided a promising avenue of research for the

investigation of intra-specific reproductive cheating (Bourke and Franks 1991; Nonacs

and Tobin 1992; Rüppell et al. 2001b; Wolf and Seppä 2016). Small queen morphs,

referred to as 'microgynes', are in many species associated with alternative dispersal

strategies and polygyny itself, and in some cases microgynes were shown to favor the

production of sexual offspring over workers, providing the basis for reproductive

cheating (Rüppell et al. 2002). In some cases, microgynes are in fact considered intra-

specific inquiline parasites (Elmes 1973; Leppänen et al. 2016), whereas in other cases

microgynous forms were raised to the species level as obligate inquiline parasites (Seifert

1993; Feitosa et al. 2008). Queen size polymorphism is heavily associated with the evolution of social parasitism based on two major lines of evidence. First, size reduction is a trait observed in most inquiline social parasites, and part of the inquiline syndrome (Wilson 1984; Bourke and Franks 1991; Buschinger 2009; Rabeling and Bacci 2010). Second, body size is known to affect developmental trajectory at the larval stages (Nonacs and Tobin 1992; Aron et al. 2004; Trible and Kronauer 2017). From these observations, it has been hypothesized that a shift of the size threshold for queen development, which would result in microgyne morphs, may also be associated with reproductive cheating, in which larvae being allocated resource which would usually lead them toward worker development would instead develop as queens (the so-called 'selfish brood hypothesis' (Nonacs and Tobin 1992; Wolf and Seppä 2015)). From there, selection might favor the assortative mating of selfish lineages, resulting in genetic divergence and eventually speciation, resulting from alternative adaptation (West-Eberhard 1986; Bourke and Franks 1991). Natural history observations reinforce this second line of evidence, as some inquiline species are known to produce a worker caste, and also display a less extreme degree of size reduction compared to their hosts (e.g. the leaf-cutting ant parasite *Acromyrmex insinuator* (Schultz et al. 1998)). It is important to note that the appearance and maintenance of these small queen morphs may also be adaptive to the colonies producing them, as they may evolve as dispersal or polygynous morphs in ecologically saturated habitats with limited space for new colony founding and nest establishment (Rüppell and Heinze 1999; Rüppell et al. 2001a; Hakala et al. 2019). Further investigations of possible sources of reproductive isolation may yield further

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support for this hypothesis and provide more examples of speciation in sympatry (Leppänen et al. 2011, 2015, 2016; Rabeling et al. 2014b).

In contrast, other inquiline species likely followed alternative evolutionary routes to workerlessness. In the formicine genus *Nylanderia*, for example, the hosts of inquiline species may be strictly monogynous (Frumhoff and Ward 1992; Messer et al. 2016, 2020), possibly indicating that the inquiline social parasites originated via a different evolutionary model. Across social insects, Emery's rule is only strictly observed in a few inquiline social parasites whereas many non-inquiline ant social parasites and parasitic wasps are not the closest relatives of their hosts (Huang and Dornhaus 2008; Lopez-Osorio et al. 2015; Rabeling 2020). These diverse phylogenetic relationships between hosts and parasites suggest that social cheating evolved convergently along different pathways in convergently evolved host parasite systems. In at least two different systems, inquiline social parasites are nested in clades featuring other socially parasitic life histories. For example, molecular phylogenies suggest that the socially parasitic *Temnothorax corsicus* species group, a clade formerly known as *Myrmoxenus* (Ward et al. 2015; Prebus 2017), which contains primarily dulotic species, also contain a number of independently evolved so-called 'murder-parasites' or 'degenerate dulotic' species, which are workerless or nearly-workerless species that display morphological and behavioral traits generally associated with inquiline social parasitism (Heinze et al. 2015). These origins are thought to likely emerge as a loss of the worker caste in dulotic species and demonstrate that some inquiline social parasites may evolve from a dulotic ancestor (Suefuji and Heinze 2015). Similarly, *Formica talbotae*, the only inquiline

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species in the wood ant genus *Formica*, likely evolved from a temporary social parasitic ancestor in the *F. difficilis* group (Talbot 1976; Wilson 1976; Hölldobler and Wilson 1990; Borowiec et al. 2020). In both cases, sympatric speciation is unlikely, and these species provide evidence for inquiline social parasites evolving from either a dulotic or potentially a temporary social parasitic ancestor instead of a free-living, polygynous ancestor. Furthermore, these *Temnothorax* and *Formica* social parasite species suggest evolutionary transitions between socially parasitic life history strategies (Wilson 1971; Buschinger 1986, 2009; Hölldobler and Wilson 1990; Rabeling 2020). Lastly, an 'inter-specific' model may be considered in which facultative parasitism evolves from inter closely related free-living species, followed by the disappearance of free-living forms resulting in an obligate social parasite (Hölldobler and Wilson 1990). While this model might explain the evolution of inquiline social parasitism in monogynous species not associated with other types of social parasitism (e.g. in *Nyalanderia*; Messer et al. 2020), little evidence has been found supporting this model of evolution of inquiline social parasitism (Buschinger 2009; Rabeling 2020). Further research in those systems might reveal novel mechanisms of evolution for workerless social parasitism in ants, and contrast the many distinct ways this type of parasitism evolved convergently in eusocial insects.

*Conclusion*

In conclusion, I found a significant association between secondary polygyny and inquiline social parasitism. However, this association is imperfect in that it affects species

with obligate or facultative polygyny equally. Our results support the previously formulated hypothesis that polygyny is an important trait for the origin of inquiline social parasitism in some, if not most cases. Secondary evolutionary transitions such as host shifts, speciation and extinction events, and changes in colony organization in either the host or parasite clades may erode the signal of the original condition under which social parasitism originated. Co-evolutionary arms race dynamics between host and parasite may also result in secondarily monogynous hosts of inquiline social parasites. However, obligate inquiline social parasitism has evolved many times independently in social insects, and it seems unrealistic that every emergence of this phenotype might have evolved following the same trajectory. I discussed potential avenues to investigate and contrast different mechanisms through which obligate workerless inquiline parasitism might have evolved in social insects, both in sympatry and in allopatry. Overall, our results support the viewpoint that a change in social structure can have significant consequences in the life history and social evolution of cooperating eusocial insects.

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APPENDIX A

SUPPLEMENTAL INFORMATION FOR CHAPTER 2:

DECOUPLED EVOLUTION OF MATING BIOLOGY AND SOCIAL STRUCTURE

IN *ACROMYRMEX* LEAF-CUTTING ANTS

Supplementary Methods

*Setting a threshold to validate multi-queen nest structures*

In several cases, the recovered parental genotypes in a colony inferred small sibship

groups, where a matriline would contribute a small number of offspring from our

sampling. While it is possible that a queen could contribute very little to a colony's

reproductive output, it is also possible that these "outlier offspring" were the result of

foreign worker adoption, genotyping error, or collecting error in the field. Therefore, I

decided to exclude matrilines contributing less than 10% of offspring to our samples

(four in colonies with 40+ offspring sampled, 3 in colonies with 30+ offspring sampled, 2

in colonies with 20+ worker sampled). The probability of sampling at least $x$ offspring

out of a sample of 48 from a queen contributing 10% of the offspring in a colony was

calculated from a binomial distribution. It is assumed that the probability of sampling

workers from given matrilines is independent for each sampled workers (that is, sibling

and half-sibling workers do not cluster together in a colony chamber). Table S5 shows

the probabilities of sampling *at least* three, four, and five offspring laid by a queen

contributing 10% of the colony's reproductive output under a binomial distribution.

Supplementary tables

Table S1: Colonies sampled for the microsatellite studies. Colony IDs refer to their deposition record ID at SIBR. Sample size refers to the number of offspring sampled from the colony.

| Species | Collection code | Colony ID | Country | Sampling location | GPS Lat | GPS Lon |
|---|---|---|---|---|---|---|
| *A. ambiguus* | CR131031-19 | Aamb_1 | Uruguay | Tucarembó | -32.8162 | -56.4999 |
| *A. ambiguus* | CR120407-11 | Aamb_2 | Uruguay | Río Negro | -32.638 | -58.1482 |
| *A. ambiguus* | CR120407-12 | Aamb_3 | Uruguay | Río Negro | -32.638 | -58.1482 |
| *A. ambiguus* | CR120405-01 | Aamb_4 | Uruguay | Tucarembó | -31.6419 | -55.9675 |
| *A. crassispinus* | CR131103-03 | Acras_1 | Uruguay | Florida | -33.8236 | -55.4888 |
| *A. crassispinus* | CR131103-06 | Acras_2 | Uruguay | Florida | -33.8236 | -55.4888 |
| *A. crassispinus* | CR131103-09 | Acras_3 | Uruguay | Florida | -33.8236 | -55.4888 |
| *A. crassispinus* | CR131103-15 | Acras_4 | Uruguay | Florida | -33.8236 | -55.4888 |
| *A. crassispinus* | CR131105-17 | Acras_5 | Uruguay | Florida | -33.8236 | -55.4888 |
| *A. crassispinus* | CR131031-04 | Acras_6 | Uruguay | Tucarembó | -32.1243 | -56.1142 |
| *A. crassispinus* | CR131031-20 | Acras_7 | Uruguay | Tucarembó | -32.8162 | -56.842 |
| *A. crassispinus* | CR120331-03 | Acras_8 | Brazil | Santa Catarina | -26.8199 | -51.9901 |
| *A. crassispinus* | CR120331-13 | Acras_9 | Brazil | Santa Catarina | -27.3531 | -51.0254 |
| *A. crassispinus* | 11 Sept. 18 B2 | Acras_10 | Uruguay | Tucarembó | -32.2102 | -56.1747 |
| *A. crassispinus* | 08 Sept. 18 C4 | Acras_11 | Uruguay | Tucarembó | -32.2378 | -56.2049 |
| *A. crassispinus* | 09 Sept. 18 B1 | Acras_12 | Uruguay | Tucarembó | -31.5642 | -55.8359 |
| *A. crassispinus* | 08 Sept. 18 C1 | Acras_13 | Uruguay | Tucarembó | -32.2378 | -56.2049 |
| *A. crassispinus* | 09 Sept. 18 C2 | Acras_14 | Uruguay | Rivera | -31.319 | -55.8563 |
| *A. crassispinus* | 09 Sept. 18 B2 | Acras_15 | Uruguay | Tucarembó | -31.5642 | -55.8359 |
| *A. crassispinus* | 08 Sept. 18 C2 | Acras_16 | Uruguay | Tucarembó | -32.2378 | -56.2049 |
| *A. crassispinus* | 11 Sept. 18 B4 | Acras_17 | Uruguay | Tucarembó | -32.2102 | -56.1747 |
| *A. crassispinus* | 08 Sept. 18 B3 | Acras_18 | Uruguay | Tucarembó | -32.6291 | -56.4595 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. heyeri* | CR130225-01 | Ahey_4 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130225-03 | Ahey_5 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-20 | Ahey_27 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-22 | Ahey_29 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-24 | Ahey_31 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-25 | Ahey_32 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-26 | Ahey_34 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-27 | Ahey_35 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-29 | Ahey_37 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR130227-31 | Ahey_39 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR131031-06 | Ahey_51 | Uruguay | Tucarembó | -32.1243 | -56.1142 |
| *A. heyeri* | CR131031-08 | Ahey_53 | Uruguay | Tucarembó | -32.1243 | -56.1142 |
| *A. heyeri* | CR131104-03 | Ahey_65 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR131104-04 | Ahey_67 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | CR131104-10 | Ahey_69 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. heyeri* | LMB131003-01 | Ahey_70 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-02 | Ahey_71 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-03 | Ahey_72 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-04 | Ahey_73 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-05 | Ahey_74 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-06 | Ahey_75 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-07 | Ahey_76 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-08 | Ahey_77 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-09 | Ahey_78 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. heyeri* | LMB131003-10 | Ahey_79 | Uruguay | Montevideo | -34.838 | -56.2206 |
| *A. lobicornis* | CR120406-01 | Alob_1 | Uruguay | Artigas | -30.4473 | -56.5923 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. lobicornis* | CR120406-02 | Alob_2 | Uruguay | Artigas | -30.4473 | -56.5923 |
| *A. lobicornis* | CR130227-41 | Alob_3 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lobicornis* | CR131031-05 | Alob_4 | Uruguay | Tucarembó | -32.1243 | -56.1142 |
| *A. lobicornis* | CR131103-23 | Alob_5 | Uruguay | Florida | -33.8811 | -55.6048 |
| *A. lobicornis* | 11 Sept. 17 B3 | Alob_6 | Uruguay | Trenta y Tres | -33.214 | -55.1267 |
| *A. lobicornis* | 13 Sept. 17 D2 | Alob_7 | Uruguay | Tucarembó | -32.5399 | -56.393 |
| *A. lobicornis* | 30 Sept. 17 C1 | Alob_8 | Uruguay | Paysandu | -31.8956 | -56.8198 |
| *A. lobicornis* | 11 Sept. 17 D1 | Alob_9 | Uruguay | Trenta y Tres | -32.8473 | -54.8108 |
| *A. lobicornis* | 28 Sept. 17 B1 | Alob_10 | Uruguay | Maldonado | -34.7827 | -55.312 |
| *A. lobicornis* | 13 Sept. 17 C1 | Alob_11 | Uruguay | Tucarembó | -32.2373 | -56.2041 |
| *A. lobicornis* | 13 Sept. 17 C2 | Alob_12 | Uruguay | Tucarembó | -32.2373 | -56.2041 |
| *A. lobicornis* | 13 Sept. 17 C3 | Alob_13 | Uruguay | Tucarembó | -32.2373 | -56.2041 |
| *A. lobicornis* | 13 Sept. 17 E3 | Alob_16 | Uruguay | Tucarembó | -32.7264 | -56.5063 |
| *A. lobicornis* | 30 Sept. 17 B1 | Alob_17 | Uruguay | Paysandu | -31.9647 | -57.3523 |
| *A. lobicornis* | 30 Sept. 17 B2 | Alob_18 | Uruguay | Paysandu | -31.9647 | -57.3523 |
| *A. lobicornis* | 30 Sept. 17 E3 | Alob_19 | Uruguay | Paysandu | -31.8282 | -56.344 |
| *A. lobicornis* | 01 Oct. 17 A2 | Alob_20 | Uruguay | Tucarembó | -31.5878 | -55.7766 |
| *A. lundii* | CR120407-05 | Alun_1 | Uruguay | Paysandu | -32.3531 | -58.0507 |
| *A. lundii* | CR120407-13 | Alun_2 | Uruguay | Rio Negro | -32.638 | -58.1482 |
| *A. lundii* | CR120406-09 | Alun_3 | Uruguay | Artigas | -30.2016 | -57.6418 |
| *A. lundii* | CR131025-08 | Alun_16 | Brazil | Rio Grade do Sul | -30.0517 | -51.1772 |
| *A. lundii* | CR131025-09 | Alun_17 | Brazil | Rio Grade do Sul | -30.0517 | -51.1772 |
| *A. lundii* | CR131025-11 | Alun_18 | Brazil | Rio Grade do Sul | -30.0521 | -51.2215 |
| *A. lundii* | CR131104-36 | Alun_19 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR120408-03 | Alun_20 | Uruguay | Soriano | -33.4944 | -58.3355 |
| *A. lundii* | CR131028-01 | Alun_21 | Uruguay | Rivera | -30.902 | -55.5464 |

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| *A. lundii* | CR120407-07 | Alun_22 | Uruguay | Rio Negro | -32.638 | -58.1482 |
|---|---|---|---|---|---|---|
| *A. lundii* | CR131103-22 | Alun_23 | Uruguay | Florida | -33.8811 | -55.6048 |
| *A. lundii* | CR131104-31 | Alun_24 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131104-32 | Alun_25 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131104-33 | Alun_26 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131104-35 | Alun_27 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131104-37 | Alun_28 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131105-06 | Alun_29 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131105-08 | Alun_31 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131105-09 | Alun_32 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131105-10 | Alun_33 | Uruguay | Florida | -33.9042 | -55.5942 |
| *A. lundii* | CR131105-12 | Alun_35 | Uruguay | Florida | -34.1002 | -56.2036 |
| *A. lundii* | CR131105-13 | Alun_36 | Uruguay | Florida | -34.1002 | -56.2036 |
| *A. lundii* | CR131105-14 | Alun_37 | Uruguay | Florida | -34.1002 | -56.2036 |
| *A. lundii* | CR120406-10 | Alun_41 | Uruguay | Artigas | -30.2016 | -57.6418 |
| *A. lundii* | CR120406-11 | Alun_42 | Uruguay | Artigas | -30.2016 | -57.6418 |
| *A. lundii* | CR131105-15 | Alun_47 | Uruguay | Florida | -34.1002 | -56.2036 |
| *A. lundii* | CR131105-20 | Alun_49 | Uruguay | Florida | -34.1002 | -56.2036 |
| *A. lundii* | CR131105-21 | Alun_50 | Uruguay | Florida | -34.1002 | -56.2036 |

Table S2: Results of the pairwise Mann-Whitney U tests of differences in empirical mating frequency (M) between four *Acromyrmex* species. The values in each cell represent the FDR-corrected p-value for the given test.

| | *A. crassispinus* | *A. heyeri* | *A. lobicornis* |
|---|---|---|---|
| *A. heyeri* | 0.330 | - | |
| *A. lobicornis* | $6.86 \times 10^{-3**}$ | $1.40 \times 10^{-4***}$ | - |
| *A. lundii* | 0.048 | 0.065 | $6.86 \times 10^{-3**}$ |

Table S3: Results of the pairwise Mann-Whitney U tests of differences in effective mating frequency ($m_e$) between four *Acromyrmex* species. The values in each cell represent the FDR-corrected p-value for the given test.

| | *A. crassispinus* | *A. heyeri* | *A. lobicornis* |
|---|---|---|---|
| *A. heyeri* | 0.886 | - | |
| *A. lobicornis* | $0.030^*$ | $2.90 \times 10^{-4***}$ | - |
| *A. lundii* | 0.196 | 0.091 | $0.018^*$ |

Table S4: Results of the Tukey HSD testing for pairwise differences in average intra-colonial relatedness between four *Acromyrmex* species. The values in each cell represent the adjusted p-value for each comparison.

| | *A. crassispinus* | *A. heyeri* | *A. lobicornis* |
|---|---|---|---|
| *A. heyeri* | 0.073 | - | |
| *A. lobicornis* | 1.000 | 0.068 | - |
| *A. lundii* | 0.999 | $0.025^*$ | 1.000 |

Table S5: Binomial probabilities of sampling at least 3, 4, or 5 offspring from a queen with a 10% reproductive share in a colony.

| Number of offspring in an "outlier" cluster | 3/48 | 4/48 | 5/48 |
|---|---|---|---|
| Probability of sampling at least x offspring | 0.87 | 0.72 | 0.53 |

Table S6: Results of the pairwise Mann-Whitney U tests of differences in empirical mating frequency (M) between five *Acromyrmex* species. The values in each cell represent the FDR-corrected p-value for the given test. The tests may be unreliable due to unbalanced sampling in *A. ambiguus*.

| | *A. ambiguus* | *A. crassispinus* | *A. heyeri* | *A. lobicornis* |
|---|---|---|---|---|
| *A. crassispinus* | 0.744 | - | | |
| *A. heyeri* | 0.739 | 0.412 | - | |
| *A. lobicornis* | $5.31 \times 10^{-3**}$ | $8.24 \times 10^{-3**}$ | $2.40 \times 10^{-4***}$ | - |
| *A. lundii* | 0.111 | 0.064 | 0.091 | $8.57 \times 10^{-3**}$ |

Table S7: Results of the pairwise Mann-Whitney U tests of differences in effective mating frequency ($m_e$) between five *Acromyrmex* species. The values in each cell represent the FDR-corrected p-value for the given test. The tests may be unreliable due to unbalanced sampling in *A. ambiguus*.

| | *A. ambiguus* | *A. crassispinus* | *A. heyeri* | *A. lobicornis* |
|---|---|---|---|---|
| *A. crassispinus* | 0.402 | - | | |
| *A. heyeri* | 0.204 | 0.886 | - | |
| *A. lobicornis* | $6.73 \times 10^{-3**}$ | $0.037^{*}$ | $4.80 \times 10^{-4***}$ | - |
| *A. lundii* | $0.038^{*}$ | 0.204 | 0.101 | $0.020^{*}$ |
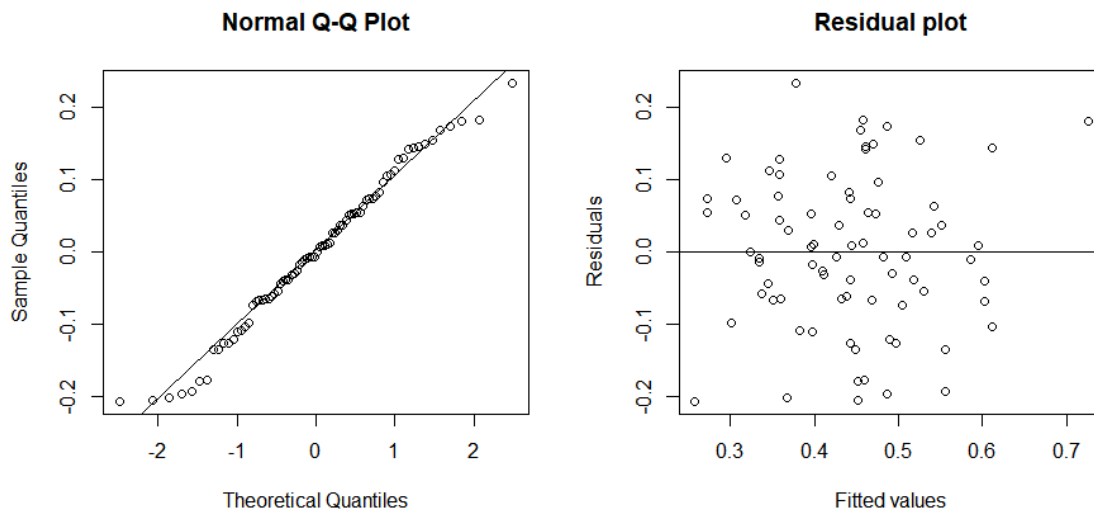


Figure S1: Normal probability (Quantile-Quantile) plot (left), and residual plot (right) of the linear regression model fitting average intra-colonial relatedness against average colony effective mating frequency, social structure, and species.

APPENDIX B

SUPPLEMENTAL INFORMATION FOR CHAPTER 3:

MULTI-QUEEN BREEDING IS ASSOCIATED WITH THE ORIGIN OF

INQUILINE SOCIAL PARASITISM IN ANTS

Table S9: List of the species curated in the dataset and their reported social structures. The evidences categories are summarized in table S12.

| Species | Gyny[1] | Type[2] | | In phylogeny | Evidence |
| | | Host status | | | |
| --- | --- | --- | --- | --- | --- |
| *Acromyrmex ambiguus* | P | Host | F | y | I |
| *Acromyrmex crassispinus* | P | Host | F | y | I |
| *Acromyrmex echinatior* | P | Host | F | y | I |
| *Acromyrmex heyeri* | P | Host | F | y | I |
| *Acromyrmex landolti* | M | Non-host | NA | y | I |
| *Acromyrmex lobicornis* | M | Non-host | NA | y | I |
| *Acromyrmex lundii* | M | Host | NA | y | I |
| *Acromyrmex octospinosus* | M | Host | NA | y | I |
| *Acromyrmex subterraneus* | P | Host | U | y | II |
| *Anoplolepis gracilipes* | P | Non-host | O | y | VI |
| *Aphaenogaster cockerelli* | M | Non-host | NA | y | VI |
| *Aphaenogaster japonica* | M | Non-host | NA | y | III |
| *Aphaenogaster rudis* | P | Non-host | F | y | III |
| *Aphaenogaster senilis* | M | Non-host | NA | y | III |
| *Aphaenogaster subterranea* | M | Non-host | NA | y | V |
| *Aphaenogaster treatae* | M | Non-host | NA | y | III |
| *Apterostigma dentigerum* | M | Non-host | NA | y | II |
| *Apterostigma mayri* | M | Non-host | NA | y | III |
| *Atta colombica* | M | Non-host | NA | y | I |
| *Atta sexdens* | M | Non-host | NA | y | I |
| *Azteca longiceps* | M | Non-host | NA | y | VI |
| *Brachymyrmex depilis* | M | Non-host | NA | y | III |
| *Camponotus americanus* | M | Non-host | NA | n | VI |
| *Camponotus consobrinus* | P | Non-host | F | n | I |
| *Camponotus festinatus* | P | Non-host | F | y | I |
| *Camponotus floridanus* | M | Non-host | NA | y | VI |
| *Camponotus herculaneus* | P | Non-host | U | n | VI |
| *Camponotus impressus* | M | Non-host | NA | n | VI |
| *Camponotus ligniperda* | P | Non-host | U | n | VI |
| *Camponotus nawai* | M | Non-host | NA | n | III |
| *Camponotus nearcticus* | M | Non-host | NA | y | VI |
| *Camponotus novaeboracensis* | M | Non-host | NA | y | VI |
| *Camponotus ocreatus* | M | Non-host | NA | y | III |

| | | | | | |
|---|---|---|---|---|---|
| *Camponotus pennsylvanicus* | M | Non-host | NA | y | III |
| *Camponotus planatus* | P | Non-host | U | n | VI |
| *Camponotus subbarbatus* | M | Non-host | NA | n | VI |
| *Camponotus tortuganus* | M | Non-host | NA | n | VI |
| *Camponotus werthi* | M | Non-host | NA | n | III |
| *Camponotus yamaokai* | P | Non-host | O | n | III |
| *Cardiocondyla atalanta* | P | Non-host | F | y | V |
| *Cardiocondyla batesii* | M | Non-host | NA | y | V |
| *Cardiocondyla elegans* | M | Non-host | NA | y | V |
| *Cardiocondyla emeryi* | P | Non-host | F | y | V |
| *Cardiocondyla kagutsuchi* | P | Non-host | F | y | V |
| *Cardiocondyla mauritanica* | P | Non-host | F | y | V |
| *Cardiocondyla minutior* | P | Non-host | F | y | V |
| *Cardiocondyla nigra* | M | Non-host | NA | y | V |
| *Cardiocondyla nuda* | P | Non-host | U | y | VI |
| *Cardiocondyla obscurior* | P | Non-host | F | y | V |
| *Cardiocondyla shuckardi* | P | Host | U | y | III |
| *Cardiocondyla strigifrons* | P | Non-host | F | n | V |
| *Cardiocondyla ulianini* | M | Non-host | NA | y | V |
| *Cardiocondyla wroughtonii* | P | Non-host | F | y | V |
| *Cataglyphis bicolor* | M | Host | NA | y | VI |
| *Cataglyphis bombycinus* | M | Non-host | NA | y | I |
| *Cataglyphis emmae* | M | Non-host | NA | n | I |
| *Cataglyphis hispanica* | M | Non-host | NA | y | I |
| *Cataglyphis livida* | M | Non-host | NA | n | I |
| *Cataglyphis mauritanica* | P | Non-host | O | y | I |
| *Cataglyphis niger* | P | Non-host | U | y | I |
| *Cataglyphis sabulosa* | M | Non-host | NA | n | I |
| *Cataglyphis savigny* | M | Non-host | NA | n | I |
| *Cataglyphis theryi* | M | Non-host | NA | n | I |
| *Cataglyphis velox* | P | Non-host | F | y | I |
| *Cephalotes atratus* | M | Non-host | NA | y | VI |
| *Cephalotes minutus* | M | Non-host | NA | y | VI |
| *Cephalotes setulifer* | M | Non-host | NA | y | VI |
| *Colobopsis nipponica* | M | Non-host | NA | y | VI(I) |
| *Crematogaster abstinens* | M | Non-host | NA | n | III |
| *Crematogaster ashmeadi* | M | Non-host | NA | y | VI |
| *Crematogaster atkinsoni* | P | Non-host | U | y | VI |
| *Crematogaster biroi* | M | Non-host | NA | y | III |

| | | | | | |
|---|---|---|---|---|---|
| *Crematogaster carinata* | P | Non-host | O | y | V |
| *Crematogaster cerasi* | M | Non-host | NA | y | VI |
| *Crematogaster crinosa* | P | Non-host | F | y | V |
| *Crematogaster emeryana* | M | Non-host | NA | y | VI |
| *Crematogaster erecta* | P | Non-host | F | y | V |
| *Crematogaster gerstaeckeri* | P | Non-host | U | y | VI |
| *Crematogaster laeviuscula* | M | Non-host | NA | y | VI |
| *Crematogaster limata* | P | Non-host | F | y | V |
| *Crematogaster lineolata* | M | Non-host | NA | y | VI |
| *Crematogaster longispina* | P | Non-host | U | y | V |
| *Crematogaster marioni* | M | Non-host | NA | y | VI |
| *Crematogaster minutissima* | P | Non-host | U | y | VI |
| *Crematogaster missouriensis* | M | Non-host | NA | y | I |
| *Crematogaster monteverdensis* | P | Non-host | O | y | V |
| *Crematogaster nigropilosa* | P | Non-host | F | y | V |
| *Crematogaster pygmaea* | P | Non-host | O | y | III |
| *Crematogaster schimmeri* | M | Non-host | NA | y | III |
| *Crematogaster scutellaris* | M | Non-host | NA | y | VI |
| *Crematogaster sumichrasti* | P | Non-host | U | y | V |
| *Cyphomyrmex costatus* | M | Non-host | NA | y | III |
| *Cyphomyrmex longiscapus* | M | Non-host | NA | y | III |
| *Cyphomyrmex rimosus* | P | Non-host | F | y | II |
| *Dolichoderus mariae* | P | Non-host | O | y | III |
| *Dolichoderus plagiatus* | P | Non-host | F | y | III |
| *Dolichoderus pustulatus* | P | Non-host | F | y | III |
| *Dolichoderus quadripunctatus* | M | Non-host | NA | y | III |
| *Dorylus nigricans molestus* | P | Non-host | F | y | I |
| *Dorymyrmex bicolor* | P | Non-host | F | y | I |
| *Dorymyrmex flavopecta* | M | Non-host | NA | y | III |
| *Dorymyrmex grandula* | M | Non-host | NA | y | VI |
| *Dorymyrmex insana* | M | Non-host | NA | y | I |
| *Dorymyrmex smithi* | P | Non-host | O | y | VI |
| *Labidus coecus* | M | Non-host | NA | n | III |
| *Eciton burchellii* | M | Non-host | NA | y | VI |
| *Eciton dulcium crassinode* | M | Non-host | NA | y | VI |
| *Eciton hamatum* | M | Non-host | NA | y | VI |
| *Eciton lucanoides* | M | Non-host | NA | y | VI |
| *Eciton mexicanum* | M | Non-host | NA | y | VI |
| *Eciton vagans angustatum* | M | Non-host | NA | y | VI |

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|---|---|---|---|---|---|
| *Ectatomma ruidum* | P | Non-host | F | y | III |
| *Ectatomma tuberculatum* | P | Host | F | y | II |
| *Forelius pruinosus* | P | Non-host | F | y | I |
| *Formica accreta* | P | Non-host | U | y | VI |
| *Formica aquilonia* | P | Non-host | O | n | VI |
| *Formica argentea* | P | Non-host | U | n | I |
| *Formica cinerea* | P | Non-host | O | y | VI |
| *Formica exsecta* | P | Non-host | F | y | III |
| *Formica exsectoides* | P | Non-host | U | n | III |
| *Formica fusca* | P | Non-host | F | y | VI |
| *Formica incerta* | P | Non-host | F | y | III |
| *Formica japonica* | P | Non-host | F | n | II |
| *Formica lugubris* | P | Non-host | O | y | VI |
| *Formica montana* | P | Non-host | F | y | III |
| *Formica neogagates* | P | Non-host | U | y | VI |
| *Formica obscuripes* | P | Host | O | y | III |
| *Formica obscuriventris* | M | Non-host | NA | y | VI |
| *Formica opaciventris* | P | Non-host | O | y | III |
| *Formica pallidefulva* | M | Non-host | NA | y | III |
| *Formica paralugubris* | P | Non-host | O | n | III |
| *Formica podzolica* | P | Non-host | F | n | III |
| *Formica polyctena* | P | Non-host | F | y | IV |
| *Formica pressilabris* | P | Non-host | U | n | III |
| *Formica rufa* | P | Non-host | F | y | III |
| *Formica selysi* | P | Non-host | F | n | I |
| *Formica subsericea* | P | Non-host | U | y | VI |
| *Formica transkaucasica* | P | Non-host | F | n | III |
| *Formica truncorum* | P | Non-host | U | y | III |
| *Formica ulkei* | P | Non-host | O | n | III |
| *Formica yessensis* | P | Non-host | O | n | III |
| *Gnamptogenys striatula* | P | Non-host | O | y | V |
| *Hypoponera ergatandria* | P | Non-host | F | y | II |
| *Iridomyrmex purpureus* | P | Non-host | F | y | I |
| *Lasius alienus* | M | Non-host | NA | y | VI |
| *Lasius flavus* | P | Non-host | F | y | I |
| *Lasius neglectus* | P | Non-host | O | y | III |
| *Lasius neoniger* | M | Non-host | NA | n | VI |
| *Lasius niger* | M | Non-host | NA | y | III |
| *Lasius pallitarsis* | M | Non-host | NA | n | III |

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|---|---|---|---|---|
| *Lasius sakagamii* | P | Non-host | O | y | II |
| *Leptogenys intermedia* | M | Non-host | NA | y | III |
| *Leptothorax acervorum* | P | Host | F | y | I |
| *Leptothorax gredleri* | M | Non-host | NA | y | VI |
| *Leptothorax muscorum* | P | Host | F | y | III |
| *Leptothorax retractus* | P | Non-host | F | n | III |
| *Leptothorax scamni* | M | Non-host | NA | n | II |
| *Linepithema humile* | P | Non-host | O | y | III |
| *Manica bradleyi* | P | Host | F | y | VI |
| *Manica rubida* | P | Non-host | U | y | I |
| *Megalomyrmex modestus* | P | Non-host | U | y | V |
| *Megalomyrmex silvestrii* | P | Non-host | F | y | V |
| *Messor ebeninus* | M | Non-host | NA | y | III |
| *Monomorium cyaneum* | P | Host | F | y | III |
| *Monomorium ebeninum* | P | Non-host | F | y | III |
| *Monomorium emarginatum* | P | Non-host | F | y | VI |
| *Monomorium ergatogyna* | P | Non-host | F | y | III |
| *Monomorium floricola* | P | Host | U | y | VI |
| *Monomorium minimum* | P | Host | F | y | III |
| *Monomorium pharaonis* | P | Non-host | O | y | III |
| *Monomorium salomonis* | P | Host | O | y | VI |
| *Monomorium viridum* | P | Non-host | F | y | III |
| *Mycetophylax conformis* | M | Non-host | NA | y | III |
| *Mycetophylax morschi* | M | Non-host | NA | y | III |
| *Mycetophylax simplex* | M | Non-host | NA | n | III |
| *Mycocepurus goeldii* | P | Host | F | y | I |
| *Myrmecia brevinoda* | P | Non-host | F | n | I |
| *Myrmecia dispar* | M | Non-host | NA | y | IV |
| *Myrmecia gulosa* | M | Non-host | NA | y | VI |
| *Myrmecia nigriceps* | M | Non-host | NA | y | Vi |
| *Myrmecia pilosula* | P | Non-host | F | y | I |
| *Myrmecia vindex* | M | Host | NA | y | VI |
| *Myrmecina graminicola* | P | Non-host | F | y | II |
| *Myrmecina nipponica* | P | Non-host | F | y | II |
| *Myrmecocystus depilis* | M | Non-host | NA | y | I |
| *Myrmica  obscura* | P | Non-host | F | n | VI |
| *Myrmica alaskensis* | P | Host | U | y | VI |
| *Myrmica americana* | P | Non-host | F | y | III |
| *Myrmica cagnianti* | P | Host | U | n | III |

| | | | | | |
|---|---|---|---|---|---|
| *Myrmica constricta* | P | Non-host | U | y | V |
| *Myrmica gallienii* | P | Host | F | n | I |
| *Myrmica hellenica* | P | Non-host | F | y | V |
| *Myrmica incompleta* | P | Non-host | U | y | VI |
| *Myrmica latifrons* | P | Non-host | F | | III |
| *Myrmica lobicornis* | P | Host | F | y | III |
| *Myrmica lonae* | P | Host | U | y | V |
| *Myrmica pinetorum* | M | Non-host | NA | n | VI |
| *Myrmica punctiventris* | P | Non-host | F | y | III |
| *Myrmica rubra* | P | Host | F | y | I |
| *Myrmica ruginodis* | P | Host | F | y | V |
| *Myrmica rugulosa* | P | Host | U | y | V |
| *Myrmica sabuleti* | P | Host | F | y | I |
| *Myrmica scabrinodis* | P | Host | F | y | I |
| *Myrmica schenki* | P | Non-host | F | y | III |
| *Myrmica serica* | P | Non-host | F | n | V |
| *Myrmica specioides* | P | Non-host | F | n | V |
| *Myrmica spinosior* | P | Host | U | y | V |
| *Myrmica sulcinodis* | P | Non-host | F | y | III |
| *Myrmica tahoensis* | P | Non-host | F | y | I |
| *Myrmicocrypta ednaella* | M | Non-host | NA | y | II |
| *Myrmica stangeana* | P | Non-host | F | n | V |
| *Neivamyrmex carolinensis* | P | Non-host | F | y | I |
| *Neivamyrmex nigrescens* | M | Non-host | NA | y | III |
| *Neivamyrmex opacithorax* | M | Non-host | NA | y | III |
| *Nothomyrmecia macrops* | M | Non-host | NA | y | I |
| *Nylanderia arenivaga* | M | Non-host | NA | y | VI |
| *Nylanderia flavipes* | M | Non-host | NA | y | III |
| *Nylanderia fulva* | P | Non-host | O | y | III |
| *Nylanderia parvula* | M | Host | NA | y | VI |
| *Odontomachus bauri* | M | Non-host | NA | y | III |
| *Odontomachus cephalotes* | P | Non-host | U | y | VI |
| *Odontomachus rixosus* | P | Non-host | O | y | II |
| *Odontomachus ruginodis* | M | Non-host | NA | y | VI |
| *Odontomachus troglodytes* | P | Non-host | F | y | III |
| *Oecophylla longinoda* | M | Non-host | NA | y | III |
| *Paltothyreus tarsatus* | M | Non-host | NA | y | III |
| *Paratrechina longicornis* | P | Non-host | U | y | III/VI |
| *Pheidole cockerelli* | M | Non-host | NA | y | VI |

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|---|---|---|---|---|---|
| *Pheidole desertorum* | M | Non-host | NA | y | I |
| *Pheidole floridana* | M | Non-host | NA | y | VI |
| *Pheidole megacephala* | P | Host | U | y | VI |
| *Pheidole pallidula* | P | Non-host | F | y | I |
| *Pheidole pilifera* | M | Host | NA | y | VI |
| *Pheidole tysoni* | M | Non-host | NA | y | VI |
| *Plagiolepis pygmaea* | P | Host | F | y | III |
| *Plagiolepis vindobonensis* | P | Host | U | y | VI |
| *Pogonomyrmex badius* | M | Non-host | NA | y | VI |
| *Pogonomyrmex barbatus* | M | Host | NA | y | I |
| *Pogonomyrmex desertorum* | M | Non-host | NA | y | VI |
| *Pogonomyrmex maricopa* | M | Non-host | NA | y | VI |
| *Pogonomyrmex montanus* | M | Non-host | NA | y | III |
| *Pogonomyrmex occidentalis* | M | Non-host | NA | y | I |
| *Pogonomyrmex pima* | P | Non-host | F | y | I |
| *Pogonomyrmex rugosus* | M | Host | NA | y | III |
| *Pogonomyrmex subnitidus* | M | Non-host | NA | y | III |
| *Ponera coarctata* | P | Non-host | F | y | III |
| *Ponera pennsylvanica* | P | Non-host | U | y | III |
| *Prenolepis imparis* | P | Non-host | F | y | VI |
| *Proatta butelli* | P | Non-host | O | y | III |
| *Proceratium silaceum* | P | Non-host | U | y | VI |
| *Proformica longiseta* | P | Non-host | F | y | I |
| *Pseudomyrmex apache* | P | Non-host | F | y | III |
| *Pseudomyrmex ejectus* | P | Host | F | n | VI |
| *Pseudomyrmex ferrugineus* | P | Non-host | F | n | V |
| *Pseudomyrmex flavicornis* | M | Non-host | NA | y | V |
| *Pseudomyrmex janzeni* | P | Non-host | F | n | V |
| *Pseudomyrmex mixtecus* | M | Non-host | NA | y | V |
| *Pseudomyrmex nigrocinctus* | M | Non-host | NA | y | V |
| *Pseudomyrmex pallidus* | P | Host | F | y | III |
| *Pseudomyrmex peperi* | P | Non-host | F | y | I |
| *Pseudomyrmex satanicus* | P | Non-host | O | y | V |
| *Pseudomyrmex seminole* | M | Non-host | NA | n | III |
| *Pseudomyrmex simplex* | P | Non-host | F | y | IV |
| *Pseudomyrmex spinicola* | M | Non-host | NA | y | V |
| *Pseudomyrmex veneficus* | P | Non-host | O | y | V |
| *Rhytidoponera chalybaea* | P | Non-host | F | y | I |
| *Rhytidoponera confusa* | P | Non-host | F | y | I |

| | | | | |
|---|---|---|---|---|
| *Rhytidoponera purpurea* | P | Non-host | F | y | I |
| *Sericomyrmex amabilis* | P | Non-host | F | y | I |
| *Solenopsis carolinensis* | P | Non-host | U | y | VI |
| *Solenopsis corticalis* | P | Non-host | U | y | V |
| *Solenopsis germinata* | P | Non-host | F | y | II/III |
| *Solenopsis invicta* | P | Non-host | F | y | II/III |
| *Solenopsis molesta* | P | Non-host | U | y | VI |
| *Solenopsis picta* | P | Non-host | U | y | VI |
| *Solenopsis quinquecuspis* | P | Host | F | y | II |
| *Solenopsis richteri* | P | Host | F | y | II |
| *Solenopsis texana* | P | Non-host | U | y | VI |
| *Stenamma brevicorne* | P | Non-host | F | n | IVb |
| *Stenamma debile* | P | Non-host | F | y | II |
| *Stenamma diecki* | P | Non-host | F | n | IVb |
| *Stigmatomma pallipes* | P | Non-host | F | y | II/III |
| *Strumigenys gundlachi* | M | Non-host | NA | y | VI |
| *Tapinoma erraticum* | P | Host | F | y | III |
| *Tapinoma litorale* | P | Non-host | U | y | VI |
| *Tapinoma melanocephalum* | P | Non-host | F | y | II |
| *Tapinoma minutum* | P | Non-host | F | y | IV |
| *Tapinoma sessile* | P | Non-host | U | y | VI |
| *Technomyrmex albipes* | P | Non-host | U | y | II |
| *Temnothorax affinis* | P | Non-host | F | n | III |
| *Temnothorax allardycei* | M | Non-host | NA | y | VI |
| *Temnothorax ambiguus* | P | Non-host | F | y | III |
| *Temnothorax angustulus* | M | Non-host | NA | y | VI |
| *Temnothorax ariadnae* | M | Non-host | NA | n | III |
| *Temnothorax aveli* | M | Non-host | NA | n | VI |
| *Temnothorax carinatus* | M | Non-host | NA | y | V |
| *Temnothorax crassispinus* | M | Non-host | NA | n | III |
| *Temnothorax curvispinosus* | P | Host | F | y | III |
| *Temnothorax exilis* | P | Host | F | y | V |
| *Temnothorax helenae* | M | Non-host | NA | n | V |
| *Temnothorax lichtensteini* | M | Non-host | NA | n | VI |
| *Temnothorax longispinosus* | P | Non-host | F | y | III |
| *Temnothorax neomexicanus* | M | Non-host | NA | y | VI |
| *Temnothorax nigriceps* | P | Non-host | F | y | II |
| *Temnothorax nylanderi* | P | Non-host | F | y | V |
| *Temnothorax obliquicanthus* | M | Non-host | NA | y | VI |

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|---|---|---|---|---|---|
| *Temnothorax obturator* | M | Non-host | NA | y | VI |
| *Temnothorax parvulus* | M | Non-host | NA | n | VI |
| *Temnothorax pastinifer* | M | Non-host | NA | y | VI |
| *Temnothorax pergandei* | M | Non-host | NA | y | VI |
| *Temnothorax recedens* | P | Non-host | U | n | V |
| *Temnothorax rugatulus* | P | Host | F | y | I |
| *Temnothorax spinosior* | P | Non-host | F | y | I |
| *Temnothorax tuberum* | P | Host | F | n | II |
| *Temnothorax tuscaloosae* | P | Non-host | F | y | II |
| *Temnothorax unifasciatus* | M | Non-host | NA | y | IVb |
| *Tetramorium alpestre* | P | Host | F | y | II |
| *Tetramorium bicarinatum* | P | Non-host | U | y | VI |
| *Tetramorium caespitum* | M | Host | NA | y | V |
| *Tetramorium caldarium* | P | Non-host | U | y | VI |
| *Tetramorium impurum* | P | Host | F | y | VI |
| *Tetramorium moravicum* | P | Non-host | F | y | VI |
| *Tetramorium simillimum* | P | Non-host | U | y | VI |
| *Tetraponera anthracina* | P | Non-host | F | y | III |
| *Trachymyrmex isthmicus* | M | Non-host | NA | y | III |
| *Trachymyrmex septentrionalis* | M | Non-host | NA | y | VI |
| *Trachymyrmex turrifex* | M | Non-host | NA | y | V |
| *Vollenhovia emeryi* | P | Host | U | y | VI |
| *Wasmannia auropunctata* | P | Non-host | O | y | V |
| *Xenomyrmex floridanus* | M | Non-host | NA | y | VI |
| *Leptanella japonica* | M | Non-host | NA | y | III |

[1]M = Monogynous; P = Polygynous

[2]F = Facultative; O = Obligate; U = Unknown

Table S10: References for the social structure recovered in table S9

| Species | Reference |
| --- | --- |
| *Acromyrmex ambiguus* | Chapter 2 |
| *Acromyrmex crassispinus* | Chapter 2 |
| *Acromyrmex echinatior* | Bekkevold et al. 1999 |
| *Acromyrmex heyeri* | Diehl et al. 2001; Chapter 2 |
| *Acromyrmex landolti* | Keller & Reeve 1994 |
| *Acromyrmex lobicornis* | Chapter 2 |
| *Acromyrmex lundii* | Chapter 2 |
| *Acromyrmex octospinosus* | Boomsma et al 1999 |
| *Acromyrmex subterraneus* | De Souza et al. 2004; 2005 |
| *Anoplolepis gracilipes* | Drescher et al. 2007 |
| *Aphaenogaster cockerelli* | Hölldobler & Carlin 1989 |
| *Aphaenogaster japonica* | Mizutani & Immamura 1980 |
| *Aphaenogaster rudis* | Boulay et al. 2007 |
| *Aphaenogaster senilis* | Cheron et al. 2009; Boulay et al. 2007 |
| *Aphaenogaster subterranea* | Stukalyuk & Radchenko 2011 |
| *Aphaenogaster treatae* | Talbot 1954 |
| *Apterostigma dentigerum* | Forsyth 1981 |
| *Apterostigma mayri* | Murakami et al. 2000 |
| *Atta colombica* | Fjerdingstad et al. 1998 |
| *Atta sexdens* | Fjerdingstad & Boomsma 2000 |
| *Azteca longiceps* | Frumhoff & Ward 1992 |
| *Brachymyrmex depilis* | Keller & Reeve 1994 |
| *Camponotus americanus* | Frumhoff & Ward 1992 |
| *Camponotus consobrinus* | Fraser et al. 2000 |
| *Camponotus festinatus* | Goodisman & Hahn 2005 |
| *Camponotus floridanus* | Frumhoff & Ward 1992 |
| *Camponotus herculaneus* | Hölldobler 1961 |
| *Camponotus impressus* | Frumhoff & Ward 1992 |
| *Camponotus ligniperda* | Hölldobler 1961 |
| *Camponotus nawai* | Satoh 1989; 1991 |
| *Camponotus nearcticus* | Frumhoff & Ward 1992 |
| *Camponotus novaeboracensis* | Frumhoff & Ward 1992 |
| *Camponotus ocreatus* | Goodisman & Hahn 2004 |
| *Camponotus pennsylvanicus* | Pricer 1908 |

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|---|---|
| *Camponotus planatus* | Frumhoff & Ward 1992 |
| *Camponotus subbarbatus* | Frumhoff & Ward 1992 |
| *Camponotus tortuganus* | Frumhoff & Ward 1992 |
| *Camponotus werthi* | Skaife 1961 |
| *Camponotus yamaokai* | Terayama & Satoh 1990; Satoh 1989 |
| *Cardiocondyla atalanta* | Heinze et al. 2005 |
| *Cardiocondyla batesii* | Heinze et al. 2005 |
| *Cardiocondyla elegans* | Heinze et al. 2005 |
| *Cardiocondyla emeryi* | Heinze et al. 2005 |
| *Cardiocondyla kagutsuchi* | Heinze et al. 2005 |
| *Cardiocondyla mauritanica* | Heinze et al. 2005 |
| *Cardiocondyla minutior* | Heinze et al. 2005 |
| *Cardiocondyla nigra* | Heinze et al. 2005 |
| *Cardiocondyla nuda* | Frumhoff & Ward 1992 |
| *Cardiocondyla obscurior* | Heinze et al. 2005 |
| *Cardiocondyla shuckardi* | Heinze et al 2014 |
| *Cardiocondyla strigifrons* | Heinze et al. 2005 |
| *Cardiocondyla ulianini* | Heinze et al. 2005 |
| *Cardiocondyla wroughtonii* | Heinze et al. 2005 |
| *Cataglyphis bicolor* | Wehner et al. 1994 |
| *Cataglyphis bombycinus* | Leniaud et al. 2013 |
| *Cataglyphis emmae* | Jowers et al. 2013 |
| *Cataglyphis hispanica* | Leniaud et al. 2012 |
| *Cataglyphis livida* | Timmermans et al. 2010 |
| *Cataglyphis mauritanica* | Eyer et al 2013 |
| *Cataglyphis niger* | Leniaud et al. 2011 |
| *Cataglyphis sabulosa* | Timmermans et al. 2008 |
| *Cataglyphis savigny* | Leniaud et al 2011 |
| *Cataglyphis theryi* | Leniaud et al 2013 |
| *Cataglyphis velox* | Eyer et al 2013 |
| *Cephalotes atratus* | Corn 1980 |
| *Cephalotes minutus* | Frumhoff & Ward 1992 |
| *Cephalotes setulifer* | Frumhoff & Ward 1992 |
| *Colobopsis nipponica* | Hasegawa 1994 |
| *Crematogaster abstinens* | Martins Segundo et al. 2017 |
| *Crematogaster ashmeadi* | Frumhoff & Ward 1992 |
| *Crematogaster atkinsoni* | Frumhoff & Ward 1992 |
| *Crematogaster biroi* | Peeters et al. 2013 |
| *Crematogaster carinata* | Longino 2003 |

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|---|---|
| *Crematogaster cerasi* | Frumhoff & Ward 1992 |
| *Crematogaster crinosa* | Longino 2003 |
| *Crematogaster emeryana* | Frumhoff & Ward 1992 |
| *Crematogaster erecta* | Longino 2003 |
| *Crematogaster gerstaeckeri* | Stanton et al 2002 |
| *Crematogaster laeviuscula* | Frumhoff & Ward 1992 |
| *Crematogaster limata* | Longino 2003 |
| *Crematogaster lineolata* | Frumhoff & Ward 1992 |
| *Crematogaster longispina* | Longino 2003 |
| *Crematogaster marioni* | Frumhoff & Ward 1992 |
| *Crematogaster minutissima* | Frumhoff & Ward 1992 |
| *Crematogaster missouriensis* | Heinze et al 2000 |
| *Crematogaster monteverdensis* | Longino 2003 |
| *Crematogaster nigropilosa* | Longino 2003 |
| *Crematogaster pygmaea* | Quinet et al 2009 |
| *Crematogaster schimmeri* | Peeters et al. 2013 |
| *Crematogaster scutellaris* | Frumhoff & Ward 1992 |
| *Crematogaster sumichrasti* | Longino 2003 |
| *Cyphomyrmex costatus* | Murakami et al. 2000 |
| *Cyphomyrmex longiscapus* | Mueller & Wcislo 1998 |
| *Cyphomyrmex rimosus* | Murakami et al. 2000 |
| *Dolichoderus mariae* | Laskis & Tchinkel 2009 |
| *Dolichoderus plagiatus* | Kannowski 1967 |
| *Dolichoderus pustulatus* | Kannowski 1967 |
| *Dolichoderus quadripunctatus* | Torossian 1960 |
| *Dorylus nigricans molestus* | Kronauer et al. 2004 |
| *Dorymyrmex bicolor* | Berkelhamer 1984 |
| *Dorymyrmex flavopecta* | Nickerson et al. 1975 |
| *Dorymyrmex grandula* | Frumhoff & Ward 1992 |
| *Dorymyrmex insana* | Berkelhamer 1984; Nickerson et al 1975 |
| *Dorymyrmex smithi* | Wagner & Fleur Nicklen 2006 |
| *Labidus coecus* | Rettenmeyer & Watkins 1978 |
| *Eciton burchellii* | Rettenmeyer & Watkins 1978 |
| *Eciton dulcium crassinode* | Rettenmeyer & Watkins 1978 |
| *Eciton hamatum* | Rettenmeyer & Watkins 1978 |
| *Eciton lucanoides* | Rettenmeyer & Watkins 1978 |
| *Eciton mexicanum* | Rettenmeyer & Watkins 1978 |
| *Eciton vagans angustatum* | Rettenmeyer & Watkins 1978 |
| *Ectatomma ruidum* | Pratt 1989; Breed et al 1990 |

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|---|---|
| *Ectatomma tuberculatum* | Hora et al. 2005 |
| *Forelius pruinosus* | Holldobler 1982; Berkelhamer 1984 |
| *Formica accreta* | Frumhoff & Ward 1992 |
| *Formica aquilonia* | Rosengren et al. 1993 |
| *Formica argentea* | Snyder 1993 |
| *Formica cinerea* | Rosengren et al. 1993 |
| *Formica exsecta* | Pisarski 1972; 1973; Pamilo & Rosengen 1984 |
| *Formica exsectoides* | Scherba 1961 |
| *Formica fusca* | Rosengren et al. 1993 |
| *Formica incerta* | Talbot 1948 |
| *Formica japonica* | Masuko et al 1998 |
| *Formica lugubris* | Rosengren et al. 1993 |
| *Formica montana* | Henderson & Jeanne 1992 |
| *Formica neogagates* | Frumhoff & Ward 1992 |
| *Formica obscuripes* | Finnegan 1977 |
| *Formica obscuriventris* | Frumhoff & Ward 1992 |
| *Formica opaciventris* | Scherba 1961 |
| *Formica pallidefulva* | Talbot 1948 |
| *Formica paralugubris* | Chapuisat et al 1999 |
| *Formica podzolica* | Deslippe & Savolainen 1995 |
| *Formica polyctena* | Seifert 1991; Pamilo 1982 |
| *Formica pressilabris* | Pamilo & Rosengren 1984 |
| *Formica rufa* | Seifert 1991 |
| *Formica selysi* | Purcell et al 2015 |
| *Formica subsericea* | Frumhoff & Ward 1992 |
| *Formica transkaucasica* | Mabelis & Chardon 2005; Pamilo 1982 |
| *Formica truncorum* | Rosengren et al. 1985; Sundstrom 1993; Elias et al. 2004 |
| *Formica ulkei* | Scherba 1961 |
| *Formica yessensis* | Higashi 1983 |
| *Gnamptogenys striatula* | Blatrix & Jaisson 2000 |
| *Hypoponera ergatandria* | Yamauchi et al. 1996; Seifert 2003 |
| *Iridomyrmex purpureus* | Halliday 1983 |
| *Lasius alienus* | Frumhoff & Ward 1992 |
| *Lasius flavus* | Steinmeyer et al. 2012 |
| *Lasius neglectus* | Van Loon et al. 1990 |

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|---|---|
| *Lasius neoniger* | Frumhoff & Ward 1992 |
| *Lasius niger* | Gaspar 1965 |
| *Lasius pallitarsis* | Nonacs 1990 |
| *Lasius sakagamii* | Yamauchi et al. 1981; 1982 |
| *Leptogenys intermedia* | Villet et al. 1991 |
| *Leptothorax acervorum* | Douwes et al. 1987 |
| *Leptothorax gredleri* | Buschinger 1968 |
| *Leptothorax muscorum* | Henize & Buschinger 1988; Buschinger 1979 |
| *Leptothorax retractus* | Heinze & Buschinger 1988 |
| *Leptothorax scamni* | Heinze & Gratiashvili 2015 |
| *Linepithema humile* | Tsutsui & Case 2001; Newell & Barber 1913 |
| *Manica bradleyi* | Wheeler & Wheeler 1970 |
| *Manica rubida* | Cammaerts & Cammaerts 1987; Lenoir et al. 2010 |
| *Megalomyrmex modestus* | Boudinot et al. 2013 |
| *Megalomyrmex silvestrii* | Boudinot et al. 2013 |
| *Messor ebeninus* | Tohme 1975 |
| *Monomorium cyaneum* | DuBois 2000 |
| *Monomorium ebeninum* | DuBois 2000 |
| *Monomorium emarginatum* | Frumhoff & Ward 1992 |
| *Monomorium ergatogyna* | DuBois 2000 |
| *Monomorium floricola* | Frumhoff & Ward 1992 |
| *Monomorium minimum* | DuBois 2000 |
| *Monomorium pharaonis* | DuBois 2000 |
| *Monomorium salomonis* | Forel 1928 |
| *Monomorium viridum* | DuBois 2000 |
| *Mycetophylax conformis* | Klingenberg et al. 2007 |
| *Mycetophylax morschi* | Klingenberg et al. 2007 |
| *Mycetophylax simplex* | Klingenberg et al. 2007 |
| *Mycocepurus goeldii* | Rabeling & Bacci 2010; Rabeling et al. in prep |
| *Myrmecia brevinoda* | Qian et al. 2011 |
| *Myrmecia dispar* | Gray 1971 |
| *Myrmecia gulosa* | Haskins & Haskins 1980 |
| *Myrmecia nigriceps* | Haskins & Haskins 1980 |
| *Myrmecia pilosula* | Craig & Crozier 1979 |
| *Myrmecia vindex* | Keller & Genoud 1997; Haskins & Haskins 1980 |

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|---|---|
| *Myrmecina graminicola* | Buschinger & Schreiber  2002 |
| *Myrmecina nipponica* | Okhawara et al. 1993 |
| *Myrmecocystus depilis* | Hölldobler et al. 2011 |
| *Myrmica  obscura* | Radchenko & Elmes 2010 |
| *Myrmica alaskensis* | Glasier et al. 2014 |
| *Myrmica americana* | Keller & Reeve 1994 |
| *Myrmica cagnianti* | Espadaler 1996 |
| *Myrmica constricta* | Czekes et al 2012 |
| *Myrmica gallienii* | Elmes & Petal 1990; Seppä 1996 |
| *Myrmica hellenica* | Czekes et al 2012 |
| *Myrmica incompleta* | Buschinger et al. 1980; Lenoir et al. 1992 |
| *Myrmica latifrons* | Kannowski 1970 |
| *Myrmica lobicornis* | Kannoski 1970; Seppä 1994 |
| *Myrmica lonae* | Czekes et al 2012 |
| *Myrmica pinetorum* | Frumhoff & Ward 1992 |
| *Myrmica punctiventris* | Bansbach and Herbers 1996 |
| *Myrmica rubra* | Elmes & Petal 1990; Leppannen et al 2014 |
| *Myrmica ruginodis* | Czekes et al 2012 |
| *Myrmica rugulosa* | Czechowski 1979; Czekes et al 2012 |
| *Myrmica sabuleti* | Seppä 1996 |
| *Myrmica scabrinodis* | Seppä 1996 |
| *Myrmica schenki* | Elmes 1980 |
| *Myrmica serica* | Radchenko & Elmes 2010 |
| *Myrmica specioides* | Jansen & Radchenko 2009 |
| *Myrmica spinosior* | Radchenko & Elmes 2010 |
| *Myrmica sulcinodis* | Radchenko & Elmes 2010; Elmes 1987 |
| *Myrmica tahoensis* | Evans 1998 |
| *Myrmicocrypta ednaella* | Murakami & Higashi 1997 |
| *Myrmica stangeana* | Radchenko & Elmes 2010 |
| *Neivamyrmex carolinensis* | Rettenmeyer & Watkins 1978; Kronauer & Boomsma 2007 |
| *Neivamyrmex nigrescens* | Rettenmeyer & Watkins 1978 |
| *Neivamyrmex opacithorax* | Rettenmeyer & Watkins 1978 |
| *Nothomyrmecia macrops* | Sanetra & Crozier 2001 |
| *Nylanderia arenivaga* | Frumhoff & Ward 1992 |
| *Nylanderia flavipes* | Ichinose 1994 |

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|---|---|
| *Nylanderia fulva* | Arcila et al. 2002; McDonald 2012 |
| *Nylanderia parvula* | Frumhoff & Ward 1992 |
| *Odontomachus bauri* | Ehmer & Hölldobler 1995 |
| *Odontomachus cephalotes* | Peeters 1987; Ito et al 1996 |
| *Odontomachus rixosus* | Ito et al 1996 |
| *Odontomachus ruginodis* | Frumhoff & Ward 1992 |
| *Odontomachus troglodytes* | Colombel 1970 |
| *Oecophylla longinoda* | Hölldobler & Wilson 1983 |
| *Paltothyreus tarsatus* | Braun et al. 1994 |
| *Paratrechina longicornis* | Yamauchi & Ogata 1995 |
| *Pheidole cockerelli* | Frumhoff & Ward 1992 |
| *Pheidole desertorum* | Helms 1999 |
| *Pheidole floridana* | Frumhoff & Ward 1992 |
| *Pheidole megacephala* | Hoffmann 1998 |
| *Pheidole pallidula* | Fournier et al 2002 |
| *Pheidole pilifera* | Frumhoff & Ward 1992 |
| *Pheidole tysoni* | Frumhoff & Ward 1992 |
| *Plagiolepis pygmaea* | Passera et al. 2001 |
| *Plagiolepis vindobonensis* | Faber 1969; Buschinger 1990 |
| *Pogonomyrmex badius* | Keller & Reeve 1994 |
| *Pogonomyrmex barbatus* | Suni et al. 2007 |
| *Pogonomyrmex desertorum* | Keller & Reeve 1994 |
| *Pogonomyrmex maricopa* | Keller & Reeve 1994 |
| *Pogonomyrmex montanus* | MacKay 1981 |
| *Pogonomyrmex occidentalis* | Wiernasz et al. 2004 |
| *Pogonomyrmex pima* | Tate Holbrook et al. 2007; Strehl 2005 |
| *Pogonomyrmex rugosus* | Gadau et al 2002 |
| *Pogonomyrmex subnitidus* | Mackay 1981 |
| *Ponera coarctata* | Liebig et al. 1995 |
| *Ponera pennsylvanica* | Pratt et al. 1994 |
| *Prenolepis imparis* | Tschinkel 1987 |
| *Proatta butelli* | Moffet 1986 |
| *Proceratium silaceum* | Frumhoff & Ward 1992 |
| *Proformica longiseta* | Seppä et al. 2008 |
| *Pseudomyrmex apache* | Ward 1985 |
| *Pseudomyrmex ejectus* | Ward 1985 |
| *Pseudomyrmex ferrugineus* | Ward 1993 |
| *Pseudomyrmex flavicornis* | Ward 1993 |

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|---|---|
| *Pseudomyrmex janzeni* | Ward 1993; Janzen 1973 |
| *Pseudomyrmex mixtecus* | Ward 1993 |
| *Pseudomyrmex nigrocinctus* | Ward 1993 |
| *Pseudomyrmex pallidus* | Ward 1985 |
| *Pseudomyrmex peperi* | Kautz et al. 2009 |
| *Pseudomyrmex satanicus* | Ward 1993; Janzen 1974 |
| *Pseudomyrmex seminole* | Ward 1985 |
| *Pseudomyrmex simplex* | Ward 1985 |
| *Pseudomyrmex spinicola* | Ward 1993 |
| *Pseudomyrmex veneficus* | Ward 1993 |
| *Rhytidoponera chalybaea* | Ward 1980 |
| *Rhytidoponera confusa* | Ward 1980 |
| *Rhytidoponera purpurea* | Ward 1980 |
| *Sericomyrmex amabilis* | Villesen et al 2002 |
| *Solenopsis carolinensis* | Frumhoff & Ward 1992 |
| *Solenopsis corticalis* | Thompson 1989 |
| *Solenopsis germinata* | Adams et al 1976 |
| *Solenopsis invicta* | Greenberg et al 1985 |
| *Solenopsis molesta* | Frumhoff & Ward 1992 |
| *Solenopsis picta* | Frumhoff & Ward 1992 |
| *Solenopsis quinquecuspis* | Jouvenaz et al 1989 |
| *Solenopsis richteri* | Jouvenaz et al 1989 |
| *Solenopsis texana* | Frumhoff & Ward 1992 |
| *Stenamma brevicorne* | Talbot 1974 |
| *Stenamma debile* | Buschinger 1999 |
| *Stenamma diecki* | Talbot 1974 |
| *Stigmatomma pallipes* | Traniello 1982 |
| *Strumigenys gundlachi* | Frumhoff & Ward 1992 |
| *Tapinoma erraticum* | Cournault & Aron 2009 |
| *Tapinoma litorale* | Frumhoff & Ward 1992 |
| *Tapinoma melanocephalum* | Bustos & Cherix 1998 |
| *Tapinoma minutum* | Herbers 1991 |
| *Tapinoma sessile* | Buczkowski & Bennett 2008 |
| *Technomyrmex albipes* | Yamauchi et al. 1991 |
| *Temnothorax affinis* | Buschinger 1968 |
| *Temnothorax allardycei* | Frumhoff & Ward 1992 |
| *Temnothorax ambiguus* | Alloway et al 1982 |
| *Temnothorax angustulus* | Frumhoff & Ward 1992 |
| *Temnothorax ariadnae* | Salata et al 2018 |

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|---|---|
| *Temnothorax aveli* | Keller 1998 |
| *Temnothorax carinatus* | MacKay 2000 |
| *Temnothorax crassispinus* | Ticha & Stys 2002; Ticha 1992; Ticha 2002 |
| *Temnothorax curvispinosus* | Alloway et al. 1982 |
| *Temnothorax exilis* | Salata et al. 2018 |
| *Temnothorax helenae* | Salata et al. 2018 |
| *Temnothorax lichtensteini* | Keller 1998 |
| *Temnothorax longispinosus* | Alloway et al 1982 |
| *Temnothorax neomexicanus* | Frumhoff & Ward 1992 |
| *Temnothorax nigriceps* | Buschinger 1968 |
| *Temnothorax nylanderi* | Buschinger 1968 |
| *Temnothorax obliquicanthus* | Frumhoff & Ward 1992 |
| *Temnothorax obturator* | Frumhoff & Ward 1992 |
| *Temnothorax parvulus* | Stukalyduk & Radchenko 2011 |
| *Temnothorax pastinifer* | Frumhoff & Ward 1992 |
| *Temnothorax pergandei* | Frumhoff & Ward 1992 |
| *Temnothorax recedens* | Salata et al 2018 |
| *Temnothorax rugatulus* | Rüppell et al. 1998; Rüppell et al 2002 |
| *Temnothorax spinosior* | Hamaguchi et al. 1993 |
| *Temnothorax tuberum* | Buschinger 1968 |
| *Temnothorax tuscaloosae* | Guenard et al. 2016 |
| *Temnothorax unifasciatus* | Buschinger 1968 |
| *Tetramorium alpestre* | Steiner 2003; Wagner et al. 2017 |
| *Tetramorium bicarinatum* | Astruc et al. 2001 |
| *Tetramorium caespitum* | Wagner et al. 2017 |
| *Tetramorium caldarium* | Frumhoff & Ward 1992 |
| *Tetramorium impurum* | Steiner et al 2003; Buschinger 1974 |
| *Tetramorium moravicum* | Shlick-Steiner et al. 2005 |
| *Tetramorium simillimum* | Frumhoff & Ward 1992 |
| *Tetraponera anthracina* | Terron 1977 |
| *Trachymyrmex isthmicus* | Murakami et al. 2000 |
| *Trachymyrmex septentrionalis* | Frumhoff & Ward 1992 |
| *Trachymyrmex turrifex* | Rabeling et al. 2007 |
| *Vollenhovia emeryi* | Kinomura & Yamauchi 1994; Okamoto & Ohkawara 2010 |
| *Wasmannia auropunctata* | Ulloa-Chacon & Cherix 1988 |

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|---|---|
| *Xenomyrmex floridanus* | Frumhoff & Ward 1992 |
| *Leptanella japonica* | Masuko 1990 |

Table S11: Phylogenetic information and references used for the reconstruction of a cladogram. Only genera retained after filtering the data for maximum statistical power are shown. Taxa with only one species in the social structure dataset were equally omitted.

| Group | Level | Source |
|---|---|---|
| Formicidae | Sub-Families | (Borowiec et al. 2019) |
| Ponerinae | Genera | (Schmidt and Shattuck 2014) |
| Dorylinae | Genera | (Borowiec 2016) |
| Dolichoderinae | Genera | (Ward et al. 2010) |
| Pseudomyrmecinae | Genera | (Ward and Downie 2005) |
| Myrmecinae | Genera | (Ward et al. 2015) |
| Formicinae | Genera | (Ward et al. 2016) |
| Amblyoponinae | Genera | (Ward and Fisher 2016) |
| *Myrmecia* | Species | (Hasegawa and Crozier 2006) |
| *Lasius* | Species | (Maruyama et al. 2008) |
| *Pseudomyrmex* | Species | (Gómez-Acevedo et al. 2010) |
| *Myrmica* | Species | (Jansen et al. 2010) |
| *Cataglyphis* | Species | (Knaden et al. 2012) |
| *Formica* | Species | (Blaimer et al. 2015) |
| *Anochetus* | Species | (Larabee et al. 2016) |
| *Odontomachus* | Species | (Larabee et al. 2016) |
| *Temnothorax* | Species | (Prebus 2017) |

Table S12: Evidence categories used in table S9.

| Evidence class | Evidence |
|---|---|
| I | Molecular analyses |
| II | Queen dissections |
| III | >10% polygyny, or 100% monogyny among field colonies |
| IV | 5%<X<10% polygyny |
| IVb | <5% polygyny |
| V | Taxonomic monograph |
| VI | Mentioned in passing/Personal Communications |