Faculty of Biological and Environmental Sciences
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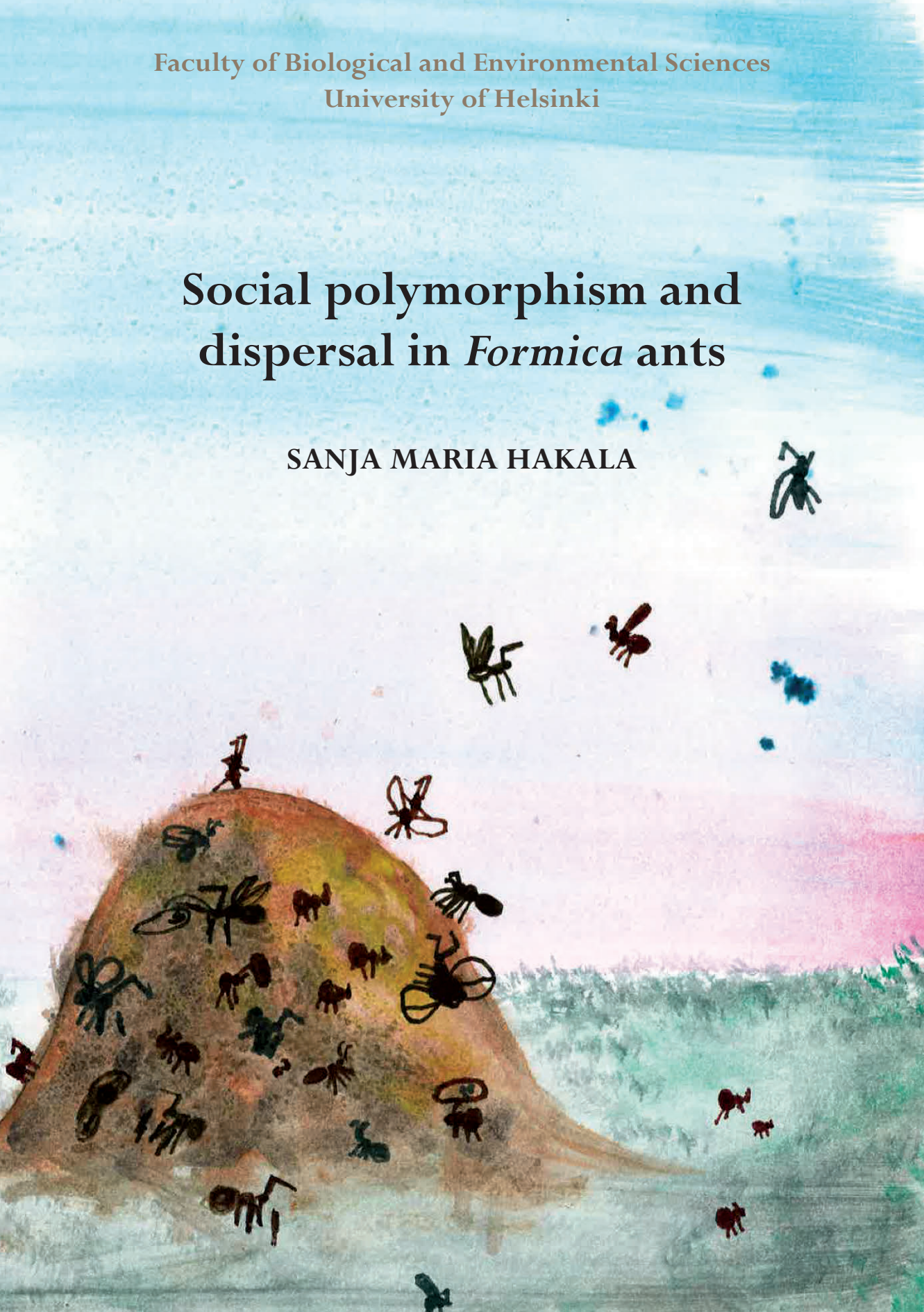
SANJA MARIA HAKALA



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Kuule istuta vielä se omenapuu
vaikka tuli jo tukkaasi nuolee
vaikka huomenna saaste jo laskeutuu
vaikka huomenna aurinko kuolee

Juice Leskinen (1981)

CONTENTS / SISÄLLYS

* Jokainen kappale alkaa suomenkielisellä tiivistelmällä.
Every chapter begins with a Finnish translation of its abstract.

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following articles, which are referred to in the text by their Roman numerals:

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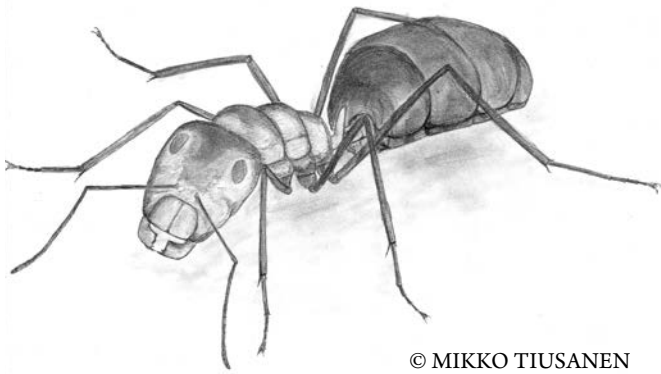

ABSTRACT

There are intricate links between the evolution of sociality and the spatial population structures created by dispersal. In ants, the evolution of the most complex societies, supercolonies, is strongly connected to limited dispersal. The supercolonies consist of hundreds of interconnected nests where thousands of queens and their workers cooperate over large areas. Supercolonies arise from simpler family units when large proportions of daughter queens are philopatric and stay in their natal colony as additional reproducing queens instead of dispersing by wing. This allows the colonies to grow quickly and colonize large areas, but also increases social conflicts due to very low local relatedness in these societies.

In this thesis I inspect the evolution and maintenance of ant supercolonies, by focusing on dispersal traits and the consequences of dispersal in socially polymorphic *Formica* ants. *Formica* have both simple family-based monodomous colonies and complex supercolonies, and some species have also intraspecific variation. To lay a sound theoretical background for my work, I began by synthesizing current knowledge of dispersal evolution in ants. In my literature review I showed how eco-evolutionary feedbacks link the evolution of ant dispersal strategies and social organization, and pinpointed the most relevant future research directions. Next, to be able to inspect some of the hypotheses formulated in the review, I clarified the species identification of one of my intended study species, *Formica fennica* with molecular methods, and concluded that the populations I studied should be treated as conspecific to *Formica exsecta*.

I analyzed the individual dispersal traits of six socially variable species of *Formica* ants to assess whether dispersal ability varies between monodomous and supercolonial societies in accordance to the observed behavioral difference. According to my results the dispersal behavior of these species is likely not restricted by their morphology or resources the individuals get from their natal colonies. The traits of all species and both sexes indicate good flight ability, with overall male bias and large variation both among and within species. The increased philopatry in supercolonial species and populations is more a behavior change: the queens are philopatric even though the society provides them resources for dispersal. However, I observed a small decrease of male flight muscle ratio in supercolonial species, which indicates strong coevolution of the sexes.

In order to better understand the outcomes of limited dispersal in supercolonial *Formica*, I analyzed the behavioral and genetic structure of a dense supercolonial population of *Formica pressilabris*. The population is genetically viscous at a small spatial scale, but still not genetically structured by location on a larger spatial scale. This indicates that although dispersal is limited within the population, a large enough proportion of individuals do disperse to keep the local populations connected. Interestingly, when analyzing worker behavior among the polydomous nests, the observed aggression pattern indicates that they are not a single cooperative unit – but also not clearly separate colonies. The sensitive behavioral assay developed for this study shows that workers allow a proportion of individuals from outside nests to enter their own nest material, but are slightly more aggressive towards individuals from further away. This indicates the population is only partially cooperative over the whole nest aggregation, and shows that the inner structures of supercolonies should be analyzed in more detail.

This thesis sheds light on ants' dispersal ability and behavior, and demonstrates the crucial role of dispersal in the evolution of their different social structures. My results also raise new questions about possible conflicts over dispersal in ant societies.

TIIVISTELMÄ

Sosiaalisuuden evoluutio linkittyy vahvasti siihen, kuinka eliöt hajaantuvat elinympäristöönsä levittäytymisen seurauksena. Muurahaisten monimutkaisimpien yhteiskuntien, superkolonioiden, evoluutio on yhteydessä rajoittuneeseen levittäytymiseen. Superkoloniat koostuvat sadoista toisiinsa yhteydessä olevista pesistä, joissa tuhannet kuningattaret ja niiden työläiset tekevät yhteistyötä laajoilla alueilla. Superkoloniat syntyvät yksinkertaisemmista perheyksiköistä, kun suuri osuus tytärkuningattarista jää synnyinkoloniaansa ylimääräisiksi lisääntyviksi kuningattariksi sen sijaan, että lentäisivät muualle. Tämän seurauksena yhteiskunnat voivat kasvaa nopeasti ja suurille alueille, mutta samalla sosiaaliset ristiriidat lisääntyvät, koska paikallisesti sukulaisuus laskee hyvin alhaiseksi.

Tässä väitöskirjassa tutkin superkolonioiden evoluutiota ja toimintaa sosiaalisesti monimuotoisilla suomumuurahaisilla (*Formica*), keskittyen levittäytymiseen liittyviin ominaisuuksiin ja levittäytymisen seurauksiin. *Formica*-muurahaisilla on sekä yksipesäisiä perheryhmiin perustuvia kolonioita että monimutkaisia superkolonioita, ja joillakin lajeilla on myös lajinsisäistä vaihtelua. Luodakseni työlleni vahvan teoreettisen pohjan, aloitin kokoamalla yhteen nykytietämyksen muurahaisten levittäytymisen evoluutiosta. Katsausartikkelissani osoitan kuinka eko-evolutiiviset takaisinkytkennät liittävät levittäytymisstrategioiden evoluution ja sosiaalisen rakenteen toisiinsa. Hahmottelen myös tärkeimpiä tulevaisuuden tutkimussuuntia. Voidakseni tutkia katsausartikkelissa muotoilemiani hypoteeseja, varmistin suomenloviniskan (*Formica fennica*) lajintunnistuksen molekulaarisin menetelmin. Totesin, että geneettisen samankaltaisuuden vuoksi tutkimuspopulaatioitani tulee käsitellä karvaloviniskan (*Formica exsecta*) kanssa samaan lajiin kuuluvina.

Analysoin kuuden sosiaalisesti erilaisen *Formica*-lajin yksilöiden levittäytymisominaisuuksia selvittääkseni, vaihteleeko niiden yksipesäisten ja superkoloniaalisten yhteiskuntien levittäytymiskyky samalla tavalla kuin niiden levittäytymiskäyttäytymisen tiedetään vaihtelevan. Osoitin, että näiden lajien käyttäytyminen ei todennäköisesti riipu yksilöiden morfologiasta tai resursseista, joita ne saavat synnyinkolonioiltaan. Jokaisen tutkimani lajin ominaisuudet viittaavat siihen, että sekä koiraat että kuningattaret osaavat lentää hyvin. Koirailla on parempi lentokyky kuin kuningattarilla, ja sekä lajien välillä että lajien sisällä yksilöiden välillä on suurta vaihtelua. Se, että superkoloniaalisten lajien kuningattaret jäävät synnyinkoloniaan on siis käyttäytymispiirre: ne jäävät, vaikka yhteiskunta antaa niille tarvittavat resurssit levittäytymiseen. Havaitsin kuitenkin, että superkoloniaalisilla lajeilla koiraiden lentolihasten koko on pienentynyt, mikä viittaa sukupuolten yhteisevoluutioon.

Ymmärtääkseni rajoittuneen levittäytymisen seurauksia superkoloniaalisilla *Formica*-muurahaisilla, analysoin kaljuloviniskan (*Formica pressilabris*) tiiviin, superkoloniaalisen populaation käyttäytymistä ja geneettistä rakennetta. Osoitin, että populaation sisällä läheiset pesät ovat geneettisesti samankaltaisempia kuin kaukaisemmat pesät, mutta että suuremmassa mittakaavassa populaatiot eivät silti suuresti eroa geneettisesti toisistaan. Tämä viittaa siihen, että vaikka levittäytyminen on rajoittunutta populaation sisällä, tarpeeksi suuri joukko yksilöitä silti lentää alueelta toiselle, ja eri paikallispopulaatiot ovat yhteydessä toisiinsa. Superkolonian sisäisen käyttäytymisen analysoiminen osoitti mielenkiintoisen seikan: työläisten vaihtelevasti aggressiivinen käytös viittaa siihen, että kaikki pesät eivät kuulu samaan yhteistyössä olevaan yksikköön – mutta pesien välille on silti mahdoton osoittaa selkeitä rajoja. Tätä tutkimusta varten kehitetty herkkä käyttäytymisanalyysi osoitti, että työläiset sallivat muiden pesien työläisten tulla omalle pesämateriaalilleen, mutta ovat hiukan aggressiivisempia mitä kauempaa vierailijat ovat peräisin. Tämä viittaa siihen, että populaatio on vain osittain yhteistyössä koko pesärykelmän alalla. Superkolonioiden sisäistä rakennetta kannattaa jatkossa tutkia aiempaa yksityiskohtaisemmin.

Tämä väitöskirja valottaa muurahaisten levittäytymiskykyä ja -käyttäytymistä, sekä näyttää levittäytymisen linkittyvän vahvasti sosiaalisten rakenteiden evoluutioon. Tulokseni herättävät myös uusia kysymyksiä levittäytymiseen mahdollisesti liittyvistä ristiriidoista muurahaisyhteiskunnissa.

SUMMARY

Sanja Maria Hakala

1 INTRODUCTION

1.1 KIN SELECTION EXPLAINS SOCIAL EVOLUTION

Understanding social evolution is fundamental for our understanding of most biological systems, from genomes to eukaryote cells, and from multicellularity to social groups and societies. The increasing complexity of biological organization has evolved through major evolutionary transitions, where originally independent units come together and evolve to function as a single entity (Szathmáry and Maynard Smith 1995, Szathmáry 2015). The major evolutionary transitions, and the evolution of cooperation and altruism that are needed for them, can be explained with kin selection (inclusive fitness) and multilevel selection frameworks, from which the kin selection framework is more formally constructed and more widely accepted (Abbot et al. 2011, Kramer and Meunier 2016, Birch 2017).

The kin-selection theory explains how altruistic behavior can evolve even though it is harmful for the actor's direct fitness: generalized Hamilton's rule states that an altruistic trait can be selected for if the benefit to others (b) multiplied by relatedness between the two individuals (r) is higher than costs to self (c) (Hamilton 1963; Hamilton 1964 a & b; Maynard Smith 1964, Queller 1992a).

$$b * r > c$$

The benefits and costs are measured as changes in fitness – in practice usually as the number or biomass of offspring produced. In the kin selection framework, an organism's fitness is divided into two components: direct fitness that the organism gets through producing own offspring, and indirect fitness that it gets by helping other individuals produce more offspring that share some of their genes (or alleles) with it. These two fitness components produce inclusive fitness, a much used concept for understanding social adaptations. At its extreme, kin selection can lead to completely forgoing one's own reproduction in order to help relatives to reproduce. There is overwhelming evidence for the notion that social behavior correlates with relatedness across all domains of life, providing empirical support for kin selection theory (Bourke 2011). All interactions between two organisms can be analyzed under the kin selection framework – not just those that are harmful for the actor and beneficial for the recipient (altruism), but also those that are beneficial for both (cooperation), harmful for both (spite) or beneficial for the actor but harmful for the recipient (selfishness) (Hamilton 1970, 1972).

In the multilevel selection framework the focus is, instead of individual actors and their inclusive fitness, on the different levels of natural selection, from cells to individuals to social groups, and on the notion that there can be adaptations on all these levels (Okasha 2006). Especially when discussing the major evolutionary transitions and the evolution of individuality on different levels of organization, this way of framing the discussion has some benefits (Szathmáry and Maynard Smith 1995, Okasha 2006, Szathmáry 2015). Despite the sometimes heated debate, these two frameworks are not mutually exclusive and can be used as complementary (Kramer and Meunier 2016). In this thesis, I follow the framework of kin selection theory, while discussing natural selection acting on multiple levels.

1.2 DISPERSAL AND SOCIALITY

Any movement of individuals or their propagules that can lead to gene flow is dispersal (Ronce 2007). If an organism does not disperse away from its relatives, it will compete for resources with them, which is usually

harmful for its inclusive fitness and considered one of the main selective pressures for the existence of dispersal (Hamilton and May 1977, West et al. 2002). However, this problem is partially overcome if staying close to relatives brings some additional benefits through social interactions. Limited dispersal and the resulting genetically viscous population structures often promote altruism (Taylor 1992, Lehmann et al. 2008, Kümmerli et al. 2009, Platt and Bever 2009, Gardner 2010, Van Dyken and Wade 2012), although the exact relationship between limited dispersal and altruism is far from simple and depends greatly on both ecological and social factors (Queller 1992b, Kümmerli et al. 2009, Gardner 2010).

Natal philopatry, where individuals forgo or delay dispersal and instead stay in their birth site or social group, is common in social animals (Greenwood 1980). This means they are balancing the benefits arising from philopatry (cooperation and altruism), and the negative effects of limited dispersal: increased kin competition (Hamilton and May 1977, West et al. 2002), increased inbreeding that leads to reduced genetic diversity and inbreeding depression (Bengtsson 1978, Motro 1991, Perrin and Goudet 2001), and increased risk of local extinction (Van Valen 1971, Bowler and Benton 2004, Burgess et al. 2016). Because changes in dispersal strategies can kindle both cooperation and conflict among individuals, it is beneficial to analyze dispersal in the light of social evolution (Chapter I).

1.3 ANT COLONY AS A SUPERORGANISM

Ants and other eusocial hymenopterans (Wilson 1971) can be considered to be superorganisms that have evolved individuality at the society level (Wheeler 1911, Boomsma and Gawne 2018). In these societies, only queens and males reproduce and can thus be considered equivalent to germ line cells in multicellular organisms, whereas female workers help them reproduce, equivalent to somatic cells. Workers pass their genes to future generations by helping related queens reproduce and kin selection maintains altruistic traits in the population (Hamilton 1964 a & b). Ant colonies are more or less sessile, and the superorganism disperses only through its daughter queens and males (Figure 1), who in most species leave the colony for mating flights, after which the males die and the daughter queens found new colonies (Wilson 1971; Hölldobler and Wilson 1990).

Eusociality has always evolved through parental care in nesting animals, where some of the offspring stay and help their parents (Wheeler 1928, Nowak et al. 2010, Socias-Martínez and Kappeler 2019). Phylogenetic reconstructions show that in ancestral hymenopteran societies there was only one singly mated queen, and all the helpers were her daughters (Hughes et al. 2008, Boomsma 2009). Thus, relatedness within a colony was high, making altruism beneficial for all members of the society in a wide spectrum of ecological conditions. This explains the evolution of obligate altruism and

Figure 1. *Formica pratensis* sexuals at the time of dispersal. On the left a winged daughter queen walking on the nest surface. On the right a male that has climbed on the tree branches immediately above the nest. In monogynous and monodomous *F. pratensis*, both sexes leave the nest and mating happens somewhere outside the nest.

Figure 2. A highly polydomous *Formica exsecta* colony. Each nest is highlighted with an orange circle, and only a fraction of the nests is shown in the picture. The workers move freely among the nests.

permanently physically differentiated castes that we see in today's social Hymenoptera, although multiple mating and other relatedness-decreasing behaviors have since evolved (Hughes et al. 2008, Boomsma 2009, Boomsma et al. 2014, Boomsma and Gawne 2018). Traditionally eusociality has been considered "the highest type of social organization" (Allee 1927), and this idea is still often enforced (see for example Kappeler 2019). However, myrmecologists have long known there are different levels of social organization in ants, reaching beyond the family-based societies described above (Heinze 2007).

1.4 SOCIAL ORGANIZATION IN ANTS

Several queens cooperating and sharing their resources – including the colony and worker forces – is common in ants: 23% of the species have at least occasionally polygynous societies (societies with multiple queens), and in many species polygyny is more the rule than exception (Boomsma et al. 2014). Ant polygyny commonly arises through natal philopatry: some of the daughter queens stay in their natal colony as extra queens, instead of dispersing away (Boomsma et al. 2014). This is beneficial when long-range dispersal is risky due to ecological reasons such as nest-site limitation, highly variable environment, high predation and low success of nest founding: the staying daughter queens benefit from the existing colony that allows them to reproduce immediately, and the whole colony benefits from the competitive advantage they gain from the extra egg-layers and increased size (Hölldobler and Wilson 1977; Keller 1993; Bourke and Franks 1995; Keller 1995; Bonte et al. 2012; Boomsma et al. 2014). The workers allow daughter queens to stay, because it is beneficial for them, too (Rosengren et al. 1993; Bourke and Franks 1995).

Additionally, many polygynous ant taxa have a derived nesting strategy where instead of a single nest, the society divides to multiple interconnected nests, forming a polydomous colony where individuals move among the nests (Figure 2, Debout et al. 2007, Robinson 2014). Polydomy increases foraging efficiency and allows the society to gather more resources, enabling it to grow even larger (Debout et al. 2007; Robinson 2014). Polydomy is not always connected to polygyny, but when it is, the colonies can grow very big given that the habitat patch is good enough to support the growing society (Boomsma et al. 2014).

There is a drawback to high polygyny and social complexity, though. All cooperative organisms are vulnerable to evolutionary conflicts arising from different individuals having different optimal strategies (Parker 2006, Cant 2012). Selfish behavior and even cheating may evolve as a result of these conflicts (Ghoul et al. 2014). In ant societies, when relatedness among colony members is low, as it is when there are more egg-laying queens, social conflicts increase (Bourke and Franks 1995, Crozier and Pamilo 1996). Ant societies have conflicts over sex- and caste allocation, over who gets to reproduce, and over brood rearing, but they usually also have good kin-selected conflict resolution mechanisms that keep the societies functional (Bourke and Franks 1995, Crozier and Pamilo 1996, Ratnieks et al. 2006).

If the polygynous and polydomous colonies grow very big and consist of hundreds of interconnected nests where thousands of queens and their workers cooperate, they are called supercolonies (Helanterä et al. 2009). By some definitions, a colony larger than the distance an individual worker can cross, is a supercolony (Pedersen et al. 2006), whereas others set size limits such as over a million workers (Moffett 2012). Supercolonies seem to grow with no internal control of their size, restricted only by ecological factors (Moffett 2012). All these definitions apply to the most studied supercolonial species, Argentine ants (*Linepithema humile*), whose invasive supercolony has spread over the whole Mediterranean coast, or even through the whole globe (Giraud et al. 2002, Wetterer et al. 2009, Van Wilgenburg et al. 2010). However, Lester and Gruber (2012) point out that the most important defining factor of any ant colony should be the functional connection of nests: if all the nests do not share resources and workers in natural landscapes, they should not be considered a single colony. Supercoloniality has been reported in a wide variety of ant taxa (Helanterä et al. 2009), but not all of them have been tested for all aspects of connectedness and functionality (Hoffmann 2014).

Because of the extremely high numbers of egg-laying queens and worker movement among the interconnected nests, the members of supercolonies are not closely related, but instead the average relatedness may approach zero when measured at local scale within the supercolony (Bourke and Franks 1995, Giraud et al. 2002, Helanterä et al. 2009). The relatedness can be estimated by comparing genes shared among nestmates to these genes' average frequencies in the population, which makes the measure sensitive to inbreeding and spatial genetic structures such as population viscosity (Pamilo and Rosengren 1984; Queller and Goodnight 1989; Crozier and Pamilo 1996). This means the relatedness should be analyzed on relevant spatial scales that correspond to the true scales of competition (Chapuisat et al. 1997). Supercolonies present an evolutionary paradox, because under such low local relatedness altruistic individuals do not gain indirect fitness benefits (Helanterä et al. 2009). Selection for worker traits is relaxed because their altruism is not directed towards close relatives that could pass on their genes, and additionally selection for selfish behavior is expected to increase (Helanterä et al. 2009). Supercoloniality has likely evolved by overshooting of the kin-selected superorganismality and altruism, but it remains unclear how supercolonies are maintained in evolutionary time scales – or whether they are evolutionary dead ends (Helanterä et al. 2009, Boomsma et al. 2014).

Regardless, supercolonies are ecologically very successful and typically dominate large habitat patches, and several of the supercolonial species have become harmful invasive pests all over the globe (ISSG 2015). This is in line with the notion that increased social complexity increases ecological success in wide variety of animal taxa (Brooks et al. 2017, Cornwallis et al. 2017, Kappeler 2019). Many ant taxa are socially polymorphic and have different types of colonies, likely because social polymorphism is adaptive in changing environments (Schradin et al. 2018). Because ecological and social adaptations are closely linked in such a way, studying social polymorphism will aid in understanding the ecological success of social taxa.

1.5 DISPERSAL AND SUPERCOLONIALITY

Ants have very variable dispersal strategies, derived from the ancestral strategy of natal dispersal by winged young males and queens, combined with independent colony founding by the queens (Hölldobler and Wilson 1990, Bourke and Franks 1995, Heinze and Tsuji 1995, Heinze 2007, Keller et al. 2014). I review this diversity in detail in Chapter I. Ant dispersal coevolves with mate localization, and the last phase of dispersal, colony founding, sets important constraints

on dispersal evolution especially for the queens (Hölldobler and Bartz 1985, Heinze and Tsuji 1995, Helms and Kaspari 2015, Peeters and Aron 2017). I hypothesize that male-biased dispersal is likely common in ants because there is a strong difference in the trade-offs between dispersal and reproduction between the sexes (Zera and Denno 1997; Marden 2000; Perrin and Goudet 2001, Helms 2018, Chapter I). The nest founding phase and the subsequent society life set more restrictions on the queen life history, and possibly due to this, most of the derived ant dispersal strategies are characterized by limited queen dispersal (Heinze and Tsuji 1995). So far, the existence of overall male bias in ant dispersal has not been conclusively tested.

Limited dispersal is an inbuilt part of supercoloniality, as supercolonies arise through increased philopatry of the daughter queens (Helanterä et al. 2009; Boomsma et al. 2014, Chapter I). All supercolonial ants show a tendency to poor dispersal, and some supercolonial species have lost their flight ability partially or completely: In Argentine ants only the males fly (Wilmon and Barber 1913), whereas is *Monomorium pharaonis* both sexes are flightless (Bolton 1986, Fowler et al. 1993). In *Technomyrmex albipes*, a colony is founded by a flying queen, but the subsequent generations that help it grow into a supercolony are produced by flightless intercaste females and flightless males (Yamauchi et al. 1991). Also supercolonial *Formica* have frequently been described as poor dispersers (e.g. Chapuisat et al. 1997; Seppä et al. 2012; Schultner et al. 2016), and some *Formica* supercolonies have been suggested to be sink populations with very little dispersal outwards (Seppä et al. 2004). However, unlike in some of the other supercolonial taxa that are physically unable to fly, in supercolonial *Formica* it is unclear whether the increased queen philopatry reflects their dispersal ability and resources they get from their natal colonies, or whether it is a behavioral change.

It has been theorized that frequency-dependent selection should maintain dispersal even when a large proportion of queens are philopatric (Rosengren et al. 1993). According to this theory, queen philopatry should be favored only when the habitat patch is good enough to support the growing society, when there would be no inclusive fitness costs through increased competition for any members of the society – as may be the case in the beginning of supercolony formation (Rosengren et al. 1993; Boomsma et al. 2014). The workers should raise their threshold for accepting young queens back in the society, when the society gets overcrowded (Rosengren et al. 1993). However, this theory has not been tested, and it is not clear whether dispersal in supercolonies is regulated this way. Instead supercolonies seem to grow with no internal control of their size, which may be one of the main attributes separating them from other ant colonies (Moffett 2012). In this thesis I analyze the connection of supercoloniality and dispersal in socially polymorphic *Formica* ants.

2 AIMS OF THE THESIS

This PhD thesis deals with the evolution of social polymorphism in ants. The overall aim of my work is to provide new insights into evolution and maintenance of the most complex ant societies, highly polygynous and polydomous supercolonies where relatedness is very low (Helanterä et al. 2009). My theoretical work and empirical study questions focus on the phenomenon that has created these complex societies in the first place: limited dispersal (Table 1).

In Chapter I, I synthesize the current knowledge of dispersal evolution in ants, focusing on the interplay of social evolution and dispersal evolution. Additionally, I outline a research program to fill the knowledge gaps about ant dispersal.

In Chapter II, in order to analyze the evolutionary patterns in the later chapters, I clarify the species relationships and identification of two of the intended study species and populations, *F. exsecta* and *F. fennica*.

In Chapter III, I test a hypothesis of the connection of social organization and dispersal ability in six species of *Formica* ants with differing social organizations, and two socially polymorphic species. With analyses on the individual dispersal traits I assess whether dispersal ability is lowered in the species and populations whose queens are often philopatric. I also analyze whether dispersal ability is better in males than in queens.

In Chapter IV, I inspect the outcome of high philopatry in *Formica* by inspecting the behavioral and genetic structure of a supercolonial population of *F. pressilabris*.

Table 1. Summary of the main study questions and results of the chapters of this thesis.

Chapter	Study questions	Main results
I	What are the causes and consequences of the different dispersal strategies in ants? Where are the biggest knowledge gaps?	Ant dispersal evolution and social evolution are strongly linked through eco-evolutionary feedbacks. Ant dispersal strategies are very variable and there are a lot of knowledge gaps, especially about male strategies.
II	Are the currently identified *F. fennica* populations genetically differentiated from *F. exsecta*?	The studied *F. fennica* populations should be considered morphs of *F. exsecta*.
III	Does individual dispersal ability reflect the dispersal behavior and social structure of the species? Is *Formica* dispersal ability sex biased?	Queen morphology does not reflect the social organization, but surprisingly male morphology does. Males have better dispersal ability overall.
IV	Are there signs of limited dispersal within and away from a *Formica* supercolony? Is the population truly a single cooperative unit?	Dispersal is limited within the supercolony field, but there are signs of dispersal away from it. The population is not a single cooperative unit, but also does not have clearly defined internal borders.

3 MATERIALS AND METHODS

3.1 FORMICA ANTS

Formica is a key ant genus in boreal habitats, with extreme variation in social systems and dispersal strategies both among and within species (Table 2, Collingwood 1979; Rosengren and Pamilo 1983; Pamilo and Rosengren 1984, Seifert 2000). Unlike most other supercolonial ant genera, *Formica* has several closely related supercolonial or potentially supercolonial species (Helanterä et al. 2009, Schultner et al. 2014). This allows me to compare species with evolutionarily young polydomous and supercolonial societies to closely related monodomous species. All of my study species have winged sexuals regardless of their social organization (Figure 1, Table 2), but their flight ability or dispersal behavior has not been studied in detail. However, the overall gene flow patterns of *Formica* ants can be inferred from the large number of population genetic studies done in earlier decades with several Palearctic species (Sundström et al. 2005). Thus, *Formica* ants are a perfect study system of the interactions between the evolution of social organization and dispersal.

3.2 FIELD SAMPLING

I did all my field work in the vicinity of Tvärminne Zoological station in southern Finland, during summers 2015-2017, mapping and monitoring the field colonies and collecting samples (Figure 3). When the ants were brought to the laboratory for either maturation (Chapter III) or behavioral assays (Chapter IV) they were kept in room temperature and natural light cycle in plastic boxes coated with fluon, and the nests were watered daily and fed with Bhatkar & Whitcomb diet (1970).

For Chapter II, I used worker samples collected during 2005–2015 from 128 *Formica* (*Coptofomica*) nests all over Finland. These samples were obtained from the Natural Resources Institute Finland, and other collaborators. For Chapter III, I sampled altogether 1580 young males and queens from six different species, representing two different social organizations: simple monodomous societies (*F. pratensis, F. exsecta, F. fusca*) and complex supercolonial societies (F. *aquilonia, F. pressilabris, F. cinerea*). For socially polymorphic *F. exsecta* and *F. pressilabris* I collected additional

Table 2. Formica species sampled for the chapters of this thesis.

Species	Taxonomic group	Nest founding *	Social structure **				Chapter		
			mg	pg	pd	s	II	III	IV
F. fusca	*Serviformica*	i	x	x				x	
F. cinerea	*Serviformica*	i,d		x	x	x		x	
F. pratensis	*F. rufa* group	p	x	(x)	(x)			x	
F. aquilonia	*F. rufa* group	d, p		(x)	(x)	x		x	
F. pressilabris	*Coptoformica*	d, p		x	x	x	x	x	x
F. exsecta	*Coptoformica*	d, p	x	x	x	x	x	x	x
F. fennica / exsecta"rubens"	*Coptoformica*	d, p	x	x	x	x	x	x	
F. suecica	*Coptoformica*	d, p	x	x	x		x		
F. forsslundi	*Coptoformica*	d, p	x	?			x		

* Queen's ability to found new nest: i=independent (completely alone), d=dependent (with help of workers from their natal colony), p=parasitism (queen enters an unrelated colony as a social parasite).

** The number of queens in a society: mg=monogyny (one queen in a colony), pg=polygyny (several queens in a colony), pd=polydomy (several nests in a colony), s=supercoloniality (huge polygynous and polydomous colonies).

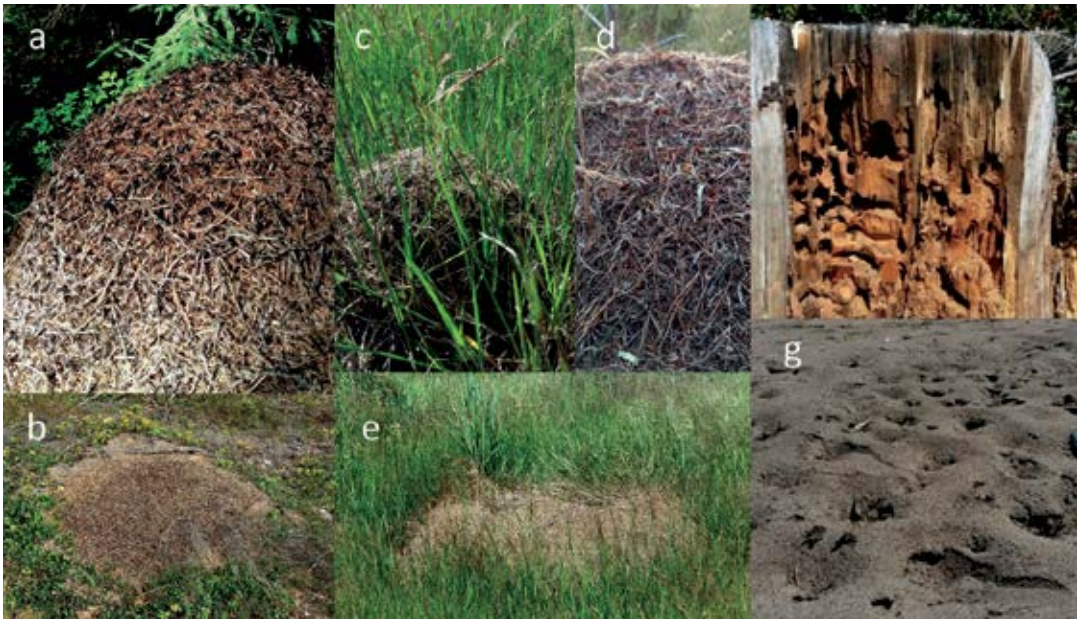

Figure 3. Nests of *Formica* ants. a) *F. aquilonia* nest constructed from pine needles; b) *F. pratensis*, characteristically low nest mound; c) *F. pressilabris*, a small monodomous nest (width ~10 cm); d) *F. exsecta* nest constructed from grass; e) *F. pressilabris*, a highly polygynous nest from a polydomous population (width ~1m); f) excavated *F. fusca* nest in a tree stump; g) entrances of polydomous *F. cinerea* nests.

samples from populations representing their rarer social organization: polydomous and monodomous, respectively (Collingwood 1979; Seifert 2000). All individuals were collected when they appeared on top of their colonies, which signals their willingness to mate and disperse. This was the best way to control their age and maturation and ensure that comparisons between species are physiologically sound. For Chapter IV, I mapped a supercolonial *F. pressilabris* population consisting of more than 1,300 nests, and sampled workers from 285 nests for population genetic analyses and 16 nests for behavioral assays. Additionally, some *F. exsecta* workers were collected for the behavioral assays.

3.3 OVERVIEW OF THE METHODS

As dispersal is a complex process strongly affected by behavioral ecology, population structures and individual physiology (Bowler and Benton 2004, Ronce 2007, Saastamoinen et al. 2017), no single research method is sufficient for understanding its evolution as a whole. Thus, in this thesis I use multiple methods to approach my study questions (Table 3). The following sections discuss and justify the chosen methods, while detailed descriptions of the protocols and statistical analyses are presented in the corresponding chapters.

3.4 SPECIES IDENTIFICATION

Species identification is often poorly reported in ecological studies, if at all (Bortolus 2008), even though mistakes in species identification are very common (Conn et al. 2013, Pante et al. 2015, Groom and Whild 2017, Packer et al. 2018). This is unfortunate, as inferring evolutionary patterns is impossible without reliably knowing which of the studied organisms belong to independently evolving lineages (de Queiroz 2007). Comparing related species allows us to inspect evolutionary change in longer time scales, whereas intraspecific comparisons are ideal for studying plasticity and evolutionary change in shorter time scales at the population level, where gene flow among populations can still counter the evolutionary process. As I aimed to do both, I needed to be very sure of my species identifications.

All species were initially identified with morphological characters (Seifert 2000, Czechowski et al. 2012). In most cases, the identifications of the long-lived study populations and nests had already been done prior to my work (e.g. Sundström et al. 2005; Martin et al. 2008; Seppä et al. 2011; Schultner et al. 2014; Helanterä et al. 2016), and I only verified them to still remain accurate. Identification for one of my intended study populations of the species *F. fennica*, had also been done

Table 2. Methods used in each chapter of this thesis.

Method	Chapter			
	I	II	III	IV
Narrative literature review	x			
COI barcoding		x		
Microsatellite genotyping		x		x
Morphological measurements		x	x	
Transmission electron microscopy for flight muscles			x	
Colorimetric assays for protein, glycogen and triglycerides			x	
Assay for aggression and regognition behavior				x

prior to my work (e.g. Helanterä and d'Ettorre 2015). However, the accuracy of the key presented in (Seifert 2000) has been questioned since the publication of these earlier works, as *F. fennica* greatly resembles a pilosity-reduced "*rubens*" morph of a more commons species *F. exsecta* – but this morph is not described in detail in the identification key (Ødegaard 2013). Thus, I checked the identification of my *F. fennica* / *F. exsecta "rubens"* samples, along with samples from most other known Finnish *F. fennica* populations (Punttila and Kilpeläinen 2009) with molecular markers: COI barcoding and DNA microsatellites (Chapter II, Chapuisat et al. 1997; Pamilo et al. 1997; Trontti et al. 2003; Hasegawa and Imai 2004; Seppä et al. 2011). Molecular methods have proven to be useful for aiding in difficult species identifications and revealing cryptic diversity (Bickford et al. 2007).

3.5 FLIGHT MUSCLES AND BODY PROPORTIONS

To analyze *Formica* flight ability in relation to their social organization, I compared individuals from monodomous species and populations to individuals from supercolonial species and populations (Chapter III). As my study species have very different total body masses (Collingwood 1979; Seifert 2000, Chapter III), I consistently analyzed ratios of body proportions: thorax mass divided by body mass (thorax ratio), abdomen mass divided by body mass (abdomen ratio) and flight muscle mass divided by body mass (muscle ratio, Marden 1989). The first two are standardly used in insect flight research to roughly estimate the resource allocation to flight (thorax) and reproductive organs (abdomen) (e. g. (Thomas et al. 1998, Berwaerts et al. 2002, Helms and Kaspari 2014). Flight muscle ratio correlates very well with flight ability across a wide range of animal taxa (Marden 1987, 1989, 2000). Thus, I treated flight muscle ratio as the most important measure of the flight ability, and the other measures as supporting data.

3.6 MICROSCOPICAL FLIGHT MUSCLE STRUCTURES

To assess the functionality of the flight muscles of the six *Formica* species, I inspected the muscle microscopical structures, mainly mitochondrion size, shape and density; and myofibril size, organization and density (Chapter III). These structures correlate with flight ability in insects (Sohal et al. 1972, Fernandes et al. 1991, Marden 2000, Rauhamäki et al. 2014). While light microscopy would be well sufficient for overall analysis of mitochondria and myofibril areas in the flight muscles, only transmission electron microscopy (TEM) has enough resolution for inspecting the inner structures of mitochondria, such as cristae (Sacktor 1972). High cost and labor-intensity of TEM limited my sample size to only nine individuals per species and sex. However, for a TEM study this dataset is already very large, and allowed me to infer the overall functionality and major interspecific differences of the flight muscle structures of these species.

3.7 METABOLIC RESOURCES

To analyze individual dispersal and nest founding resources (Chapter III), I measured glycogen, triglyceride and protein concentrations of the six *Formica* species by using standard colorimetric assays (Tennessen et al. 2014), modified to obtain all measurements from the same specimen. I standardized all measures with the body size. Glycogen is the main fuel ants use for flight (Peakin 1964, Toom et al. 1976, Passera and Keller 1990a) and triglycerides are the main energetic resource for nest founding in the queens (Keller and Passera 1989, Wheeler and Buck 1996). I analyzed total protein concentration as it is a quick and low cost substitute for analyzing directly the storage proteins the queens also use for nest founding (Wheeler and Buck 1995, 1996, Wheeler and Martínez 1995). In males, the total protein amount correlates with the sperm amount they store in their abdomens during dispersal, and thus helps analyzing their resource allocation trade-offs during flight (Avila et al. 2011, Stürup et al. 2011, Helms 2018).

3.8 GENETIC ANALYSES

I used DNA microsatellites (Chapuisat 1996, Gyllenstrand et al. 2002, Trontti et al. 2003, Hasegawa and Imai 2004) and population genetic methods to infer the dispersal history, genetic structure and genetic differentiation within a dense nest aggregation of *F. pressilabris* and its surrounding population, both of which were also carefully mapped for their spatial

structure (Chapter IV). DNA microsatellites are powerful for analyzing fine-scale population structures (Defaveri et al. 2013), and gene flow inferred from genetic markers correlates with dispersal in animal populations (Bohonak 1999).

3.9 BEHAVIORAL ASSAYS

Ants are aggressive toward intruders from outside their colony, and their recognition behavior and colony identity can be studied with aggression assays (Roulston et al. 2003, Ellis et al. 2017). I analyzed how connected the different nests are within a supercolonial population of *F. pressilabris* with an assay where 15 workers meet an intruding worker on their own nest material. The assay is more sensitive than most assays previously used for ants, as it inspects the nest-defense behavior instead of behavior on neutral arenas (e.g. Giraud et al. 2002; Chapuisat et al. 2005; Holzer et al. 2006; Björkman-Chiswell et al. 2008; Vogel et al. 2009; Chen et al. 2018). If supercolonial workers let visitors from other parts of the field enter their nest, it indicates high potential for cooperation. I measured the aggressive and inspective behaviors of supercolonial workers against their own nests mates, workers from nests close by, workers from far away on the same supercolonial field, workers from a different field, and allospecific workers (*F. exsecta*). Each of the tests between different nests were repeated five times to assess whether the behavioral patterns are constant or whether they vary between trials. (Figure 4)

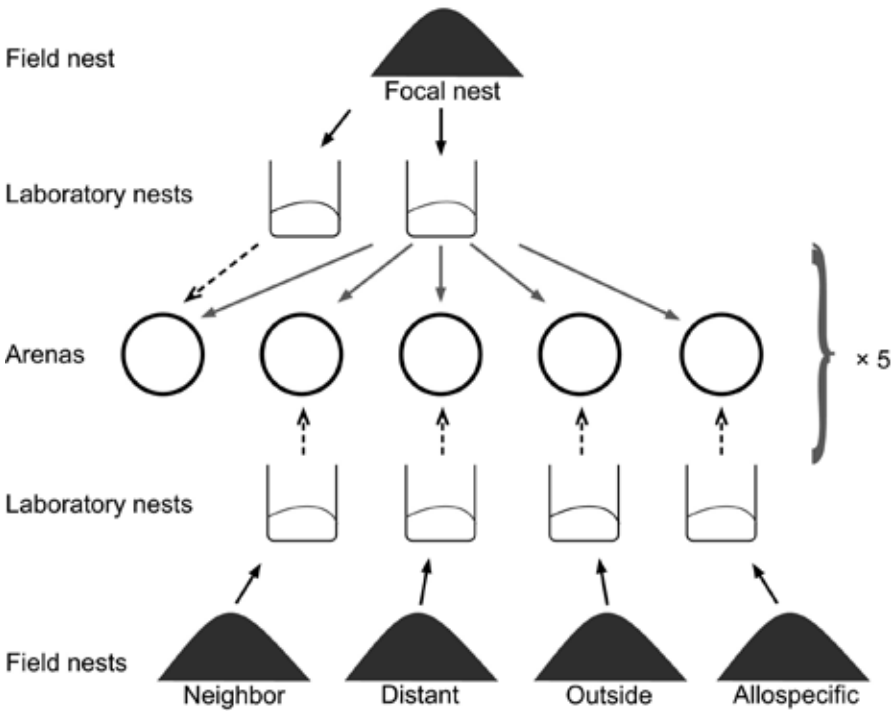

Figure 4. The design of the behavioral experiment. The reactions of 15 ants from a focal nest toward one ant from another laboratory nest box were observed. Control = introduced ant from the same original field nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta.* For each focal nest and each of the five tests, the assays were replicated five times with new ants and arenas. © Mats Ittonen.

4 MAIN RESULTS AND DISCUSSION

4.1 STRONG FEEDBACKS BETWEEN SOCIAL EVOLUTION AND DISPERSAL EVOLUTION

In Chapter I, I discuss the interplay between social evolution and dispersal evolution in ants across all of their nesting and dispersal strategies, and point out relevant future research directions. Overall it became clear that our knowledge on ant dispersal is rather limited, and mostly based on research done on a few well known species with certain life history aspects such as polygynous societies or dependent colony founding (Cronin et al. 2013, Boomsma et al. 2014). Especially data on male dispersal is very limited. Due to species specific selection pressures and evolutionary histories, dispersal evolution is best studied by observing trends in broad phylogenetic comparisons. Thus the future research focus should be on collecting more life history, behavioral and gene flow data without the current research biases. Additionally, more work on the genetic architecture of dispersal traits (Linksvayer 2015, Saastamoinen et al. 2017) and careful theoretical work on co-evolving traits and eco-evolutionary feedbacks are needed.

Studying the eco-evolutionary feedbacks between dispersal strategies and social organization would give us a better understanding of the evolution of social polymorphism. Through such feedbacks, evolution of new dispersal strategies may facilitate further evolutionary changes in ant societies. Generally, when organisms evolve new traits they may consequently transform their environments in a way that leads to new selection pressures, as explained by the niche construction theory (Odling-Smee et al. 1996). It is possible that social niche construction (Ryan et al. 2016) plays a role in the evolution of supercoloniality: The occasional queen philopatry that originally was beneficial for all members of the society and led to polygyny, leads to lower relatedness that in turn may lead to selection for more philopatric queens, because staying no longer increases kin competition with close relatives (Hamilton and May 1977, West et al. 2002, Boomsma et al. 2014). Even if the society as a whole would suffer from extensive numbers of daughter queens staying, it might be beneficial for the individual daughter queens. Thus increased philopatry could be one of the selfish traits that are predicted to increase in low-relatedness supercolonial societies (Helanterä et al. 2009), and selfish philopatry can be part of the eco-evolutionary feedback loops leading to the evolution of supercoloniality (Chapter I).

If selfish philopatry indeed increases in supercolonies, this may create new or strengthened social conflicts over dispersal: the workers, egg-laying queens and the young dispersing individuals may all have different optimal strategies, in a similar way as the classical parent-offspring conflict over dispersal (Motro 1983). The conflict is likely to be stronger the lower the relatedness within the society is: dispersing individuals are expected to value their direct fitness more, when indirect fitness benefits are low due to low relatedness.

4.2 SPECIES IDENTIFICATION OF FORMICA EXSECTA "RUBENS" / F. FENNICA

My results do not confirm the hypothesis of assumed *F. fennica* samples as a separate species, but instead strongly support grouping them with *F. exsecta* (Figure 5). Since the publication of my article (Chapter II), Seifert has further analyzed *F. fennica* and *F. exsecta* samples and published a more detailed identification key including the "*rubens*" morph of *F. exsecta* (Seifert 2018, 2019). These recent works confirm my results. Thus, my study population that was originally identified as *F. fennica* is now identified to belong to the "*rubens*" morph of *F. exsecta*, and analyzed as such in Chapter III.

4.3 DISPERSAL ABILITY AND INTRASPECIFIC VARIATION

Overall, *Formica* males have bigger flight muscle ratios than queens (Figure 6, Chapter III), indicating that the males are stronger flyers than the queens. This can be seen regardless of the social organization of the species, confirming my hypothesis of overall male-bias

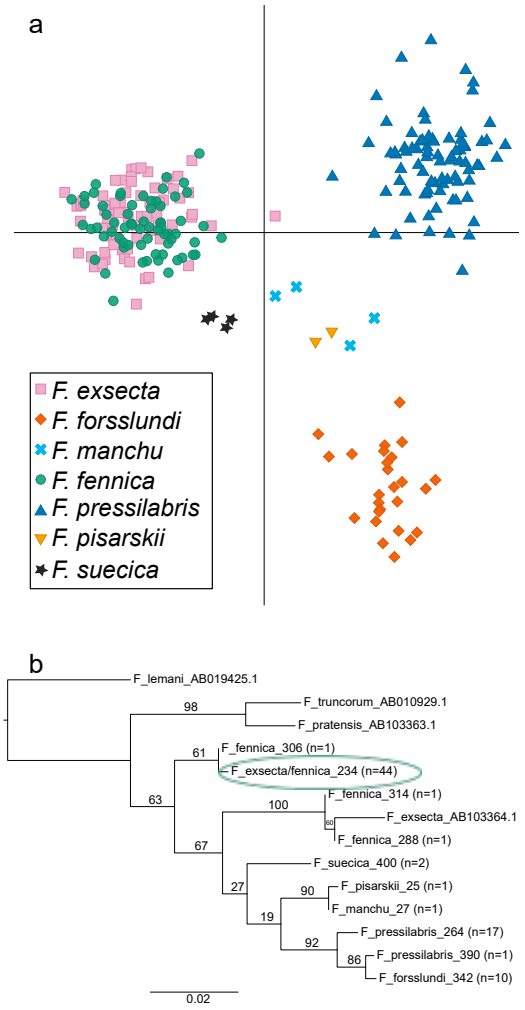

Figure 5. Data used to check the species identifications for (*Formica*) *Coptoformica*. Specimens identified as *F. fennica* mostly group together with *F. exsecta*, indicating the samples are conspecific regardless of their differing morphologies. Based on these data, these populations are treated as a single species, *F. exsecta*, in further analyses in chapter III.
a) Discriminant analysis of principal components for the microsatellite data (with 24 principal components).
b) Maximum likelihood tree based on mitochondrial COI gene sequences for *Coptoformica* and three outgroup *Formica* species. Samples with codes starting 'AB…' are from Genebank. The clade including the supercolonial *Formica exsecta* '*rubens*' population used in chapter III is circled. Note that some of the branches do not have sufficient bootstrap support (values next to the nodes) and the tree should not be used to interpret the phylogeny.

in dispersal. The microscopical muscle structures show that the flight muscles are fully functional in all of the species and both sexes, and resemble those of other flying insects (Figure 7, Sohal 1976; Marden 2000). The concentrations of biochemical resources vary greatly among species, showing that species-specific ecological selection pressures are important. However, no clear evolutionary patterns can be seen in the biochemical resources regarding social organization or nest founding strategies of these species, somewhat contrary to earlier studies (Keller and Passera 1989, Passera and Keller 1990b, Sundström 1995, Hahn et al. 2004).

According to my results, the within-species variation in dispersal traits is substantial (Chapter III). It is likely to affect dispersal decisions at the individual level, because individuals often make their decisions based on their condition (Clobert et al. 2009, Lowe and McPeek 2014). In *F. truncorum*, social organization correlates with differences in individual traits, such as size and fat content, between monodomous and polydomous populations (Sundström 1995, Johnson et al. 2005). My results from *F. exsecta* are similar: both males and queens from the polydomous population have smaller flight muscle ratio than those from the monodomous populations. Yet the direction of causation is not clear. Sundström (1995) suggests that intraspecific social polymorphism could arise due to initial intraspecific variation connected to differences in dispersal propensities among individuals. After social polymorphism arises, selection may accelerate the initial differences, and result in bigger differences between the population types, as we see in *F. truncorum* and *F. exsecta*. However, the result is not repeated between the two population types of *F. pressilabris*, possibly because this species is more commonly polydomous than the other polymorphic species (Collingwood 1979; Seifert 2000) and may have lost some of its original variation. Clearly species-specific evolutionary histories and ecology have a big role in these kinds of eco-evolutionary feedbacks.

4.4 SOCIAL ORGANIZATION AND COEVOLUTION OF SEXES

Although the difference between monodomous and supercolonial social organization is caused by different levels of queen philopatry, my results do not show

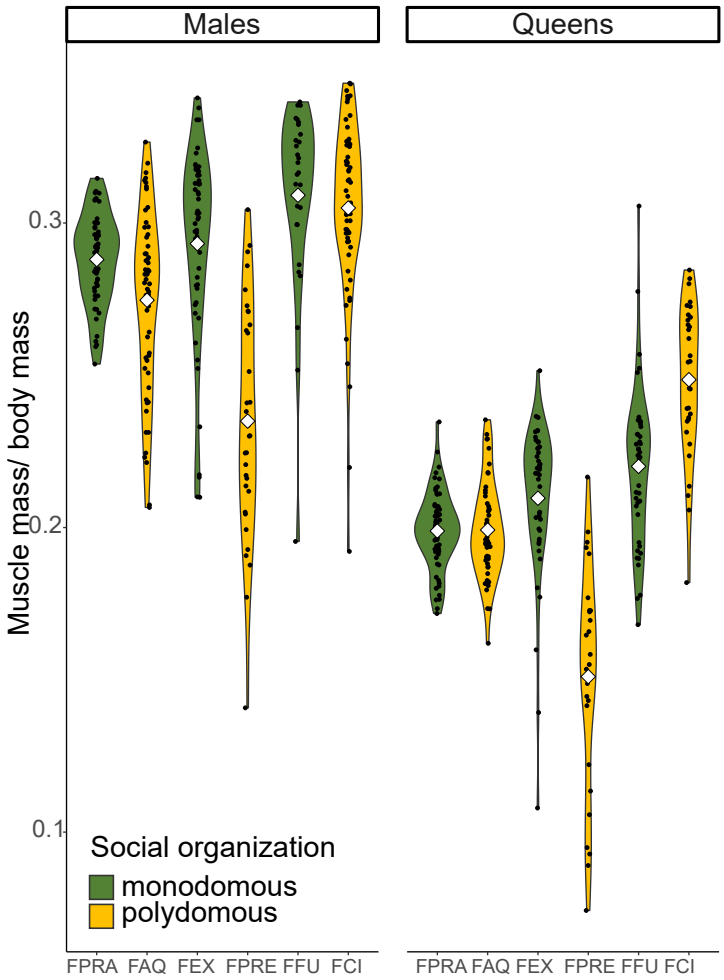

Figure 6. Flight muscle ratios of six *Formica* species with different social organizations. The males have larger muscle ratios than the queens in all of the species, and the monodomous males have larger muscle ratios than the polydomous males. All data are visualized as data points and density plots, the mean is visualized with a diamond shape. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*. The data of *F. exsecta* and *F. pressilabris* used for this figure include only the main populations that have the typical social organization. For within-species variation of these two species, see Chapter III.

any overall differences in the queen dispersal abilities between the two social organizations at the species level (Chapter III). The flight muscle ratios are not consistently smaller in the supercolonial queens, compared to the closely related monodomous queens (Figure 6). These results indicate that the higher levels of queen philopatry in supercolonies are not caused by morphological differences or differing resources the queens get from their natal colonies, but are behavioral decisions at the individual level. This strengthens the hypothesis of the role of selfish philopatry in the evolution of supercoloniality (Chapter I): even though these societies provide the daughter queens with resources to leave, many of them stay.

Contrary to queens, the males show a trend towards lower resource allocation to flight in supercolonial species: they have significantly smaller muscle ratios than the monodomous species (Figure 6). This indicates that male dispersal strategies have evolved to match the philopatric strategies of the polydomous queens – revealing coevolution between sexes. When local mating with the philopatric queens is possible, as it has been shown to be for example in supercolonial *F. paralugubris* (Chapuisat et al. 1997; Chapuisat and Keller 1999), some males may be selected to allocate less resources to flight muscles.

There is an interesting contrast in the actualized dispersal patterns, inferred from gene flow (Sundström et al. 2005), and the measurements of flight ability presented in this thesis. The supercolonial species, that have less sex difference in their flight muscle ratio, still show more sex bias in gene flow: gene flow is male

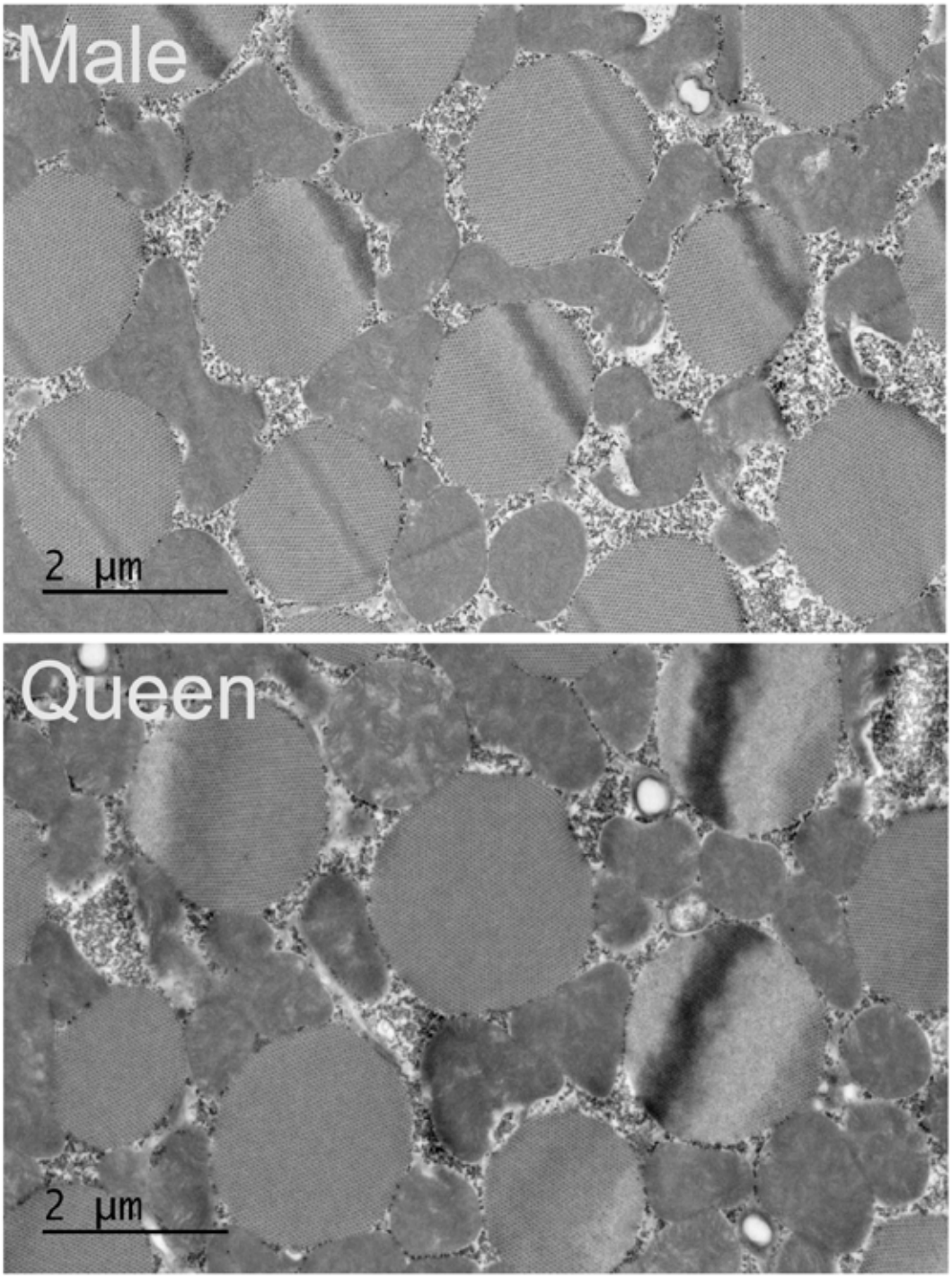

Figure 7. Typical transmission electron microscopy images of the flight muscle tissue cross-sections for both sexes of *F. exsecta*. The structures indicate good function of flight muscles, and the muscle structures are similar in all species studied, with no significant differences between the sexes. The round, equally distributed shapes in the images are myofibrils. The oval shapes are mitochondria, with cristae visible inside. Glycogen is visible as black granules between the cell organelles.

biased in *Formica* in general, but especially in the highly polygynous and polydomous species (Sundström et al. 2005). This confirms that queen philopatry must be more common in supercolonial species than their measured flight ability shows.

4.5 FORMICA SUPERCOLONIES

As a result of limited dispersal, the dense *F. pressilabris* nest aggregation inspected in Chapter IV is genetically viscous, although it is only 700 m long. Such population viscosity is common in polydomous and supercolonial *Formica* (e.g. Chapuisat et al. 1997; Sundström et al. 2005; Seppä et al. 2012). To get any values of population viscosity in such a small spatial scale, the dispersal is likely limited in both sexes, in accordance to this species having the poorest dispersal traits of the six *Formica* species I studied (Chapter III). When population genetic structuring is observed at a larger spatial scale by including adjacent subpopulations, the supercolony does not cluster separately from the other areas (Figure 8). The FST values among different areas within the supercolony field (FST 0.02-0.07), are at the same scale as the values among it and the other local subpopulations (FST 0.03-0.09). These results suggest that even though the species has somewhat limited dispersal on small spatial scale, long-range dispersal by wing still happens.

It is possible that in supercolonial *Formica* ants, the increased behavioral queen philopatry and the observed small decrease in male dispersal ability may reflect a change in the shape of their dispersal kernel rather than change in the maximum dispersal distances. In natural populations, most individuals disperse only short distances, and long range dispersal is rare (Lowe and McPeek 2014, Steyn et al. 2016). In polydomous *Formica*, there may be more philopatric individuals than in monodomous *Formica*, but the rare dispersers may still fly as far. Especially if they disperse with the help of wind (Helms 2018), insects capable of flight will occasionally disperse over long distances.

At a closer inspection, the supercolonial *F. pressilabris* population is not a textbook example of a supercolony (Pedersen et al. 2006, Helanterä et al. 2009, Moffett 2012), but rather a fluid mosaic population where lack of

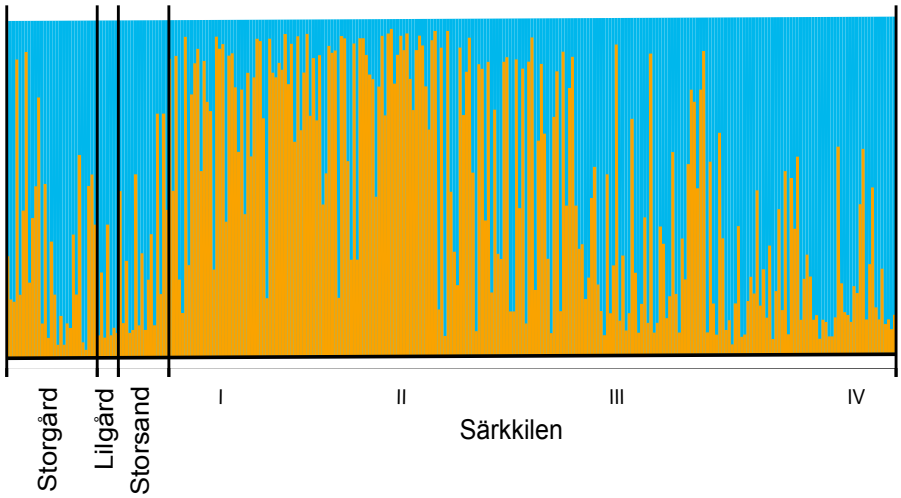

Figure 8. Bayesian clustering of all genotyped individuals from the four study fields with the software STRUCTURE (Pritchard et al. 2000). The codes I-IV within Särkkilen indicate the four different parts of the supercolony field, used for grouping workers for behavioral assays. Each colored bar represents a single genotyped individual, with samples organized from west to east. Optimal K = 2 with strong admixture indicates there is no clear genetic differentiation among the subpopulations.

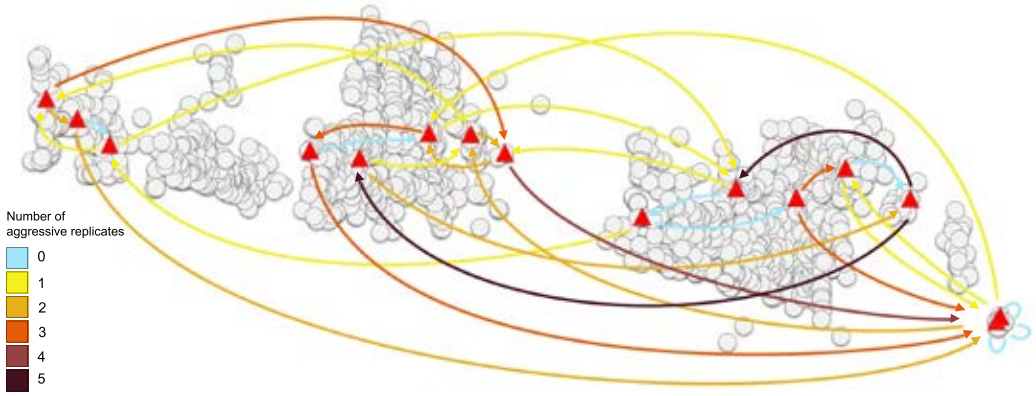

Figure 9. Complex behavioral patterns within the F. pressilabris supercolonial population. The population consisting of more than 1300 nests in an area ~700 m long. The nests used in the behavioral assays are shown as red triangles, other nests as grey circles. The different arrow colors show the number of aggressive replicates (see legend), and arrowheads show the direction of aggression, pointing toward the nest of the introduced ant. Overall the level of aggression is low, showing that workers mostly let foreign workers enter their nest material, which creates potential for cooperation among the nests. © Mats Ittonen.

aggression creates plenty of potential for cooperation, but all individuals do not seem to cooperate (Chapter IV). The aggression level is still overall low and shows that the workers allow individuals from other nests to enter their nest material, with different worker sets from the same nest behaving differently in the five replicate trials (Figure 9). However, as there is some aggression within the supercolony field, instead of a single supercolony the population could be interpreted a combination of several supercolonies with no clear boundaries. The aggression increases with distance, in accordance to the genetic viscosity, supporting the notions that genetic viscosity may be one of the mechanisms maintaining supercolonies as somewhat stable social structures (Chapuisat, et al. 1997; Helanterä et al. 2009; Holzer et al. 2009).

5 CONCLUSIONS AND FUTURE DIRECTIONS

My work increases the overall knowledge of ant dispersal strategies and ability. I show that the great social polymorphism and the corresponding differences in the rate of queen philopatry in *Formica* ants is foremost a behavioral difference. Interestingly, the biggest physiological effect according to my results is seen in males rather than queens, which indicates strong coevolution of the sexes (Bonduriansky 2009). The large intraspecific variation of dispersal ability and resources may contribute to the existence of different levels of social organization within species, but this does not directly translate to big overall differences between monodomous and supercolonial species. The evolutionary pathway towards increased philopatry and social complexity seems to start from behavior, rather than from resource allocation decisions at the society level.

This raises questions about the role of worker behavior in the dispersal decisions and the maintenance of these societies. The workers may have power to decide how many and which queens they let stay in the society, and which queens have to leave (Beekman and Ratnieks 2003, Chapter I). The possibility of dispersal conflict in low-relatedness ant societies should be carefully analyzed both theoretically and empirically. The existence of supercolonies ultimately depends on worker behavior: their lack of aggression against other members of the society, acceptance of philopatric queens, and acceptance of males wanting to mate with these queens. Thus, although dispersal is performed by the sexual castes, the worker genomes likely control it strongly through indirect genetic effects (Linksvayer 2015). In supercolonial societies where local relatedness is very low, the interactions

of the unrelated genomes may get complex. However, genetic viscosity within the supercolony may give the workers some inclusive fitness benefits, if the daughter queens even occasionally move to other nests within the supercolony to compete with less-related queens, and especially if they fly away from the supercolony and compete in the larger spatial scale (Chapuisat, et al. 1997; Chapuisat and Keller 1999).

To understand the evolution and maintenance of supercolonies we should better understand the scales of competition. Although relatedness within supercolonies is low, at large spatial scale they are still genetically differentiated from each other (Pamilo et al. 2005, Schultner et al. 2016). Based on these observations, selection maintaining supercolonies could exist at this higher level, through competition among separate supercolonies (Pedersen et al. 2006, Kennedy et al. 2014). According to my results, however, the large nest networks of *Formica* ants may not be single cooperative units but rather more complex nest mosaics and only partially cooperative. Even if they are theoretically single units due to lack of clear internal borders, if they don't function as ecologically single units (Lester and Gruber 2012), it seems unlikely that colony level selection would be strong enough to maintain them. To resolve these questions of the scale of competition and levels of selection, we should study the supercolonial species and their dispersal on larger spatial scales, and also inspect the life histories of supercolonies in more detail.

Based on my results there is a need for some extra work on defining the nature of *Formica* supercolonies. At the moment supercoloniality is defined based on the invasive Argentine ant populations, although supercolonies exist also in wide range of other taxa (Helanterä et al. 2009, Lester and Gruber 2012, Moffett 2012, Hoffmann 2014). In *Formica*, it seems likely that the nature of the polydomous and supercolonial populations varies not just between populations and species, but also within a single population across different seasons (Mabelis 1979, 1984, Elias et al. 2005, Schultner et al. 2016) and according to available resources (Sorvari and Hakkarainen 2004). This kind of variability and flexibility of their social organization seems to be an overall hallmark of *Formica* ants, and possibly underlines their ecological success.

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> Freedom is a heavy load, a great and strange burden for the spirit to undertake. It is not easy. It is not a gift given, but a choice made, and the choice may be a hard one. The road goes upward towards the light; but the laden traveler may never reach the end of it.
>
> Ursula K. LeGuin (1970)

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7 REFERENCES

Abbot, P., J. Abe, J. Alcock, S. Alizon, J. A. C. Alpedrinha, J. Andre, M. Van Baalen, F. Balloux, S. Balshine, et al. 2011. Inclusive fitness theory and eusociality. Nature 471:E1–E4.

Allee, W. C. 1927. Animal aggregations. The Quarterly Review of Biology 2:367–398.

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner. 2011. Insect seminal fluid proteins: identification and function. Annual Review of Entomology 56:21–40.

Beekman, M., and F. L. W. Ratnieks. 2003. Power over reproduction in social Hymenoptera. Philosophical Transactions of the Royal Society B: Biological Sciences 358:1741–1753.

Bengtsson, B. O. 1978. Avoiding inbreeding: at what cost? Journal of Theoretical Biology 73:439–444.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly Pararge aegeria. Functional Ecology 16:484–491.

Bhatkar, A., and W. H. Whitcomb. 1970. Artificial diet for rearing various species of ants. The Florida Entomologist 53:229.

Bickford, D., D. J. Lohman, N. S. Sodhi, P. K. L. Ng, R. Meier, K. Winker, K. K. Ingram, and I. Das. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22:148–155.

Birch, J. 2017. The inclusive fitness controversy: finding a way forward. Royal Society Open Science 4:170335.

Björkman-Chiswell, B. T., E. Van Wilgenburg, M. L. Thomas, S. E. Swearer, and M. A. Elgar. 2008. Absence of aggression but not nestmate recognition in an Australian population of the Argentine ant *Linepithema humile*. Insectes Sociaux 55:207–212.

Bohonak, A. J. 1999. Dispersal, gene flow, and population structure. The Quarterly Review of Biology 74:21–45.

Bolton, B. 1986. Apterous females and shift of dispersal strategy in the *Monomorium salomonis* -group (Hymenoptera: Formicidae). Journal of Natural History 20:267–272.

Bonduriansky, R. 2009. Reappraising sexual coevolution and the sex roles. PLoS Biology 7(12): e1000255.

Bonte, D., H. Van Dyck, J. M. Bullock, M. Delgado, M. Gibbs, V. Lehouck, E. Matthysen, K. Mustin, N. Schtickzelle, V. M. Stevens, S. Vandewoestijne, M. Baguette, K. Barton, T. G. Benton, A. Chaput-bardy, C. Dytham, T. Hovestadt, C. M. Meier, C. F. Steve, C. Turlure, and J. M. J. Travis. 2012. Costs of dispersal. Biological Reviews 87:290–312.

Boomsma, J. J. 2009. Lifetime monogamy and the evolution of eusociality. Philosophical Transactions of the Royal Society B: Biological Sciences, 364(1533): 3191-3207

Boomsma, J. J., and R. Gawne. 2018. Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. Biological Reviews 93:28–54.

Boomsma, J. J., D. B. Huszár, and J. S. Pedersen. 2014. The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. Animal Behaviour 92:241–252.

Bortolus, A. 2008. Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. Ambio 37:114–118.

Bourke, A. F. 2011. Principles of social evolution. Oxford University Press, Oxford, UK.

Bourke, A. F. G., and N. R. Franks. 1995. Social Evolution in Ants. Princeton University Press, Princeton, UK.

Bowler, D. E., and T. G. Benton. 2004. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80:205–225.

Brooks, K. C., R. Maia, J. E. Duffy, K. M. Hultgren, and D. R. Rubenstein. 2017. Ecological generalism facilitates the evolution of sociality in snapping shrimps. Ecology Letters 20:1516–1525.

Burgess, S. C., M. L. Baskett, R. K. Grosberg, S. G. Morgan, and R. R. Strathmann. 2016. When is dispersal for dispersal? Unifying marine and terrestrial perspectives. Biological Reviews of the Cambridge Philosophical Society 91:867–882.

Cant, M. A. 2012. Suppression of social conflict and evolutionary transitions to cooperation. American Naturalist 179:293–301.

Chapuisat, M. 1996. Characterization of microsatellite loci in *Formica lugubris* B and their variability in other ant species. Molecular Ecology 5:599–601.

Chapuisat, M., C. Bernasconi, S. Hoehn, and M. Reuter. 2005. Nestmate recognition in the unicolonial ant *Formica paralugubris*. Behavioral Ecology 16:15–19.

Chapuisat, M., J. Goudet, and L. Keller. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. Evolution 51:475–482.

Chapuisat, M., and L. Keller. 1999. Extended family structure in the ant *Formica paralugubris* : the role of the breeding system. Behavioral Ecology and Sociobiology, 46(6): 405-412.

Chen, W., Á. O'Sullivan, and E. S. Adams. 2018. Intraspecific aggression and the colony structure of the invasive ant *Myrmica rubra*. Ecological Entomology 43:263–272.

Clobert, J., J.-F. Le Galliard, J. Cote, S. Meylan, and M. Massot. 2009. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. Ecology Letters 12:197–209.

Collingwood, C. 1979. The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica 8:64.

Conn, P. B., B. T. McClintock, M. F. Cameron, D. S. Johnson, E. E. Moreland, and P. L. Boveng. 2013. Accommodating species identification errors in transect surveys. Ecology 94:2607–2618.

Cornwallis, C. K., C. A. Botero, D. R. Rubenstein, P. A. Downing, S. A. West, and A. S. Griffin. 2017. Cooperation facilitates the colonization of harsh environments. Nature Ecology & Evolution 1:1–10.

Cronin, A. L., M. Molet, C. Doums, T. Monnin, and C. Peeters. 2013. Recurrent evolution of dependent colony foundation across eusocial insects. Annual Review of Entomology 58:37–55.

Crozier, R. H., and P. Pamilo. 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford University Press, Oxford, U.K.

Czechowski, W., A. Radchenko, W. Czechowska, and K. Vepsäläinen. 2012. The ants (Hymenoptera: Formicidae) of Poland with Reference to the Myrmecofauna of Europe. Natura Optima Dux Foundation, Warszawa.

Debout, G., B. Schatz, M. Elias, and D. Mckey. 2007. Polydomy in ants: what we know, what we think we know, and what remains to be done. Biological Journal of the Linnean Society 90:319–348.

Defaveri, J., H. Viitaniemi, E. Leder, and J. Merilä. 2013. Characterizing genic and nongenic molecular markers: Comparison of microsatellites and SNPs. Molecular Ecology Resources 13:377–392.

Van Dyken, J. D., and M. J. Wade. 2012. Origins of altruism diversity I: The diverse ecological roles of altruistic strategies and their evolutionary responses to local competition. Evolution 66:2484–2497.

Elias, M., R. Rosengren, and L. Sundström. 2005. Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant *Formica truncorum*. Behavioral Ecology and Sociobiology 57:339–349.

Ellis, S., D. S. Procter, P. Buckham-Bonnett, and E. J. H. Robinson. 2017. Inferring polydomy: a review of functional, spatial and genetic methods for identifying colony boundaries. Insectes Sociaux 64:19–37.

Fernandes, J., M. Bate, and K. Vijayraghavan. 1991. Development of the indirect flight muscles of *Drosophila*. Development 113:67–77.

Fowler, H. G., L. E. Alves, and O. C. Bueno. 1993. Reproductive strategies of the exotic Pharaoh's ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae) in Brazil. Invertebrate Reproduction and Development 23:235–238.

Gardner, A. 2010. Sex-biased dispersal of adults mediates the evolution of altruism among juveniles. Journal of Theoretical Biology 262:339–345.

Ghoul, M., A. S. Griffin, and S. A. West. 2014. Toward an evolutionary definition of cheating. Evolution 68:318–331.

Giraud, T., J. S. Pedersen, and L. Keller. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences of the United States of America 99:6075–6079.

Greenwood, P. J. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour 28:1140–1162.

Groom, Q. J., and S. J. Whild. 2017. Characterisation of false-positive observations in botanical surveys. PeerJ 5:e3324.

Gyllenstrand, N., P. J. Gertsch, and P. Pamilo. 2002. Polymorphic microsatellite DNA markers in the ant *Formica exsecta*. Molecular Ecology Notes 2:67–69.

Hahn, D., R. Johnson, N. Buck, and D. Wheeler. 2004. Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. Physiological and Biochemical Zoology: 77:100–108.

Hamilton, W. D. 1963. The evolution of altruistic behavior. The American Naturalist 97:354–356.

Hamilton, W. D. 1964a. The genetical evolution of social behaviour I. Journal of Theoretical Biology 7:1–16.

Hamilton, W. D. 1964b. The genetical evolution of social behaviour II. Journal of Theoretical Biology 7:17–52.

Hamilton, W. D. 1970. Selfish and spiteful behaviour in an evolutionary model. Nature 228:1218–1220.

Hamilton, W. D. 1972. Altruism and related phenomena, mainly in social insects. Annual Review of Ecological Systems 3:193–232.

Hamilton, W., and R. May. 1977. Dispersal in stable habitats. Nature 269:578–581.

Hasegawa, E., and S. Imai. 2004. Characterization of microsatellite loci in red wood ants *Formica* (s. str.) spp. and the related genus *Polyergus*. Molecular Ecology Notes 4:200–203.

Heinze, J. 2007. The demise of the standard ant (Hymenoptera: Formicidae). Myrmecological News 11:9–20.

Heinze, J., and K. Tsuji. 1995. Ant reproductive strategies. Researches on Population Ecology 37:135–149.

Helanterä, H., and P. d'Ettorre. 2015. A comparative study of egg recognition signature mixtures in *Formica* ants. Evolution 69:520–529.

Helanterä, H., J. Kulmuni, and P. Pamilo. 2016. Sex allocation conflict between queens and workers in *Formica pratensis* wood ants predicts seasonal sex ratio variation. Evolution 70(10), 2387-2394.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution 24:341–9.

Helms, J. 2018. The flight ecology of ants (Hymenoptera: Formicidae). Myrmecological News 26:19–30.

Helms, J. A., and M. Kaspari. 2014. Found or fly: Nutrient loading of dispersing ant queens decreases metrics of flight ability (Hymenoptera: Formicidae). Myrmecological News 19:85–91.

Helms, J. A., and M. Kaspari. 2015. Reproduction-dispersal tradeoffs in ant queens. Insectes Sociaux 62:171–181.

Hoffmann, B. D. 2014. Quantification of supercolonial traits in the yellow crazy ant, *Anoplolepis gracilipes*. Journal of Insect Science 14:1–21.

Hölldobler, B., and H. S. Bartz. 1985. Sociobiology of reproduction in ants. Pages 237–257 in Experimental Behavioral Ecology and Sociobiology (editors Hölldobler, B. and Lindauer, M.). Gustav Fischer Verlag, Stuttgart.

Hölldobler, B., and E. O. Wilson. 1977. The number of queens: An important trait in ant evolution. Naturwissenschaften 64:8–15.

Hölldobler, B., and O. E. Wilson. 1990. The ants. Belknap Press of Harvard University Press, Cambridge.

Holzer, B., M. Chapuisat, N. Kremer, C. Finet, and L. Keller. 2006. Unicoloniality, recognition and genetic differentiation in a native *Formica* ant. Journal of Evolutionary Biology 19:2031–2039.

Holzer, B., L. Keller, and M. Chapuisat. 2009. Genetic clusters and sex-biased gene flow in a unicolonial *Formica* ant. BMC Evolutionary Biology 9:69.

Hughes, W. O. H., B. P. Oldroyd, M. Beekman, and F. L. W. Ratnieks. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science 320:1213–1216.

Johnson, C. A., L. Sundström, and J. Billen. 2005. Development of alary muscles in single- and multiple- queen populations of the wood ant *Formica truncorum*. Annales Zoologici Fennici 42:225–234.

Kappeler, P. M. 2019. A framework for studying social complexity. Behavioral Ecology and Sociobiology 73(1), 13.

Katzerke, A., P. Neumann, C. W. W. Pirk, P. Bliss, and R. F. A. Moritz. 2006. Seasonal nestmate recognition in the ant *Formica exsecta* 61(1), 143-150.

Keller, L. 1993. Queen number and sociality in insects. Oxford University Press, Oxford UK.

Keller, L. 1995. Social life: The paradox of multiple-queen colonies. Trends in Ecology and Evolution 10:355–360.

Keller, L., and L. Passera. 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). Oecologia 80:236–240.

Keller, R. A., C. Peeters, and P. Beldade. 2014. Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. eLife 3, e01539 .

Kennedy, P., T. Uller, and H. Helanterä. 2014. Are ant supercolonies crucibles of a new major transition in evolution? Journal of Evolutionary Biology 27:1784–96.

Kramer, J., and J. Meunier. 2016. Kin and multilevel selection in social evolution: A never-ending controversy? F1000Research, 5:776.

Kümmerli, R., A. Gardner, S. West, and A. Griffin. 2009. Limited dispersal, budding dispersal, and cooperation: An experimental study. Evolution 63:939–949.

Lehmann, L., V. Ravigne, and L. Keller. 2008. Population viscosity can promote the evolution of altruistic sterile helpers and eusociality. Proceedings of the Royal Society B: Biological Sciences 275:1887–1895.

Lester, P., and M. A. Gruber. 2012. Comment on Moffett: "Superclonies of billions in an invasive ant: What is a soceity?" Behavioral Ecology 23:934–935.

Linksvayer, T. A. 2015. The molecular and evolutionary genetic implications of being truly social for the social insects. Advances in Insect Physiology 48:271–292.

Lowe, W. H., and M. A. McPeek. 2014. Is dispersal neutral? Trends in Ecology and Evolution 29:444–450.

Mabelis, A. 1979. The relationship between aggression and predation in the red wood ant (*Formica polyctena* Först.). Netherlands Journal of Zoology 29:451–620.

Mabelis, A. 1984. Aggression in wood ants (*Formica polyctena* Foerst., Hymenoptera, Formicidae). Aggressive Behavior 10:47–53.

Marden, J. H. 1987. Maximum lift production during takeoff in flying animals. Journal of Experimental Biology 130:235–238.

Marden, J. H. 1989. Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. Physiological Zoology 62:505–521.

Marden, J. H. 2000. Variability in the size, composition, and function of insect flight muscles. Annual Review of Physiology 62:157–178.

Martin, S. J., E. Vitikainen, H. Helanterä, and F. P. Drijfhout. 2008. Chemical basis of nest-mate discrimination in the ant *Formica exsecta*. Proceedings of the Royal Society B: Biological Sciences 275:1271–1278.

Maynard Smith, J. 1964. Group selection and kin selection. Nature 201:145–1147.

Moffett, M.W. 2012. Supercolonies of billions in an invasive ant: What is a society? Behavioral Ecology 23:925–933.

Motro, U. 1983. Optimal rates of dispersal. III. Parent-offspring conflict. Theoretical Population Biology 23:159–168.

Motro, U. 1991. Avoiding inbreeding and sibling competition: the evolution of sexual dimorphism for dispersal. The American Naturalist 137:108–115.

Nowak, M. A, C. E. Tarnita, and E. O. Wilson. 2010. The evolution of eusociality. Nature 466:1057–62.

Ødegaard, F. 2013. New and little known ants (Hymenoptera, Formicidae) in Norway. Norwegian Journal of Entomology 60:172–175.

Odling-Smee, J., K. N. Laland, and M. W. Feldman. 1996. Niche construction. The American Naturalist 147:641–648.

Okasha, S. 2006. Evolution and the levels of selection. Oxford University Press, Oxford.

Packer, L., S. K. Monckton, T. M. Onuferko, and R. R. Ferrari. 2018. Validating taxonomic identifications in entomological research. Insect Conservation and Diversityiversity 11:1–12.

Pamilo, P., P. Gertsch, P. Thorén, and P. Seppä. 1997. Molecular population genetics of social insects. Annual Review of Ecology and Systematics 28(1), 1-25.

Pamilo, P., and R. Rosengren. 1984. Evolution of nesting strategies of ants: genetic evidence from different population types of *Formica* ants. Biological Journal of the Linnean Society 21:331–348.

Pamilo, P., D. Zhu, W. Fortelius, R. Rosengren, P. Seppä, and L. Sundström. 2005. Genetic patchwork of network-building wood ant populations. Annales Zoologici Fennici 42:179–187.

Pante, E., N. Puillandre, A. Viricel, S. Arnaud-Haond, D. Aurelle, M. Castelin, A. Chenuil, C. Destombe, D. Forcioli, M. Valero, F. Viard, and S. Samadi. 2015. Species are hypotheses: Avoid connectivity assessments based on pillars of sand. Molecular Ecology 24:525–544.

Parker, G. A. 2006. Sexual conflict over mating and fertilization: An overview. Philosophical Transactions of the Royal Society B: Biological Sciences 361:235–259.

Passera, L., and L. Keller. 1990. Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). Journal of Comparative Physiology B 160:207–211.

Peakin, G. 1964. Food reserves in the reproductive castes of *Lasius flavus* Fab. (Hymenoptera). In Proceedings of the XIIth International Congress of Entomology, London (Vol. 3030).

Pedersen, J. S., M. J. B. Krieger, V. Vogel, T. Giraud, and L. Keller. 2006. Native supercolonies of unrelated individuals in the invasive argentine ant. Evolution 60:782–791.

Peeters, C., and S. Aron. 2017. Evolutionary reduction of female dispersal in *Cataglyphis* desert ants. Biological Journal of the Linnean Society 122:58–70.

Perrin, N., and J. Goudet. 2001. Inbreeding, kinship, and the evolution of natal dispersal. In Dispersal (editors Clobert, J., Danchin, E., Dhondt, A. A. and Nichols, J. D.). Oxford University Press, Oxford.

Platt, T. G., and J. D. Bever. 2009. Kin competition and the evolution of cooperation. Trends in Ecology and Evolution 24:370–377.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Punttila, P., and J. Kilpeläinen. 2009. Distribution of mound-building ant species (*Formica* spp ., Hymenoptera) in Finland : preliminary results of a national survey. Annales Zoologici Fennici 46:1–15.

de Queiroz, K. 2007. Species concepts and species delimitation. Systematic Biology 56:879–886.

Queller, D. C. 1992a. A general model for kin selection. Evolution 46:376.

Queller, D. C. 1992b. Does population viscosity promote kin selection? Trends in Ecology & Evolution 7:322–324.

Queller, D. C., and K. F. Goodnight. 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.

Ratnieks, F. L. W., K. R. Foster, and T. Wenseleers. 2006. Conflict resolution in insect societies. Annual Review of Entomology 51:581–608.

Rauhamäki, V., J. Wolfram, E. Jokitalo, I. Hanski, and E. P. Dahlhoff. 2014. Differences in the aerobic capacity of flight muscles between butterfly populations and species with dissimilar flight abilities. PLoS ONE 9:1–8.

Robinson, E. J. 2014. Polydomy: the organisation and adaptive function of complex nest systems in ants. Current Opinion in Insect Science 5:37–43.

Ronce, O. 2007. How does it feel to be like a rolling stone? Ten questions about dispersal evolution. Annual Review of Ecology, Evolution, and Systematics 38:231–253.

Rosengren, R., and P. Pamilo. 1983. The evolution of polygyny and polydomy in mound-building *Formica* ants. Acta Entomologica Fennica 42:65–77.

Rosengren, R., L. Sundström, and W. Fortelius. 1993a. Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactic. In Queen number and sociality

in insects (editor Keller, L.). Oxford Science publications, Oxford.

Roulston, T. H., G. Buczkowski, and J. Silverman. 2003. Nestmate discrimination in ants: Effect of bioassay on aggressive behavior. Insectes Sociaux 50:151–159.

Ryan, P. A., S. T. Powers, and R. A. Watson. 2016. Social niche construction and evolutionary transitions in individuality. Biology and Philosophy 31:59–79.

Saastamoinen, M., G. Bocedi, J. Cote, D. Legrand, F. Guillaume, C. W. Wheat, E. A. Fronhofer, C. Garcia, R. Henry, A. Husby, M. Baguette, D. Bonte, A. Coulon, H. Kokko, E. Matthysen, K. Niitepõld, E. Nonaka, V. M. Stevens, J. M. J. Travis, K. Donohue, J. M. Bullock, and M. del Mar Delgado. 2017. Genetics of dispersal. Biological Reviews 358:574–599.

Sacktor, B. 1972. Degenerative changes in the mitochondria of flight muscle from aging blowflies. The Journal of Cell Biology 52:465–477.

Schradin, C., L. D. Hayes, N. Pillay, and C. Bertelsmeier. 2018. The evolution of intraspecific variation in social organization. Ethology 124:527–536.

Schultner, E., A. Gardner, M. Karhunen, and H. Helanterä. 2014. Ant larvae as players in social conflict: relatedness and individual identity mediate cannibalism intensity. The American Naturalist 184:E161–E174.

Schultner, E., J. Saramäki, and H. Helanterä. 2016. Genetic structure of native ant supercolonies varies in space and time. Molecular Ecology 25:6196–6213.

Seifert, B. 2000. A taxonomic revision of the ant subgenus *Coptoformica* Mueller, 1923 (Hymenoptera, Formicidae). Zoosystema 22:517–568.

Seifert, B. 2018. The ants of Central and North Europe. Lutra Verlags- und Vertriebsgesellschaft, Tauer.

Seifert, B. 2019. The *rubens* morph of *Formica exsecta* Nylander, 1846 and its separation from *Formica fennica* Seifert, 2000 (Hymenoptera, Formicidae). Deutsche Entomologische Zeitschrift 66:55–61.

Seppä, P., N. Gyllenstrand, J. Corander, and P. Pamilo. 2004. Coexistence of the social types: genetic population structure in the ant *Formica exsecta*. Evolution 58:2462–71.

Seppä, P., H. Helanterä, K. Trontti, P. Punttila, A. Chernenko, S. J. Martin, and L. Sundström. 2011. The many ways to delimit species: Hairs, genes and surface chemistry. Myrmecological News 15:31–41.

Seppä, P., H. Johansson, N. Gyllenstrand, S. Pálsson, and P. Pamilo. 2012. Mosaic structure of native ant supercolonies. Molecular Ecology 21:5880–5891.

Socias-Martínez, L., and P. M. Kappeler. 2019. Catalyzing transitions to sociality: ecology builds on parental care. Frontiers in Ecology and Evolution 7:160.

Sohal, R. S. 1976. Aging changes in insect flight muscle. Gerontology 22:317–333.

Sohal, R. S., J. L. McCarthy, and V. F. Allison. 1972. The formation of "giant" mitochondria in the fibrillar flight muscles of the house fly, *Musca domestica* L. Journal of Ultrasructure Research 39:484–495.

Sorvari, J., and H. Hakkarainen. 2004. Habitat-related aggressive behaviour between neighbouring colonies of the polydomous wood ant *Formica aquilonia*. Animal Behaviour 67:151–153.

Steyn, V. M., K. A. Mitchell, and J. S. Terblanche. 2016. Dispersal propensity, but not flight performance, explains variation in dispersal ability. Proceedings of the Royal Society B: Biological Sciences, 283(1836), 20160905.

Stürup, M., S. P. A. den Boer, D. R. Nash, J. J. Boomsma, and B. Baer. 2011. Variation in male body size and reproductive allocation in the leafcutter ant *Atta colombica*: Estimating variance components and possible trade-offs. Insectes Sociaux 58:47–55.

Sundström, L. 1995. Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. Behavioral Ecology 6:132–139.

Sundström, L., P. Seppä, and P. Pamilo. 2005. Genetic population structure and dispersal patterns in *Formica* ants- a review. Annales Zoologici Fennici 42:163–177.

Szathmáry, E. 2015. Toward major evolutionary transitions theory 2.0. Proceedings of the National Academy of Sciences of the United States of America 112:10104–10111.

Szathmáry, E., and J. Maynard Smith. 1995. Major evolutionary transitions. Nature 374(6519): 227.

Taylor, P. D. 1992. Altruism in viscous populations - an inclusive fitness model. Evolutionary Ecology 6:352–356.

Tennessen, J. M., W. E. Barry, J. Cox, and C. S. Thummel. 2014. Methods for studying metabolism in *Drosophila*. Methods 68:105–115.

Thomas, C. D., J. K. Hill, and O. T. Lewis. 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. Journal of Animal Ecology 67:485–497.

Toom, P., C. Johnson, and E. Cupp. 1976. Utilization of body reserves during preovisposition activity by *Solenopsis invicta*. Annals of the Entomological Society of America 69:145–148.

Trontti, K., W. T. Tay, and L. Sundström. 2003. Polymorphic microsatellite markers for the ant *Plagiolepis pygmaea*. Molecular Ecology Notes 3:575–577.

Van Valen, L. 1971. Group selection and the evolution of dispersal. Evolution 25:591–598.

Vogel, V., J. S. Pedersen, P. D'Ettorre, L. Lehmann, and L. Keller. 2009. Dynamics and genetic structure of argentine ant supercolonies in their native range. Evolution 63:1627–1639.

West, S., I. Pen, and A. Griffin. 2002. Cooperation and competition between relatives. Science 296:72–75.

Wetterer, J. K., A. L. W. Wild, A. V. S. Suarez, N. R. Roura-Pscual, and X. E. Espadaler. 2009. Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). Myrmecological News 12:187–194.

Wheeler, D. E., and N. a. Buck. 1995. Storage proteins in ants during development and colony founding. Journal of Insect Physiology 41:885–894.

Wheeler, D. E., and N. A. Buck. 1996. Depletion of reserves in ant queens during claustral colony founding. Insectes Sociaux 43:297–302.

Wheeler, D. E., and T. Martínez. 1995. Storage proteins in ants (Hymenoptera: Formicidae). Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology 112:15–19.

Wheeler, W. M. 1911. The ant-colony as an organism. Journal of Morphology 22:307–325.

Wheeler, W. M. 1928. The social insects: their origin and evolution. Abingdon, Routledge.

Van Wilgenburg, E., C. W. Torres, and N. D. Tsutsui. 2010. The global expansion of a single ant supercolony. Evolutionary Applications 3:136–143.

Wilmon, N., and T. C. Barber. 1913. The Argentine ant. US Dept. Agric. Bureau of Entomology Bulletin 122.

Wilson, Edward, O. 1971. The insect societies. Harward University Press, Cambridge, Massachusetts.

Yamauchi, K., T. Furukawa, K. Kinomura, H. Takamine, and K. Tsuji. 1991. Secondary polygyny by inbred wingless sexuals in the dolichoderine ant *Technomyrmex albipes*. Behavioral Ecology and Sociobiology 29:313–319.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42:207–230.

Evolution of dispersal in ants (Hymenoptera: Formicidae): a review on the dispersal strategies of sessile superorganisms

I

Muurahaisten levittäytymisen evoluutio: katsaus paikallaan pysyvien superorganismien levittäytymisstrategioihin

Sanja Maria Hakala, Perttu Seppä & Heikki Helanterä

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TIIVISTELMÄ

Maaeläinten joukossa muurahaisten (Hymenoptera: Formicidae) levittäytymisstrategioiden monimuotoisuus on ainutlaatuisen suurta. Muurahaispesän analysoiminen sosiaalisena, monivuotisena ja paikallaan pysyvänä supereliönä auttaa tämän monimuotoisuuden ymmärtämisessä, yhdistettynä sosiaalista evoluutiota selittävän kokonaiskelpoisuuden teoriaan. Tässä katsausartikkelissa käsittelemme muurahaisten levittäytymisstrategioita tarkoituksenamme osoittaa kurssi muurahaisten levittäytymisen ja sen evoluution tulevalle tutkimukselle.

Listaamme levittäytymisominaisuuksien perimmäisiä ja välittömiä syitä sekä levittäytymisen ekologisia ja evolutiivisia seurauksia populaatiorakenteista ja -dynamiikasta eliöyhteisöihin. Hahmottelemme eko-evoluutiivisia takaisinkytkentöjä levittäytymisen evoluutioon vaikuttavien ominaisuuksien välillä, sekä ehdotamme evoluution todennäköisiä kulkusuuntia muurahaisten eri levittäytymisstrategioiden välillä. Lopuksi hahmottelemme tutkimusohjelman, jonka tavoitteena on täyttää nykytietämyksen aukot: jatkossa tarvitaan lisää vertailevaa tutkimusta eri lajien yhteiskuntien elinkaaresta ja populaatiorakenteista sekä teoreettisia malleja levittäytymisen eko-evolutiivisesta dynamiikasta kokonaiskelpoisuuden teorian puitteissa.

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Review Article

Evolution of dispersal in ants (Hymenoptera: Formicidae): a review on the dispersal strategies of sessile superorganisms

Sanja Maria Hakala, Perttu Seppä & Heikki Helanterä

Abstract

The extreme diversity of dispersal strategies in ants is unique among terrestrial animals. The nature of ant colonies as social, perennial, and sessile superorganisms is the basis for understanding this diversity, together with the inclusive-fitness framework for social evolution. We review ant dispersal strategies, with the aim of identifying future research directions on ant dispersal and its evolution. We list ultimate and proximate determinants of dispersal traits and the ecological and evolutionary consequences of dispersal for population structures and dynamics, as well as species communities. We outline the eco-evolutionary feedbacks between the multitude of traits affecting dispersal evolution and the likely evolutionary routes and ecological drivers in transitions among the diverse ant dispersal strategies. We conclude by presenting a research framework to fill the gaps in current knowledge, including comparative studies of colony life histories and population structures and theoretical models of the eco-evolutionary dynamics affecting dispersal, in an inclusive-fitness framework.

Key words: Colony founding, dispersal conflict, inbreeding, inclusive fitness, kin competition, kin selection, local resource competition, mating flight, mating system, philopatry, resource allocation, sex bias.

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Introduction

Dispersal is any movement of organisms that can potentially lead to gene flow (Ronce 2007). Movement at all spatial scales, both within and between habitat patches, can qualify as dispersal. Dispersal allows organisms to colonize new areas and thus survive environmental changes should their current habitats become unsuitable. Indeed, the ability to find new habitats and resources in variable environments is considered one of the ultimate causes for dispersal (Van Valen 1971). As natural populations are threatened by habitat fragmentation and climate change, as well as the spread of invasive species, dispersal is also a key consideration in conservation planning (Pressey & al. 2007).

Local resource competition is another ultimate cause for dispersal. From an inclusive-fitness perspective, competition with relatives is harmful, and dispersing away from them is favored by kin selection (Hamilton & May 1977). Staying close to one's relatives and mating with them may also cause inbreeding depression, and lower both direct and indirect fitness (Bengtsson 1978, Motro 1991, Perrin & Goudet 2001). This highlights that dispersal is a social trait and includes both elements of cooperation and conflict among individuals. Thus, selection for dispersal needs to be considered in an inclusive-fitness or multi-level selection framework (Poethke & al. 2007).

Dispersal is a complex process with three distinct phases: emigration (dispersal decision), movement, and immigration that includes establishment as a reproducer in the destination (Bowler & Benton 2004, Ronce 2007). Thus, dispersal comprises multiple traits, potentially responding to multiple selection pressures (Starrfelt & Kokko 2012). As these traits may be genetically corre-

Tab. 1: Overview of the ant nest founding strategies and the typical mating locations and dispersal strategies connected to them. Note that a species can use a combination of several strategies and this is very common in some cases (such as polygynous colonies having alternative strategies). The dispersal strategy mentioned here refers to the individuals, not the species. When mating happens within the gyne's natal colony, the male can be either a disperser from a foreign colony, or a philopatric individual from the same colony. * Nest refers to a single nest mound / cavity / structure, colony refers to the whole society. A colony can consist of one or several nests (monodomy or polydomy, respectively) or be a nestless one (in army ants).

lated and plastic, the genetic architecture of dispersal is potentially very complex (Saastamoinen & al. 2017). This makes predicting responses to selection difficult, especially since the evolutionary role of phenotypic plasticity may be more substantial than conventionally appreciated (Pfennig & al. 2010).

To truly appreciate dispersal and its consequences requires understanding both its ultimate and proximate causes, and their interactions (Bowler & Benton 2004). In this review, we bring together the theoretical context of dispersal evolution with empirical studies on behavior, genetics, physiology and ecology of ants. Our aim is twofold: to show that studying dispersal can further our understanding of social evolution in ants, and to show that ants offer an excellent study system for future work inferring dispersal evolution and the selection pressures affecting it.

Dispersal of the superorganism: Natal dispersal of winged young queens and males has been suggested to be the ancestral dispersal strategy in ants (Hölldobler & Wilson 1990). As colonies of most ant species are sessile and queens do not leave the established colonies (Wilson 1971), this is the only stage where dispersal happens in most ants. Thus, ant colonies are superorganisms that disperse through their mobile offspring like plants do through their pollen and seeds, and sessile marine invertebrates, such as corals, through their sperm, eggs and larvae (Hölldobler & Wilson 2008, Helanterä 2016). The young adult queens (gynes) are the propagules founding new colonies and can disperse both before and after fertilization. They mate within a short time period before colony founding, with one or several males, and store the sperm in their bodies for the rest of their lives (Boomsma 2013). The males are haploid and their sperm is clonal, so gene flow through their movement resembles pollen dispersal. In many species males are sperm limited and only mate once (Passera & Keller 1992), although in some taxa they can mate more than once and possibly in different locations (Shik & al. 2013).

Most ants have sessile colonies and are thus central place foragers. This makes escaping local competition over resources, especially nest sites and food, an important selection pressure for dispersal. Because ant workers are wingless, ants are especially effective in utilizing resources in the immediate proximity of their colonies (Peeters & Ito 2015) and central place foraging affects ants more than social insects with winged workers. Even if not all ants defend their territories (Savolainen & Vepsalainen 1988), the central place foraging lifestyle is likely to result in selection for dispersal beyond the foraging area of the natal colony. The necessity of dispersal in ants is further enhanced by competition between generations, and the low likelihood of nest site and resource inheritance. In many species, the queens are long lived and their colonies can exist in the same location up to a few decades (Wilson 1971). Colony life spans of even a few decades have been recorded in, for example, *Myrmecocystus* and *Formica* (see Chew 1987, Pamilo 1991c). Thus, ants, along with some perennial bees such as the honey bee, differ from most other social hymenopterans by controlling local resources over long timescales (Wilson 1971).

However, female offspring may skip dispersal and stay in their long-lived natal colonies as extra queens. Polygyny (see Tab. 1) is a form of cooperative breeding where multiple queens share the same colony and resources, including the worker force. It is common throughout the ant phylogeny and indeed often arises through philopatry of daughters of the colony (Keller 1995, Heinze 2007, Boomsma & al. 2014). Thus, the theoretical prediction (Kokko & Lundberg 2001) that natal philopatry and cooperation are favored in nest-site limited systems, where dispersal is risky and survival of territory owners high, seems to fit well with the evolution of secondary polygyny (Tab. 1) in ants. However, in addition to cooperation, polygyny also introduces potential for conflict among co-breeders (see sections "Social selection pressures" and "Consequences of dispersal").

In this review, we consider leaving the natal colony always as dispersal, even when the spatial scale is small. Dispersing at all spatial scales is costly and risky (Bonte & al. 2012). The abovementioned idiosyncrasies of ants further add to the risks. Especially the last phases of dispersal, including mating, colony founding and establishment as a reproducer in a competitive community, are critical phases in ant life cycles, and a stage for eco-evolutionary feedbacks where many aspects of ant lives intertwine. Research on ant dispersal has touched on many of these aspects, as demonstrated with detailed examples below. However, an overarching framework for understanding these complex interactions is still needed, and we conclude the review by proposing the building blocks of a research program aiming to better understand the evolution of ant dispersal.

Diversity of ant dispersal strategies

Here, we explain the diversity of ant dispersal strategies and show how mating and colony founding are integrally tied to dispersal. We point out some of the main constrains on ant dispersal, both on wing and by foot, and discuss how dispersal differs between the sexes. Ant flight ecology and the selection pressures affecting the movement phase

Nest / colony* founding strategy	Definition	Typical mating location	Queen dispersal	Male Dispersal	Example taxa
Independent, non-claustral (Hölldobler & Wilson 1990, Brown & Bonhoeffer 2003)	Queens forage for food during nest-founding	Away from the colony	By flight	By flight	Ponerinae, Myrmeciinae (Peeters 1997)
Independent, claustral (Peeters & Ito 2001)	Queens do not forage but stay enclosed in the nest chamber during nest-founding	Away from the colony	By flight	By flight	Prevalent in Formicinae, Myrmicinae (Hölldobler & Wilson 1990)
Pleometrosis (= primary polygyny) (Hölldobler & Wilson 1977)	Several queens found the nest together; the nest usually reverts later to a single-queen state (= monogyny)	Away from the colony	By flight	By flight	*Lasius niger* (see Sommer & Hölldobler 1995), *Pachycondyla* (see Trunzer & al. 1998), *Pogonomyrmex californicus* (see Johnson 2004)
Dependent, fissioning (Bourke & Franks 1995, Cronin & al. 2013)	Existing colony splits into two, workers carry gynes to new locations	At the gyne's colony	On foot with workers	By flight, or no dispersal	*Cataglyphis cursor* (see Lenoir & al. 1988), all army ants (Schneirla 1971)
Dependent, budding (Bourke & Franks 1995, Cronin & al. 2013)	Workers found new nests close to the original ones and carry the queens with them either as juveniles or as adults; often leads to polydomy	At the gyne's colony	On foot with workers	By flight, or no dispersal	Many *Formica* ants (Rosengren & Pamilo 1983)
Polydomy (Debout & al. 2007)	Existing nest splits by budding but the parts retain connection; does not necessarily fit the definition of dispersal, unless the colony grows very big and queens are moved from nest to nest; makes dispersal avoidance more profitable by enhancing the colony's ability to gather resources	None, or see budding	No dispersal, or see budding	No dispersal, or see budding	Many species of *Crematogaster, Leptothorax, Camponotus, Formica* (see Debout & al. 2007)
Secondary polygyny (Rosengren & al. 1993, Crozier & Pamilo 1996, Boomsma & al. 2014)	Queens seek adoption to existing colony as extra queens; the recruiting colony can be a foreign one (dispersal) or the natal colony (philopatry); often connected to budding and polydomy	Away from the colony, or at the gyne's colony	By flight, or no dispersal	By flight, or no dispersal	Many *Formica* ants (Rosengren & al. 1993), many *Myrmica* ants (Keller 1993, Seppä 1996)
Supercoloniality (Pedersen 2006, Boomsma & al. 2014)	Extreme polydomous polygyny, where colonies cover large areas; can function as a distingt dispersal strategy due to the invasive potential on continuous habitat	At the gyne's colony	No dispersal except within the colony	By flight, or no dispersal except within the colony	*Linepithema humile* (see Giraud & al. 2002), many *Formica* ants (Rosengren & al. 1993)
Temporary social parasitism (Buschinger 1986, 2009)	Queens exploit colonies of other species as stepping stones for founding their own colonies	Away from the colony	By flight	By flight	Several *Formica* and *Lasius* species (Buschinger 2009)
Inquiline parasitism (Buschinger 1986, 2009)	Queens exploit colonies of other ant species, usually without ever producing own workers	Away from the colony, or at the gyne's colony	Dispersal limited in various ways	Dispersal limited in various ways	Several species of *Leptothorax* and *Plagiolepis* (Buschinger 2009)
Xenobiosis (Buschinger 1986, 2009)	Queens found their nests inside the nests of other ant species, and exploit some of the host's resources, but produce their own workers	Away from the colony, or at the gyne's colony	Dispersal limited in various ways	Dispersal limited in various ways	*Formicoxenus* and *Polyrhachis* (Buschinger 2009)

of dispersal were recently reviewed by Helms (2018), and are outside the scope of the current review.

Colony founding as an integral part of dispersal: Colony founding by queens is the final immigration phase of dispersal, directly for the gynes and indirectly for the males. This is the phase of dispersal that has received most attention in ant research and through which ant dispersal is most commonly described. Due to these reasons, we continue using the colony founding terminology for describing ant dispersal strategies (see Tab. 1 for summary of the main colony founding strategies and their effects on dispersal, and explanation of the terms that will be used hereafter). However, dispersal is successful only after the dispersing individual reproduces in the new location, resulting in gene flow (Ronce 2007). In population genetic terms, only colonies producing sexual offspring can be considered successful, but from the ecological perspective, colonies often have an effect on local communities already during their growth phase when they concentrate on worker production (Oster & Wilson 1978). The growth phase can be considerably long, for example in *Pogonomyrmex barbatus* (Smith, 1858) it takes about five years (Gordon 1995, Gordon & Wagner 1999).

Phylogenetic reconstruction shows that ancestral ant queens used non-claustral colony founding (Keller & al. 2014). Modifications to this ancestral strategy have evolved several times in different ant taxa, most likely to reduce the high costs of dispersal (Heinze & Tsuji 1995), but the proposed evolutionary pathways between the strategies have mostly not been formally tested. In line with the general notion that dispersal has high potential for evolution (Saastamoinen & al. 2017), closely related species can use different strategies, and reversals to ancestral strategies have occurred (e.g., Keller 1991, Brown & Bonhoeffer 2003, Johnson 2010). We will discuss the possible evolutionary pathways in detail in "Evolutionary transitions in dispersal", and specify the knowledge gaps and research needs in "Conclusions and future directions."

Dispersal ability and resources: The queens and males of many ants are strong flyers (Helms 2018) and based on studies on colonization and community ecology (Vepsäläinen & Pisarski 1982, Vasconcelos 1999), it is obvious that many species are pioneers that commonly colonize new habitats and at least some individuals of these species disperse long distances. For example in *Lasius niger* (Linnaeus, 1758), populations in Northwestern Europe seem to be genetically uniform, suggesting regular long range dispersal (Boomsma & Van Der Have 1998), consistent with the pioneer lifestyle of the species.

However, measurements of flight distances have been reported for only a few species, sometimes with small sample sizes (Helms 2018). There are also plenty of taxa whose colony structures and life cycles have not been studied at all, especially among ponerine ants (Peeters 1997). Furthermore, knowing average dispersal distances is not enough. Dispersal distribution in natural populations of most organisms is fat-tailed, meaning that most individuals do not disperse much at all and the rest have large variation in their dispersal distances (Lowe & McPeek 2014). As the whole distribution of dispersal distances is likely to affect the population level consequences of dispersal, assessing also the intraspecific variation in ants is an important aspect of future research.

In the absence of direct data on flight, the flight ability can be roughly inferred from morphological traits such as the wing muscle mass to body mass ratio (Marden 1987, 2000). Flying ants carry large amounts of resources, especially the claustrally founding gynes that need fat and storage proteins for energy during colony founding (Wheeler & Buck 1995, Wheeler & Martínez 1995, Wheeler & Buck 1996). Such queens have larger abdomens and smaller muscles compared with the gynes using other nest founding methods and might therefore be less skilled in flying and less able to fly long distances (Helms & Kaspari 2014, 2015). In contrast, non-claustral gynes have worker-like large heads and strong neck muscles for foraging during colony founding and thus face restrictions in their thorax architecture and flight muscle size (Keller & al. 2014). This makes the relationship of colony founding strategy and flight ability complex in ants. Also pleometrosis and parasitic strategies can change the queen's need for resources and therefore affect wing muscle to body mass ratios.

In addition to flying, several ant species also disperse on foot, especially the queens but in some cases also the males (Heinze & Tsuji 1995). This results in shortened dispersal distance and patterns of isolation by distance across small geographical scales (Peeters & Aron 2017). Especially dependent colony founding through budding or fissioning compromises the colonization ability of a species (Peeters & Molet 2009). Transition to dependent founding changes colony resource allocation: The resources for dispersal are not only allocated to the queens, but also indirectly to the workers that assist them in colony founding (Peeters & Ito 2001, Peeters 2012). In some species budding leads to polydomy, which enhances the colony's ability to gather resources, expands the colony area, and affects local competition (Debout & al. 2007, see "Social selection pressures").

Dispersal polymorphism in queens: Variation in resource allocation leads to variation in dispersal ability among individuals. For example in fire ants, the heavier summer gynes with better resources disperse smaller distances and found nests alone, while the leaner overwintered gynes fly further, and sneak into established colonies as intraspecific parasites (Helms & Godfrey 2016). Regardless of whether this is an adaptive parasitic strategy or just starved individuals making the best of a bad job, such variation has ecological and evolutionary consequences through effects on the selective regime of dispersal traits.

Clear-cut dispersal polymorphism is widespread among insects (Zera & Denno 1997). Such polymorphism is suggested to evolve when different selection pressures select for and against dispersal at the same time (Mathias & al. 2001). Dispersal polymorphism exists in ant queens,

too. Especially in dependently founding ants there are queen polymorphic species, with flying and completely flightless morphs, convergently evolved in several taxa (Peeters 2012). The genetic and developmental basis of such polymorphism is largely unknown, but for example in *Harpagoxenus* and *Leptothorax*, polymorphism seems to be caused by a single-locus mutation (Bourke 1987, Heinze & Buschinger 1989, Heinze & Tsuji 1995). In *Myrmica* ants, several species have queen size morphs, microgynes that are more commonly philopatric or even parasitic without producing their own workers, and macrogynes that usually participate in long range dispersal. In *Myrmica rubra* (Linnaeus, 1758) the two morphs are clearly distinct in size and behavior, and also genetically partially differentiated (Vepsäläinen & al. 2009, Leppänen & al. 2015), whereas in *Myrmica ruginodis* Nylander, 1846 the correlation between dispersal strategy and size is not as strong (Wolf & al. 2018). Similar queen size dimorphism exists also in several *Leptothorax* species (Hamaguchi & Kinomura 1996, Rüppell & Heinze 1999, Rüppell & al. 2001).

Variation in dispersal strategies may also exist with no obvious external morphological differences. For example, many *Formica* species have both monogynous and polygynous, even supercolonial, populations as a result of different dispersal strategies (Rosengren & al. 1993). In the polygynous populations, some individuals are philopatric and stay in their natal colonies, while some disperse and found their nests independently or via temporary parasitism (Collingwood 1979). Recently such intraspecific variation in dispersal has been linked to genetic architecture (Libbrecht & al. 2013). So-called social chromosomes, first found in *Solenopsis invicta* Buren, 1972 (Ross & Keller 1998, Krieger & Ross 2002, Wang & al. 2013) and later in *Formica selysi* Bondroit, 1918 (Purcell & al. 2014) seem to be connected to dispersal behavior of colonies: One type of the linkage group is associated with monogynous colonies where extra queens are not accepted, and the other type with highly polygynous colonies where workers readily accept extra queens.

In general, the genetic architecture of dispersal evolution is still largely unknown, both empirically and theoretically (Saastamoinen & al. 2017), but the different types of ant dispersal polymorphisms hold promise for being good model systems for such questions in the future.

Dispersal strategies of ant males: Ant males are traditionally seen only as the vehicles of sperm, and not much more. Their role inside the colonies during their development has not evoked much interest (Schultner & al. 2017), and their life outside the colonies is usually described by a single word: short. Ant males can potentially allocate all their resources to mating and dispersal, and do not need to invest in longevity, for example through costly immune defenses (Boomsma & al. 2005, Stürup & al. 2014). Detailed studies on male ants have focused on only a few species, such as *Atta* leaf-cutter ants (e.g., Baer & Boomsma 2006, Stürup & al. 2011). As male behavior and mating strategies vary a lot in other social Hymenopterans (Alcock & al. 1978, Boomsma & al. 2005), also ant males should be investigated more.

Fig. 1: *Formica pratensis* male has left the natal nest and climbed to a tree branch above it, ready to fly. Photograph by S. Hakala.

Dispersal coevolves with mate localization in ants (Peeters & Aron 2017) and studies on mating behavior are the main source of information about male dispersal. Traditionally, two main ways of mate localization are distinguished: In the male-aggregation system, both sexes join synchronous mating swarms away from their natal colonies, whereas in the female-calling system, males find gynes that advertise themselves with pheromones near their natal nests, with no clear synchrony in the flights among sexes (Hölldobler & Bartz 1985, Kaspari & al. 2001, Peeters & Aron 2017). Female calling is associated with male biased dispersal so that gynes fly only after mating, or not at all (Peeters & Aron 2017, Helms 2018). Female calling systems sometimes mean long search times for males, which has occasionally resulted in increased male life spans, and special morphological adaptations such as functional mandibles for feeding (Shik & Kaspari 2009, Shik & al. 2012). The division between the two mating systems is not necessarily strict in all taxa. For example in some *Formica* species (Fig. 1), individual gynes have been reported to either fly away from their natal nest or wait close by and males answer this with specific patrolling behavior for locating them (Kannowski & Johnson 1969).

Even less is known about within species variation in male behavior. In supercolonial *Linepithema humile* (Mayr, 1868), males are shown to either mate at their natal colony or to disperse and mate with gynes in other colonies (Passera & Keller 1993) and similar variation in behavior exists in *Formica* (Rosengren & Pamilo 1983). Also socially polymorphic species such as *Leptothorax acervorum* (Fabricius, 1793) (Hammond & al. 2001) or several *Myrmica* species (Seppä 1996) are likely candidates for such behavioral variation. In *Formica exsecta* Nylander, 1846, males are dimorphic, with monogynous colonies predominantly producing larger males that are suggested to be better at competing over mating opportunities locally, and polygynous colonies producing smaller, possibly more dispersive males (Fortelius & al. 1987).

The most dramatic within-species variation occurs in *Cardiocondyla* ants (Stuart 1987, Heinze & al. 1998): A winged male morph disperses, and a wingless, philopatric fighter morph mates in the natal colony and fights with other wingless males for mating opportunities.

Outside *Cardiocondyla*, flightless males are rare and found mainly in highly specialized species where also queen dispersal is restricted, such as social parasites *Formicoxenus* (see Härkönen & Sorvari 2017), or supercolonial *Monomorium pharaonis* (Linnaeus, 1758), whose males do have wings but still do not fly (Bolton 1986, Fowler & al. 1993). This can be contrasted with at least 16 different subfamilies with completely flightless queens (Peeters 2012) and even more taxa with otherwise limited female dispersal (Heinze & Tsuji 1995). Such apparent difference in the dispersal ability and propensity between the sexes begs for a systematic investigation of sex-biased dispersal in ants, both in terms of population genetics and the dispersal morphology and physiology.

Sex-biased dispersal: Sex-biased dispersal strategies are expected when there are differences in the trade-offs between dispersal and reproduction between the sexes (Zera & Denno 1997, Marden 2000, Perrin & Goudet 2001). These can arise when the sexes are competing for different resources during their adult lives (Li & Kokko in press). Classical theoretical considerations (Greenwood 1980) and empirical patterns from mainly vertebrates (Trochet & al. 2016) show that dispersal is biased towards the sex that has more to gain (or less to lose) from increased dispersal, mainly driven by the number of mating partners and local resource competition connected to parental care. In insects, dispersal strategies commonly differ between the sexes (Zera & Denno 1997), but the drivers of sex bias in invertebrates have not been systematically tested.

Accordingly, male bias in dispersal can be predicted to be more common in ants, as ant queens experience resource allocation trade-offs between flight and colony founding (See section "Dispersal ability and resources"). Since ant males do not live beyond dispersal and mating, and only compete over access to matings, selection can optimize them for these functions with fewer trade-offs, making males more likely to be the more dispersing sex. Many of the derived dispersal and nest founding strategies in ants can be roughly explained by selection that reduces the relative allocation to flight in queens (Heinze & Tsuji 1995), and are associated with female-calling mating system where males fly more in search for mating partners (Peeters & Aron 2017, Helms 2018), symptomatic of coevolution between sexes.

Male-biased dispersal is indeed often reported in ants (Sundström & al. 2005, Foitzik & al. 2009), even in species like *Formica exsecta* (see Sundström & al. 2003), whose monogynous life histories are thought to correlate with male-aggregation mating systems and dispersal of both sexes. However, a clear majority of studies on sex-biased dispersal focus on species where male bias is predicted (Johansson & al. 2018), such as species with completely flightless queens [e.g., *Nothomyrmecia macrops* Clark, 1934 (Sanetra & Crozier 2003); *Proformica longiseta* Collingwood, 1978 (Seppä & al. 2006, Sanllorente & al. 2015), army ants (Berghoff & al. 2008, Barth & al. 2013, Soare & al. 2014)]. Not surprisingly, dispersal and gene flow are heavily male-biased in these species. When sex-biased gene flow was assessed in a well-dispersing pioneer species *Formica fusca* Linnaeus, 1758, a slight female bias was observed (Johansson & al. 2018). Thus, species that do not have derived dispersal strategies should be studied more in order to gain a better understanding of sex biases in ant dispersal. Considering the idiosyncrasies of ant life histories, further investigation of the theoretical basis of sex-biased dispersal in ants would produce precise, testable hypotheses for these studies.

For the rest of this review, our discussion mostly focuses on queen dispersal, merely because males are largely overlooked in literature. Nevertheless, the examples and open questions reviewed here make it clear that male dispersal behavior is worth a closer look in the future.

Is colony relocation dispersal? In addition to natal dispersal, ants can move short distances by relocating the whole colony, with workers carrying brood and queen(s) to a new location (Smallwood 1982, McGlynn 2012). Colony relocation behavior has been reported throughout the ant phylogeny (McGlynn 2012). This behavior resembles dependent colony founding, and is also comparable with it in movement scale (Bouchet & al. 2013). This kind of small-scale movement is not usually considered dispersal, and the colonization potential of relocation is obviously small. But considering the long colony lifespan in some ants, regular nest relocation can lead to covering significant distances over longer time scales and should thus not be completely dismissed as a potential form of dispersal.

Colony relocation is most commonly a response to changing environmental conditions or disturbances (McGlynn 2012), for example colonies being built in substances that do not last for long times, such as small pieces of damp rotten wood, as in *Mystrium oberthueri* Forel, 1897 (Bouchet & al. 2013). In some other species, colonies are shown to relocate whenever they find a better quality site than the one they currently occupy, e.g., *Temnothorax albipennis* (Curtis, 1854) (Dornhaus & al. 2004). The same environmental challenges that lead to relocations can be also connected to polydomy and many polydomous species readily relocate their nests, sometimes in seasonally changing nest networks (Debout & al. 2007). Some ant species even have highly specialized behavioral strategies for colony relocation, such as self-assembled waterproof rafts in *Solenopsis invicta* to survive flooding (Mlot & al. 2011).

The most conspicuous case of colony relocation are army ants, where mobility defines the entire lifestyle: Army ants are group predators whose colonies regularly move from one location to another, without building permanent nest structures. As their queens are flightless, new colonies are produced through fission (Wilson 1958, Brady 2003). They can shift their colony as an answer to unfavorable environmental conditions, just as other ants,

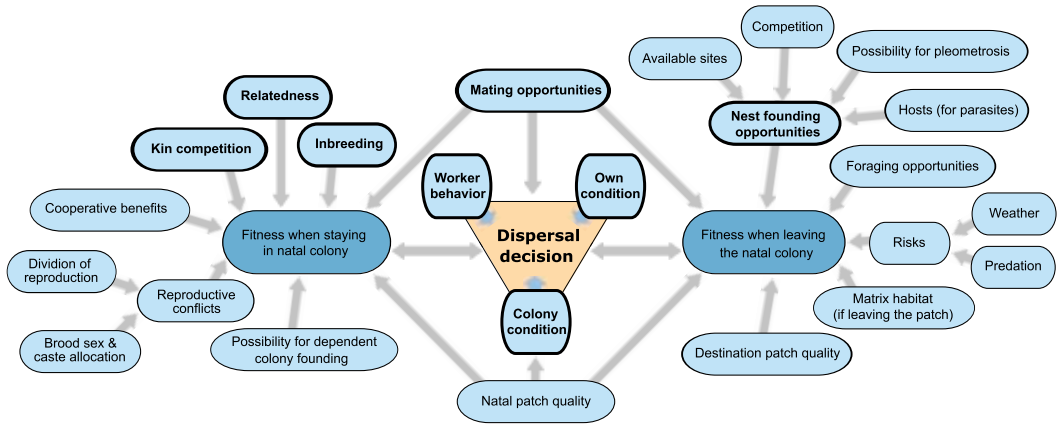

Fig. 2: Individual-level factors affecting the evolution of dispersal behavior. An individuals' decision to stay in the natal nest or to leave it – and later the decision to leave the natal habitat patch or not - depends on several factors that affect its fitness (indicated with arrows). The most influential ultimate and proximate factors are highlighted in bold. Most factors also interact with each other, and especially relatedness is a central factor that affects all social interactions. Inside the natal colony, the dispersing individual has direct information mainly about its own condition, colony condition and worker behavior (all of which closely interact). The other factors affect dispersal evolution mainly through natural selection, as ecological, social, and evolutionary feedbacks. The fitness benefits differ among the possible scenarios, and choosing one scenario has costs of lost opportunities from the other scenarios.

but they also have specialized emigration behavior. They move up to 450 meters at a time, either in a regular cycle or in a more irregular manner, depending on the taxon (Schneirla 1971). "True army ants" has been suggested to be a non-monophyletic group in the subfamily Dorylinae (Brady & al. 2006), and lifestyles with similar characteristics have also evolved in other more distantly related taxa (Kronauer 2009).

Colony relocation behavior, especially on the army ant scale, is an evolutionary transition of the superorganism from a sessile lifestyle towards a mobile one. Similar transitions have occurred in marine taxa (e.g., in feather stars (Nakano & al. 2002)), but the phenomenon has not been theoretically analyzed. The fact that such transitions in ant lifestyle and dispersal strategies are possible underlines their evolutionary flexibility.

Multiple causes and selection pressures of dispersal

Dispersal is a multicausal process, and several different selection pressures affect it, sometimes in opposite directions (Starrfelt & Kokko 2012). The importance of the three ultimate causes for dispersal (inbreeding avoidance, avoidance of kin competition, colonization of new habitats) varies between taxa and depends on their ecology and evolutionary history (Bowler & Benton 2004). Importantly, the selection pressures predicted to affect the existence, rate or the range of dispersal are partially different (Lowe & McPeek 2014). Direct selection for dispersal plays a role mostly in the onset of dispersal and less so during the movement phase (Burgess & al. 2016). Thus, whether young ant sexuals leave the natal colony or stay and mate within it, is likely under direct selection for dispersal, but the range of their movement is affected by more proximate ecological and local selection pressures.

In this section, we list and discuss the determinants of dispersal decisions of individuals (summarized in Fig. 2). We start by exploring the ultimate causes, i.e., the selection pressures for dispersal in an inclusive-fitness framework, and discuss where the power over dispersal lies in ant societies. These sections deal with theoretical predictions, and the relative importance of these causes in natural populations should be systematically investigated with comparative data, due to the multitude of ecological and proximate causes affecting dispersal (Bowler & Benton 2004). At the end of this section we briefly explain the main proximate causes that shape the realized dispersal and gene flow in natural populations, and reflect on the condition dependency of dispersal decisions.

Inbreeding avoidance: Inbreeding avoidance is likely to be an important cause selecting for dispersal in ants. As in other haplo-diploid hymenopterans with complementary sex determination system (CSD), the effect of inbreeding is considered to be particularly harmful, because it results in inviable or sterile diploid males (Cook & Crozier 1995, Zayed & Packer 2005). CSD has recently been shown to exist in *Vollenhovia emeryi* Wheeler, 1906 (Miyakawa & Mikheyev 2015) and diploid males have been reported in many other ant taxa, consistent with the existence of CSD (Cook 1993). Other costs of inbreeding have been demonstrated in *Formica exsecta* (see Haag-Liautard & al. 2009) and *Cardiocondyla obscurior*

(Wheeler, 1929) (Schrempf & al. 2006). In order to avoid inbreeding, at least one of the sexes has to leave the natal colony and mate elsewhere, which can lead to sex-biased dispersal strategies (Pusey 1987, Motro 1991, Gros & al. 2008, section "Sex-biased dispersal").

Local mating between two offspring of the same colony seems to be limited to derived strategies, such as parasitic nest founding strategies, secondary polygyny or the fighter male morphs in *Cardiocondyla*. In *Cardiocondyla elegans* (Emery, 1869), workers actively carry gynes from one colony to another to allow them to mate with unrelated males, which seems to be a behavioral adaptation to avoid inbreeding (Lenoir & al. 2007). It is likely that in some of the locally mating ant taxa either CSD based on many loci or alternative sex determination mechanisms have evolved to counter the harmful effect of CSD and inbreeding (Buschinger 1989, Schrempf & al. 2006). Also clonal reproduction contributes to inbreeding-avoidance in some ant lineages, and it has been suggested to be an important pre-adaptation to the colonization success of some of the invasive, supercolonial species (Pearcy & al. 2011, Wenseleers & Van Oystaeyen 2011). In polygyne and supercolonial ant societies, the high number and low relatedness of egg-laying queens reduces the risk of inbreeding, but the risk still remains as polygyny is often the result of the daughters of the society staying and mating locally.

Many ant species commonly produce only or mainly single sex broods (Pamilo & Rosengren 1983, Nonacs 1986, Cook & Crozier 1995), which is often explained through worker control of sex ratios in response to relatedness asymmetries (Trivers & Hare 1976, Boomsma & Grafen 1990, Boomsma & Grafen 1991, Meunier & al. 2008). However, it also contributes to inbreeding avoidance. Split sex ratios force individuals to find mating partners outside the nest, ensuring dispersal even when it would otherwise be unfavorable for the individuals.

Social selection pressures: According to general theory, resource competition with relatives is harmful and dispersing away from them is favored by kin selection (Hamilton & May 1977), and the negative effects of competition among kin can cancel out the benefits of local cooperation in simple scenarios (Taylor 1992, West & al. 2002). However, more complex models show limited dispersal and population viscosity to be beneficial for social organisms (Lehmann & al. 2008, Kümmerli & al. 2009). This complexity, together with the difficulty of specifying the spatial scale over which cooperation and competition occur in nature (West & al. 2002) makes it hard to pinpoint which selection pressures have the highest impact on dispersal.

Indeed, one of the most interesting aspects of queen philopatry is the possibility to make the colony more successful, as polygyny allows producing a larger worker force. Especially when resource competition is strong, cooperative strategies connected to better resource deployment may be favored (Van Dyken & Wade 2012). According to Rosengren & al. (1993), this could explain the prevalence of polygyny in aphid farming *Formica* ants. If their ability to attend aphid livestock increases with increasing number of worker-producing queens, they can possibly even create more resources than the habitat originally had, overcoming some of the resource limitations that would otherwise lead to harmful kin competition. Polygyny is also connected to polydomy and budding dispersal in many ants, including *Formica* (Rosengren & Pamilo 1983, Debout & al. 2007, Ellis & Robinson 2014). This has been suggested to explain why queen philopatry and polygyny are so prevalent in ants compared with all other social insect taxa (Boomsma & al. 2014).

Recruitment of new queens potentially complicates the selection pressures affecting dispersal (see also "Colony allocation decisions and conflicts"). The number of queens and the division of reproduction among them affect expected direct fitness opportunities of a philopatric young queen. For example in functionally monogynous species where only one of the nestmate queens reproduces at a time, such as *Leptothorax* sp. (Heinze & Smith 1990), direct fitness is gained only in the case of possible resource inheritance in older age. In other species, all of the queens can reproduce simultaneously, as in for example *Temnothorax* (Guénard & al. 2016) and *Myrmica* ants (Evans 1996), which could make staying a safe strategy. The division of reproduction can also be more subtle. For example, in *Solenopsis invicta* (see Ross 1988) and *Formica exsecta* (see Kümmerli & Keller 2007) some queens produce mostly workers while others concentrate on sexual production.

Furthermore, the reproductive tenure of philopatric, polygynous queens is often relatively short compared with dispersing monogynous queens (Keller & Passera 1990, Tsuji & Tsuji 1996). For example, in facultatively polygynous *Formica fusca*, queens in polygynous nests have a shorter life than queens in monogynous nests (Bargum & al. 2007). However, this is compensated for by the facts that by staying in the polygynous colony, the queen both avoids the risks of dispersal and can immediately start producing sexual offspring instead of having to produce workers first (Keller & Passera 1990, Tsuji & Tsuji 1996).

Finally, the fitness consequences of staying in the natal colony depend on multiple allocation decisions within the colony, whose evolution in turn may depend on the kin structure of the nest (Crozier & Pamilo 1996). For example, colony sex and caste allocation may be predicted to affect the dispersal decision, all else being equal: The optimal choice for a single gyne could depend on the number of other competing gynes and the choices they make (Rosengren & al. 1993). The future allocation decisions also directly affect the fitness of any queen that decides to stay in the colony. As dispersal decisions alter the social environment within the colony, it is very difficult to fully assess the fitness consequences of philopatry vs. dispersal (Keller 1993), and truly understanding the social selection pressures requires understanding the eco-evolutionary feedbacks on dispersal (see "Consequences of dispersal").

Conflict over dispersal: The optimal dispersal behavior is predicted to differ between parent and offspring perspectives. For the parents, the fitness of each offspring is equally valuable, but for the offspring their own fitness is more valuable than that of their siblings. From a parent's point of view, high levels of risk can be tolerable, but the dispersing individual's risk tolerance threshold is lower, which leads to a potential parent-offspring conflict over dispersal (Motro 1983). In addition to dispersal itself, also the distance can be under conflict, as parents favor longer distances while the dispersers themselves would rather choose to stay close (Starrfelt & Kokko 2010). The strength of the conflicts depends on the ecological setting, and the conflict is stronger when dispersal is very risky (Motro 1983).

In ants and other social insects, there is potential for a three-way conflict over dispersal, because the colony structure complicates the situation. In addition to the parent-offspring conflict (Motro 1983) between the mother queen and the dispersing sexual offspring, also the queen and the workers may have conflicting inclusive-fitness interests. As in other conflicts in insect societies, relatedness influences what are the optimal strategies for each player and whether there is potential for conflict (Ratnieks & Reeve 1992, Crozier & Pamilo 1996, Ratnieks & al. 2006). The dispersal conflict is expected to be amplified when relatedness asymmetries within the society increase. That is, the dispersing individuals are expected to value their direct fitness more, when indirect fitness effects are diluted through low relatedness to others. However, the exact shape of the potential three way conflicts and whether they manifest as actual conflicts remain to be studied.

Power over dispersal: Actualization and outcome of potential dispersal conflicts depend on which party has most power to control dispersal (Beekman & Ratnieks 2003). In some organisms, dispersal is strictly under maternal control: For example, in plants the offspring have no power over dispersal decisions (Motro 1983). In animals, the division of power is usually more equal: For example, in marine invertebrates, the parent controls the development and release of the planktonic larvae, but the larvae have power over their own behavior after that (Marshall & Morgan 2011).

In ants, workers take care of brood, and sex allocation is in many cases consistent with worker control (Meunier & al. 2008). Thus, workers may affect the dispersal patterns by controlling the sex ratios of the brood and the gyne-worker ratio of the female brood (Ratnieks & al. 2006). Workers can also indirectly affect the dispersal behavior of individual dispersers, since dispersal decisions are often condition dependent (Bowler & Benton 2004), and workers have the possibility to control larval development and thus the condition of dispersing individuals. However, the relative contribution of workers and the individuals themselves has been assessed only in a few cases. Studies on the genetic architecture behind the mass of individuals have revealed complex interactions between individual genotype and the social or indirect genetic effect

Fig. 3: (a) *Lasius flavus* gynes and workers have climbed on a rock above their nest. The workers of this subterranean species are rarely seen above ground expect at the onset of dispersal. (b) *Lasius niger* males emerging from their nest. Photographs by S. Hakala.

of rearing workers (Linksvayer 2015). In a cross-rearing experiment on *Solenopsis invicta*, the origin of rearing workers seemed to affect larval development even more than the genetic background of the larvae, which would suggest great worker power (Keller & Ross 1993), whereas in a cross-rearing experiment on *Temnothorax curvispinosus* (Mayr, 1866), there were direct (the genotype of the individual itself), maternal, and worker effects on the gyne's mass at maturation, and direct and worker effects on the male mass (Linksvayer 2006). It seems that the development of larvae is an outcome of both their own phenotype and their social environment (Fjerdingstad 2005, Linksvayer 2015, Schultner & al. 2017).

Worker control over the development of dispersers does not guarantee that the latter are willing to disperse. However, as workers outnumber the dispersing individuals, it seems likely that they have power over the actual dispersal decision as well (Beekman & Ratnieks 2003, Fig. 3a & b). Worker behavior at the onset of dispersal has not been studied quantitatively, but there are anecdotes of workers controlling the movement of winged individuals and forcing them out of the colony at the appropriate time (e.g., Talbot 1956). In mammals, forced natal dispersal is common, although aggression is not usually targeted towards relatives, but rather towards unrelated juvenile individuals competing for resources (Wolff 1993). Behavioral studies are needed to assess the role of aggression towards gynes at the onset of dispersal and the likelihood of forced dispersal in ants. In supercolonial *Linepithema humile*, execution of older egg-laying queens is common, as workers kill up to 90% of the queens in their colonies each spring, possibly to control the relatedness and queen number (Keller & al. 1989, Inoue & al. 2015), which could be a delayed manifestation of an unresolved conflict over dispersal. But with no theoretical assessment of the direct benefits of such behavior to the workers, this remains speculative and begs further investigation.

In independently founding species, gynes and males control their own movement after leaving the natal colony, and the gynes choose where they settle to found a colony. In contrast, in dependently founding species, workers have almost full control over all stages of dispersal. Workers choose which gynes (or queens, or queen-destined brood) to carry to a new location. Dependent colony founding has evolved when the success rate of independent colony founding is low due to environmental reasons (Molet & al. 2008, Cronin & al. 2013). As higher dispersal risks theoretically also result in stronger conflicts over dispersal (Motro 1983), dependent colony founding could also resolve the dispersal conflict, as it both decreases the risk for the gynes and allows workers to alleviate local competition by moving queens to new nests.

Environmental selection pressures: Evolution of dispersal, and especially of dispersal distance, is strongly linked to local environmental factors. The ecological setting affects dispersal, either immediately through facultative and condition dependent decisions based on the information individuals are able to obtain (Kokko 2003, Clobert & al. 2009), or through natural selection.

Theoretically, colonizing new habitats is an important selection pressure for dispersal (Van Valen 1971, Olivieri & al. 1995). However, the time scale for such selection is long, because it plays out only when current habitat becomes unsuitable. Thus, selection for dispersal through the need for colonizing new habitats likely depends on selection for decreasing fitness variance in a lineage, rather than increasing immediate mean fitness (i.e., bet-hedging, Starrfelt & Kokko 2012). Also the spatial scale of colonizing new habitats is large in ants: For central place foragers, even short-range dispersal is often enough to mitigate the harmful effects of kin competition, but finding new habitats requires long-range dispersal. Thus, it is not clear how strong direct selection for colonizing new habitats can be. In general, long range dispersal is probably rarely maintained purely for dispersal alone, but is often a byproduct of traits selected for other reasons, such as avoiding predators and finding mating partners (Van Dyck & Baguette 2005, Nathan & al. 2008, Burgess & al. 2016).

Fig. 4: *Crematogaster* sp. gyne after leaving the natal colony, making further dispersal decisions on the go. Note the big mesosoma with strong flight muscles. Photograph by Alejandro Santillana, published as a part of the "Insects Unlocked" project.

In general, long-range dispersal away from the current patch increases when local resource competition is high due to small size, low quality, or high competitor density of the current patch (Poethke & Hovestadt 2002, Clobert & al. 2009). To our knowledge, studies assessing the relation of habitat quality and individual dispersal decisions have not been done on ants. However, it is clear that habitat quality impacts colony condition and thus affects the overall dispersal patterns through the amount and quality of dispersers the colony produces. As an extreme example, in *Cardiocondyla* the colony condition affects which male morph it produces: Under good conditions, the less costly wingless males are produced, while the more costly, substantially larger winged males appear in unfavorable conditions (Cremer & Heinze 2003). Similar condition dependency might affect the quality of dispersers in other ant species as well. Variation in individual quality, in turn, affects single dispersal events, so that not all individuals disperse the same way (Clobert & al. 2009, Lowe & McPeek 2014). For example in *Formica truncorum* (Fabricius, 1804), the individuals in better physical condition seem to be more likely to initiate dispersal (Sundström 1995). There are no studies measuring how dispersal distances correlate with individual condition in ants.

After the decision for long range dispersal has been made, patch connectivity and quality of surrounding habitat matrix affect the success of dispersal (Hanski 1999, Fahrig 2001), as does the predation pressure (Helms

2018). Individuals are likely to base their decisions on information about their immediate surroundings and their own physical condition (Fig. 4), whereas conditions further away and at a later time point are more likely to work through eco-evolutionary feedbacks.

Consequences of dispersal

Dispersal has important consequences on different spatial and temporal scales. In dispersal, individual and population level processes are connected through ecological and evolutionary feedbacks that interact through population dynamics. Ecological feedbacks result from resource availability and social interactions, while evolutionary feedbacks result from different fitness benefits of alternative strategies (Bowler & Benton 2004). Separating causes and consequences of dispersal is partly arbitrary and full understanding of dispersal requires understanding the eco-evolutionary feedbacks at play. In this section we briefly list consequences of dispersal, but mostly discuss the potential feedbacks affecting ant dispersal, even though research on these questions still largely awaits to be done.

Colony allocation decisions and conflicts: Taking co-evolving dispersal and mating strategies into account can deepen our understanding of social conflicts within ant societies. Relatedness among different members of the colony has been shown to affect many allocation and behavioral decisions and is also predicted to affect dispersal decisions and thresholds of accepting additional philopatric queens in the colonies (Pamilo 1991a, b, Bourke & Franks 1995, Crozier & Pamilo 1996). If some of the queens avoid dispersal and stay in their natal colony, relatedness is also immediately altered, which could lead to interesting feedback loops.

The potential conflict over queen number is influenced by the dispersal optima of the parents (queen and colony) and the offspring (gynes and males). The optimal dispersal rules of gynes have not been assessed by detailed theory, but a simple prediction is that they should seek adoption more readily than the colonies are willing to allow (Motro 1983). For workers, the difference between inclusive-fitness effects of accepting or rejecting an extra queen into the colony decreases with increasing queen number (Crozier & Pamilo 1996). Thus, if the queen number increases enough (due to any reason), additional queens have only negligible effects on the relatedness between nestmate and the workers. Eventually, the selection to control queen number may be weakened or even overrun by other selection pressures. This kind of feedbacks might in part explain extremely high queen numbers per nest (tens and even hundreds), such as those found in *Formica* ants (Rosengren & al. 1993) and other supercolonial species, even though multiple other causes may explain the original switch to polygyny.

Dispersal decisions can also be predicted to affect other within-colony conflicts. The higher the number of queens per nest is, the more are workers predicted to police reproduction by other workers, which over time resolves the queen-worker conflict over male production (Ratnieks & al. 2006). Similarly, the more queens are recruited back into their natal colonies, the smaller the queen-worker conflict over sex ratio is predicted to be. This is because having multiple queens dilutes the relatedness asymmetries between workers and the male and female brood, and the sex ratio optima of both parties converge towards 1:1 (Trivers & Hare 1976, Bourke & Franks 1995). However, in practice the sex ratios in sexual brood might not reach exactly 1:1 in polygynous societies, because part of the worker force can be considered an investment in the gynes that stay and start laying eggs in the natal colony (Pamilo 1990), in a similar manner as in dependently founding species where the resource allocation for gynes happens partly through the workers that help them found colonies (Peeters 2012). This kind of indirect resource allocation makes it hard to consider the exact fitness consequences of these dispersal strategies.

There is also another potential feedback between dispersal and sex ratios in ant colonies: Local mate competition caused by philopatric males skewing optimal sex ratios towards females could explain at least part of the observed sex ratio bias (Alexander & Sherman 1977). This hypothesis has not gained large support among social insect researchers as local mate competition has been deemed unlikely in species with mating flights and male-aggregation mating system – but the hypothesis may have been dismissed prematurely (Helanterä 2016). Local mate competition theories can be useful especially when explaining female biased allocation connected to derived dispersal strategies, such as completely flightless ants (e.g., *Cardiocondyla* sp. (Schrempf & al. 2005)), social parasites (e.g., *Plagiolepis xene* Stärcke, 1936 (Aron & al. 1999)) or highly polygynous species (e.g., *Myrmica sulcinodis* Nylander, 1846 (Pedersen & Boomsma 1998)).

Population dynamics: Dispersal has the potential to alter population dynamics and different dispersal strategies may impact persistence of populations over evolutionary time scales. Population genetics offers excellent tools for inferring large-scale patterns of dispersal (Balloux & Lugon-Moulin 2002).

In most studied ant taxa the spatial scale of dispersal seems to be small and the resulting population structures genetically viscous (Rissing & Pollock 1986, Seppä & Pamilo 1995, Ross & al. 1997, Sundström & al. 2005). As suggested already by Hölldobler & Wilson (1977), this holds true especially in polygynous species and populations, showing that social structure and dispersal are tightly linked in ants (Sundström & al. 2005). Especially species using only dependent founding have very viscous populations due to reduced gyne dispersal (Sanetra & Crozier 2003, Berghoff & al. 2008, Barth & al. 2013, Sanllorente & al. 2015, Peeters & Aron 2017). However, this may be a biased view, as species using strategies with limited gyne dispersal have been studied more (Seppä 2009, Johansson & al. 2018). In contrast, lack of viscosity has been shown in a handful of species, e.g., in *Lasius ni-*

ger (see Boomsma & Van Der Have 1998), *Formica fusca* (see Johansson & al. 2018), and *Temnothorax rugatulus* (Emery, 1895) (Rüppell & al. 2001). More balanced sampling of species and careful consideration of the spatial scales used would show if short distance dispersal and population genetic viscosity are general traits in ants, and which are the correlated life history traits.

Climate change will put pressure for range shifts on natural populations (Helms & Bridge 2017), and additionally habitat fragmentation affects them (Sundström & al. 2005, Seppä 2009). The high extinction risk of isolated populations is demonstrated for example in tree-living ant communities where the ant assemblages in isolated trees are sensitive to local extinctions (Gove & al. 2009). Especially the species using strategies of limited gyne dispersal (dependent colony founding, high levels of polygyny, the flightless social parasites) are particularly at risk for facing colonization problems. Although these strategies may be beneficial locally, they can lead to extinction when the local habitat becomes unsuitable. The dispersal abilities and extinction risks of ant taxa using strategies of limited gyne dispersal should be properly assessed.

Even in well dispersing species, search efficiency for suitable habitats may affect population structure. For example, *Lasius neoniger* Emery, 1893 and *Solenopsis molesta* Emery, 1895 gynes were shown to be inefficient in returning to their preferred habitat when displaced, indicating that they cannot search for it effectively (Wilson & Hunt 1966). Ant populations indeed seem to be patchy over large spatial scales, with species often not occurring in locations with suitable habitat (Wilson 1955, Levings 1983). This indicates that ant dispersal is either not strong enough in terms of propagule pressure or not informed enough in terms of their patch-finding ability, to guarantee high occupancy everywhere. Such chance effects might lead to problems in case the suitable habitat becomes rarer.

Community dynamics: Interspecific variation in dispersal has an important role in community dynamics. Island biogeography theories (MacArthur & Wilson 1967, Kadmon & Allouche 2007) predict that more isolated or fragmented habitats are expected to have poorer ant communities. The limited dispersal of many ant species may strengthen such patterns. Brühl & al. (2003) indeed show that in Malaysian rainforest, a bigger continuous forest area has twice as diverse ant community than the fragmented areas, which is rather worrying from conservation perspective.

Ant community research has focused on two main factors: how different environmental conditions shape the communities and what is the role of competitiveness (e.g., Davidson 1980, Levings 1983, Savolainen & Vepsalainen 1988, Andersen 1992, Bestelmeyer 2000). Competitive abilities of the species already present at a location affect the success of new dispersers trying to settle (Vepsäläinen & Pisarski 1982). Colonies in ant communities are often evenly spaced both intra- and interspecifically (Levings & Traniello 1981, Levings & Franks 1982, Chew 1987), demonstrating that new colonies are founded within equal distances from the existing ones in order to minimize competition – or colonies compete until only some survive. In *Myrmecocystus mimicus* Wheeler, 1908 workers of nearby colonies are shown to prevent colony founding (Hölldobler 1981).

In this light, possible correlations between competitiveness and dispersal ability should be influential for the formation of ant communities. Overall, Vepsäläinen & Pisarski (1982) stressed how important the species' dispersal and colony founding characteristics are in the structures of ant communities: Better dispersing species generally reach new areas more easily and might get advantage for early settlement regardless of their competitive abilities, whereas, for example, social parasite species cannot settle in an area where their host species does not already exist.

Also within-species variation in dispersal behavior plays a role in community dynamics (Lowe & McPeek 2014), and since it is rather large in many ant species, and correlates with their social structures, its role in ant communities should be assessed. The dispersing individuals may have different traits or trait values than the more philopatric individuals of the same species and these traits can shape the species communities more than generally appreciated.

Evolutionary transitions in dispersal: We have argued that dispersal is both a social trait and a determinant of the kin structures that create the selective regime for social traits. Thus, dispersal is prone to eco-evolutionary feedbacks. To understand the evolutionary transitions in ant dispersal strategies (Fig. 5), we need to understand how such feedbacks affect the different aspects of dispersal. At the moment, such questions are largely unanswerable due to lack of data on dispersal traits across the whole ant phylogeny. Below, we briefly list some examples of traits and feedbacks that may prove to be important. Our speculation focuses mostly on the kin-selected adaptive consequences. As both species specific idiosyncrasies and broader ecological selection pressures undoubtedly contribute to the variation observed, we stress that the predictions we outline are best tested with observing trends in broad phylogenetic comparisons.

The first major evolutionary step in ant dispersal is the switch from non-claustral to claustral colony founding. While it is easy to see how high risks during the founding stage select for such a strategy (Hölldobler & Wilson 1990), the switch requires a large suite of changes on the metabolism and size of the queens (Brown & Bonhoeffer 2003), accompanied with miniaturization of the workers to reduce the cost of the first worker brood (Peeters & Ito 2015). In order to understand the consequences of such changes, we need to understand correlates of larger resource allocation per queen accompanied with smaller resource allocation per worker, and how they affect further evolution. Possible correlates include further changes in worker sizes and numbers (which could consequently change the ecological status of the species) and changes in mating systems, driven by changes in operative sex

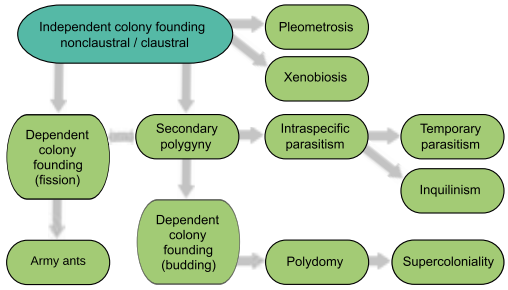

Fig. 5: The proposed evolutionary paths between colony founding strategies (see Tab. 1 for explanations of the terms). The evolutionary pathways from one dispersal and nest founding strategy to another have been widely discussed in the literature (see main text for details and references), but comprehensive phylogenetic analyses have not been made so far. Here, we present a hypothesis for the most likely evolutionary scenario for the switches between strategies. The arrows indicate the evolutionary direction from ancestral to more derived strategy, but also reverting back seems to occur commonly. The ancestral strategy in ants is independent, non-claustral colony founding, from which claustral founding has evolved (Keller & al. 2014). Army ants have arisen from non-claustral ancestors and pleometrotic species are usually claustral, but further analysis is needed to conclusively distinguish which of the two independent nest founding strategies is ancestral to some of the other strategies, and they are therefore grouped here.

ratios. As both reversals to non-claustral founding and intra-specific variation in claustrality occur (Stille 1996, Brown & Bonhoeffer 2003), it seems that claustrality is evolutionarily reversible. An important question in need of empirical and theoretical attention is whether claustral or non-claustral founding is more likely to lead to further transitions to other strategies, such as dependent colony founding.

Pleometrosis is a strategy that usually occurs combined with claustral colony founding (Bernasconi & Strassmann 1999), but is also possible in non-claustral species (e.g., *Pogonomyrmex californicus* (Buckley, 1867) Johnson 2004). This strategy requires synchronized mating flights, and large numbers of individuals to ensure colony founding partners. It remains poorly understood whether pleometrosis is associated with more or less investment per queen at the colony level and how that affects dispersal distances or the population densities. In addition to the ecological correlates for this strategy, we need to understand the selection pressures arising from social interaction among the founding queens and their first workers, such as the effects of honest or dishonest signaling of queen condition and productivity (Rissing & Pollock 1986, Nonacs 1992, Holman & al. 2010).

The next evolutionary steps towards more derived dispersal strategies, dependent colony founding and polygyny, answer similar ecological demands: They are both predicted to evolve when the dispersal risks are high and the colony-founding success is low, for example due to high competition, nest site limitation, habitat patchiness or predation (Bourke & Franks 1995, Cronin & al. 2013, Boomsma & al. 2014). Both of these strategies are very variable, and have evolved several times in vastly different ant taxa, meaning that no single explanation for their evolution is enough. It has been suggested that selection against dispersal may lead to readoption of queens in their natal colonies, which then can lead to the evolution of dependent colony founding (Bourke & Franks 1995, Heinze & Tsuji 1995, Cronin & al. 2013). Polygyny and dependent founding indeed often co-occur (Keller 1991, Cronin & al. 2013). However, this is not a general rule, and a direct causal link between these strategies does not always exist as dependent founding has evolved also independently from polygyny (Cronin & al. 2013). In the case of these strategies, potential feedbacks are less speculative, as local recruitment of queens changes the local kin structures and resource allocation that drive dispersal itself. For the gynes, it may be beneficial to exploit the colony resources instead of taking the risks of dispersal, especially when relatedness is low.

Low success of long-range dispersal leads to selection for limited dispersal and more philopatric behavior in insects and can create evolutionary prospects for morphological and behavioral changes leading to stayer morphs (Harrison 1980, Zera & Denno 1997). In ants, long distance dispersal can in extreme cases disappear completely, as in flightless dependently founding gynes (Peeters 2012) or in some of the supercolonial species (Helanterä & al. 2009). Paradoxically, supercoloniality is a successful dispersal method in continuous habitats – even though the gynes may not be good at dispersing, the colonies spread efficiently by budding. For example, wood ants have colonized Northern Eurasia very fast after the last glaciation (Pamilo & al. 2016). Even more extreme cases can be found in invasive species across their introduced habitats: For example, *Linepithema humile* has spread through the Mediterranean coast as a single supercolony since the 19th century (Giraud & al. 2002, Wetterer & al. 2009). Regardless, both supercoloniality and dependent colony founding lead to colonization problems in fragmented habitats, which may partly contribute to the notion that such lifestyles are evolutionary dead ends. Even though they are beneficial strategies locally and on shorter time scales, and seem to have evolved rather easily in several ant taxa, they do not necessarily survive and radiate on evolutionary time scales (Helanterä & al. 2009, Peeters 2012).

It has been suggested that parasitic nest founding strategies (temporary parasitism and inquilism) are an evolutionary consequence of selection for selfish philopatry within polygynous societies, leading to intraspecific parasitism and after a host shift or speciation, to interspecific parasitism (Buschinger 2009, Boomsma & al. 2014). The third type of social parasitism affecting dispersal evolution, xenobiosis, seems to follow a different evolution-

ary pathway. Xenobiotic species are not closely related to their hosts, and their development is not tied to the host resource allocation, as they take care of their own brood (Buschinger 2009). Dependency on host resources, and possible coevolution with host colony allocation decisions and caste determination, may direct also dispersal evolution of parasitic species. Small size, which is especially common among inquilines (Buschinger 2009), helps deceptive development into queens with the amount of resources the host allocates for the development of its own workers (Nonacs & Tobin 1992). As small queen size likely further selects against independent founding, this should result in the parasites being more strictly dependent on their hosts.

It is clear that all parasite species are somehow restricted in their dispersal because they can only settle on locations where one or more of their host species already live (Vepsäläinen & Pisarski 1982, Buschinger 1986). Especially inquilines and xenobiotic species can be seen as extreme habitat specialists, because the host nests are the only suitable habitat for these species. This is suggested to be a reason why these species have so often lost their flight ability: Dispersal by wing has a high risk of flying to areas without suitable hosts (Brandt & al. 2005). In contrast, temporary parasites have kept their flight ability, and consequently colonization ability, more frequently (Buschinger 2009). Connectivity and continuity of the host populations need to be considered in order to understand the evolution of the parasitic strategies, since all social parasites coevolve with their hosts, but the coevolutionary dynamics may direct the evolution of different strategies to different directions (Brandt & al. 2005).

Conclusions and future directions

We identify four key areas where further research would help to understand the causes and consequences of ant dispersal: comparative analyses on dispersal evolution and life history traits, gene flow analyses with non-biased species sampling, understanding the genetic architecture of the traits relevant for dispersal, and formulating testable theories for ant dispersal.

First, comprehensive data on colony life-history traits are needed for a wide variety of ant taxa, including at least sizes of different castes and resident queen numbers, as well as behavioral data on dispersal and mating. Ideally also details on allocation ratios and individual morphology should be documented. Importantly, these data should be collected for males, too, as they are currently seriously understudied. This dire need for comparative colony life-history data has been identified for a long time (Starr 2006) and the coordinated efforts to build databases have recently given hope for progress (Parr & al. 2017). Phylogenetic comparative analyses combined with environmental data (climate, local communities) have proven insightful in other social evolution contexts, such as understanding the relation between cooperative breeding and habitat harshness (Cornwallis & al. 2017, Griesser & al. 2017). Such analyses would allow teasing apart the crucial preadaptations and possible correlates for the evolution of different dispersal strategies, in addition to understanding the ecological drivers. Also the long-standing hypotheses of certain dispersal strategies as evolutionary dead ends (supercoloniality, dependent colony founding, parasitic strategies) should be subjected to rigorous tests.

Second, as dispersal is a multi-phase process, and the observation of movement does not comprise data on successful gene flow, descriptive population genetic structure data are needed. These data should be collected without the current biases with respect to the life histories of the taxa. While these are labor-intensive data, the increasing cost efficiency of genotyping, and the possibilities of using museum samples (Wandeler & al. 2007) means that this is achievable for a large number of species. Data on the dispersal strategies and environmental conditions should be incorporated in the analysis and studies on larger spatial scales are also needed. Modern landscape genetic methods that do not require identifying discrete populations are a useful option for analyzing this kind of data (Manel & al. 2003, Manel & Holderegger 2013).

Third, experimental and genomics approaches allow further understanding of the basis of dispersal related phenotypes and the potential constraints of adaptation (Saastamoinen & al. 2017). Investigating the relative roles of direct and indirect genetic effects (Linksvayer 2015) on developmental outcomes and behavioral decisions may shed light on how the traits potentially respond to selection. Sequencing approaches complement the picture by allowing to understand the role of plastic gene expression underlying dispersal phenotypes, possible pleiotropic constraints and elements of parallel and lineage specific evolution of the genomic underpinnings of dispersal phenotypes.

Fourth, careful theoretical work on co-evolving traits in ant dispersal is needed to make the most of the comparative data. Models of coevolution of social traits and population structures have demonstrated strong feedbacks. For example, Powers & al. (2011) show that population structure drives social evolution, but also that social behavior affects the population structure and therefore enhances the evolutionary process. Van Dyken & Wade (2012) stress how important it is to consider the connection between the evolution and the ecology of social behavior, when studying the evolution of different altruistic strategies. Similar dynamics of social niche construction (Ryan & al. 2016) are likely at work in the dispersal evolution of ants. Also the possible social conflicts over dispersal among multiple actors in the colonies should be incorporated in the future models on ant dispersal. Similarly to models of sexual selection and sexual conflict (Chapman & al. 2003), traits of one class of individuals are the key selective pressures to the other class. Testable models of sex-biased dispersal in ants, in connection to evolution of mate location, have potential to illuminate some of the open questions of the field, especially since empirical tests of kin-selection based theories of sex-biased dispersal remain surprisingly scarce overall (Li & Kokko in press).

Considering the ecological importance of ants, it is surprising how little is still known of their dispersal and colonization behavior. Even though ants are seemingly robust and numerous, their effective population sizes are often very small, which makes them more vulnerable than one might think (Seppä 2009). In this light, understanding ant dispersal has direct conservation relevance in environments undergoing rapid human induced change. In order to understand vulnerability of ant populations, dispersal is a key process at the intersection of behavior and population dynamics. To understand dispersal, we need to understand its ecological context, individual level determinants and evolutionary history.

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References

Alcock, J., Barrows, E.M., Gordh, G., Hubbard, L.J., Kirkendall, L., Pyle, D.W., Ponder, T.L. & Zalom, F. 1978: The ecology and evolution of male reproductive behaviour in the bees and wasps. – Zoological Journal of the Linnean Society 64: 293-326.

Alexander, R.D. & Sherman, P.W. 1977: Local mate competition and parental investment in social insects. – Science 194: 494-500.

Andersen, A.N. 1992: Regulation of "momentary" diversity by dominant species in exceptionally rich ant communities of the Australian seasonal tropics. – The American Naturalist 140: 401-420.

Aron, S., Passera, L. & Keller, L. 1999: Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. – Proceedings of the Royal Society B-Biological Sciences 266: 173-177.

Baer, B. & Boomsma, J.J. 2006: Mating biology of the leaf-cutting ants *Atta colombica* and *A. cephalotes*. – Journal of Morphology 267: 1165-1171.

Balloux, F. & Lugon-Moulin, N. 2002: The estimation of population differentiation with microsatellite markers. – Molecular Ecology 11: 155-165.

Bargum, K., Helanterä, H. & Sundström, L. 2007: Genetic population structure, queen supersedure and social polymorphism in a social Hymenoptera. – Journal of Evolutionary Biology 20: 1351-1360.

Barth, M.B., Moritz, R.F.A., Pirk, C.W.W. & Kraus, F.B. 2013: Male-biased dispersal promotes large scale gene flow in a subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*. – Population Ecology 55: 523-533.

Beekman, M. & Ratnieks, F.L.W. 2003: Power over reproduction in social Hymenoptera. – Philosophical Transactions of the Royal Society B-Biological Sciences 358: 1741-1753.

Bengtsson, B.O. 1978: Avoiding inbreeding: at what cost? – Journal of Theoretical Biology 73: 439-444.

Berghoff, S.M., Kronauer, D.J.C., Edwards, K.J. & Franks, N.R. 2008: Dispersal and population structure of a New World predator, the army ant *Eciton burchellii*. – Journal of Evolutionary Biology 21: 1125-1132.

Bernasconi, G. & Strassmann, J.E. 1999: Cooperation among unrelated individuals: the ant foundress case. – Trends in Ecology & Evolution 14: 477-482.

Bestelmeyer, B.T. 2000: The trade-off between thermal tolerance and behavioural dominance in a subtropical South American ant community. – Journal of Animal Ecology 69: 998-1009.

Bolton, B. 1986: Apterous females and shift of dispersal strategy in the *Monomorium salomonis*-group (Hymenoptera: Formicidae). – Journal of Natural History 20: 267-272.

Bonte, D., Dyck, H. Van, Bullock, J.M., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., Schtickzelle, N., Stevens, V.M., Vandewoestijne, S., Baguette, M., Barton, K., Benton, T.G., Chaput-bardy, A., Dytham, C., Hovestadt, T., Meier, C.M., Steve, C.F., Turlure, C. & Travis, J.M.J. 2012: Costs of dispersal. – Biological Reviews 87: 290-312.

Boomsma, J.J. 2013: Beyond promiscuity : mate-choice commitments in social breeding. – Philosophical Transactions of the Royal Society B-Biological Sciences 368: art. 20120050.

Boomsma, J.J. & Grafen, A. 1990: Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. – Evolution 44: 1026-1034.

Boomsma, J.J. & Grafen, A. 1991: Colony-level sex ratio selection in the eusocial Hymenoptera. – Journal of Evolutionary Biology 3: 383-407.

Boomsma, J.J., Baer, B. & Heinze, J. 2005: The evolution of male traits in social insects. – Annual Review of Entomology 50: 395-420.

Boomsma, J.J. & Have, T.M. Van Der 1998: Queen mating and paternity variation in the ant *Lasius niger*. – Molecular Ecology 7: 1709-1718.

Boomsma, J.J., Huszár, D.B. & Pedersen, J.S. 2014: The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. – Animal Behaviour 92: 241-252.

Bouchet, D.C., Peeters, C., Fisher, B.L. & Molet, M. 2013: Both female castes contribute to colony emigration in the polygynous ant *Mystrium oberthueri*. – Ecological Entomology 38: 408-417.

Bourke, A.F.G. 1987: Alternative reproductive strategies in workers of the slavemaking ant *Harpagoxenus sublaevis*. In: Eder, J. & Rembold, H. (Eds.): Chemistry and biology of social insects. – Verlag J. Peperny, München, pp. 259.

Bourke, A.F.G. & Franks, N.R. 1995: Social evolution in ants. – Princeton University Press, Princeton, NJ, 529 pp.

Bowler, D.E. & Benton, T.G. 2004: Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. – Biological Reviews 80: 205-225.

Brady, S.G. 2003: Evolution of the army ant syndrome: the origin and long-term evolutionary stasis of a complex of behavioral and reproductive adaptations. – Proceedings of the National Academy of Sciences of the United States of America 100: 6575-6579.

Brady, S.G., Schultz, T.R., Fisher, B.L. & Ward, P.S. 2006: Evaluating alternative hypotheses for the early evolution and diversification of ants. – Proceedings of the National Academy of Sciences of the United States of America 103: 18172-18177.

Brandt, M., Foitzik, S., Fischer-Blass, B. & Heinze, J. 2005: The coevolutionary dynamics of obligate ant social parasite systems – between prudence and antagonism. – Biological Reviews of the Cambridge Philosophical Society 80: 251-267.

Brown, M.J.F. & Bonhoeffer, S. 2003: On the evolution of claustral colony-founding in ants. – Evolutionary Ecology Research 5: 305-313.

Brühl, C.A., Eltz, T. & Linsenmair, K.E. 2003: Size does matter – effects of tropical rainforest fragmentation on the leaf litter ant community in Sabah, Malaysia. – Biodiversity and Conservation 12: 1371-1389.

Burgess, S.C., Baskett, M.L., Grosberg, R.K., Morgan, S.G. & Strathmann, R.R. 2016: When is dispersal for dispersal? Unifying marine and terrestrial perspectives. – Biological Reviews of the Cambridge Philosophical Society 91: 867-882.

Buschinger, A. 1986: Evolution of social parasitism in ants. – Trends in Ecology & Evolution 1: 155-160.

Buschinger, A. 1989: Evolution, speciation, and inbreeding in the parasitic genus *Epimyrma* (Hymenoptera, Formicidae). – Journal of Evolutionary Biology 2: 265-283.

Buschinger, A. 2009: Social parasitism among ants: a review (Hymenoptera: Formicidae). – Myrmecological News 12: 219-235.

Chapman, T., Arnqvist, G., Bangham, J. & Rowe, L. 2003: Sexual conflict. – Trends in Ecology & Evolution 18: 41-47.

Chew, R. 1987: Population dynamics of colonies of three species of ants in desertified grassland, Southeastern Arizona, 1958-1981. – The American Midland Naturalist 118: 177-188.

Clobert, J., Galliard, J.-F. Le, Cote, J., Meylan, S. & Massot, M. 2009: Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. – Ecology Letters 12: 197-209.

Collingwood, C.A. 1979: The Formicidae (Hymenoptera) of Fennoscandia and Denmark. – Scandinavian Science Press, Klampenborg, 175 pp.

Cook, J.M. 1993: Sex determination in the Hymenoptera : a review of models and evidence. – Heredity 71: 421-435.

Cook, J.M. & Crozier, R.H. 1995: Sex determination and population biology in the Hymenoptera. – Trends in Ecology & Evolution 10: 281-286.

Cornwallis, C.K., Botero, C.A., Rubenstein, D.R., Downing, P.A., West, S.A. & Griffin, A.S. 2017: Cooperation facilitates the colonization of harsh environments. – Nature Ecology & Evolution 1: art. 0057.

Cremer, S. & Heinze, J. 2003: Stress grows wings: environmental induction of winged dispersal males in *Cardiocondyla* ants. – Current Biology 13: 219-223.

Cronin, A.L., Molet, M., Doums, C., Monnin, T. & Peeters, C. 2013: Recurrent evolution of dependent colony foundation across eusocial insects. – Annual Review of Entomology 58: 37-55.

Crozier, R.H. & Pamilo, P. 1996: Evolution of social insect colonies: sex allocation and kin selection. – Oxford University Press, Oxford, UK, 306 pp.

Davidson, D.W. 1980: Some consequences of diffuse competition in a desert ant community. – The American Naturalist 116: 92-105.

Debout, G., Schatz, B., Elias, M. & Mckey, D. 2007: Polydomy in ants: what we know, what we think we know, and what remains to be done. – Biological Journal of the Linnean Society 90: 319-348.

Dornhaus, A., Franks, N.R., Hawkins, R.M. & Shere, H.N.S. 2004: Ants move to improve: Colonies of *Leptothorax albipennis* emigrate whenever they find a superior nest site. – Animal Behaviour 67: 959-963.

Dyck, H. Van & Baguette, M. 2005: Dispersal behaviour in fragmented landscapes: routine or special movements? – Basic and Applied Ecology 6: 535-545.

Dyken, J.D. Van & Wade, M.J. 2012: Origins of altruism diversity I: The diverse ecological roles of altruistic strategies and their evolutionary responses to local competition. – Evolution 66: 2484-2497.

Ellis, S. & Robinson, E.J.H. 2014: Polydomy in red wood ants. – Insectes Sociaux 61: 111-122.

Evans, J.D. 1996: Queen longevity, queen adoption, and posthumous indirect fitness in the facultatively polygynous ant *Myrmica tahoensis*. – Behavioral Ecology and Sociobiology 39: 275-284.

Fahrig, L. 2001: How much habitat is enough? – Biological Conservation 100: 65-74.

Fjerdingstad, E.J. 2005: Control of body size of *Lasius niger* ant sexuals – worker interests, genes and environment. – Molecular Ecology 14: 3123-3132.

Foitzik, S., Bauer, S., Laurent, S. & Pennings, P.S. 2009: Genetic diversity, population structure and sex-biased dispersal in three co-evolving species. – Journal of Evolutionary Biology 22: 2470-2480.

Fortelius, W., Pamilo, P., Rosengren, R. & Sundström, L. 1987: Male size dimorphism and alternative reproductive tactics in *Formica exsecta* ants (Hymenoptera, Formicidae). – Annales Zoologici Fennici 24: 45-54.

Fowler, H.G., Alves, L.E. & Bueno, O.C. 1993: Reproductive strategies of the exotic Pharaoh's ant, *Monomorium pharaonis* (L.) (Hymenoptera: Formicidae) in Brazil. – Invertebrate Reproduction and Development 23: 235-238.

Giraud, T., Pedersen, J.S. & Keller, L. 2002: Evolution of supercolonies: the Argentine ants of southern Europe. – Proceedings of the National Academy of Sciences of the United States of America 99: 6075-6079.

Gordon, D.M. 1995: The development of an ant colony's foraging range. – Animal Behaviour 49: 649-659.

Gordon, D.M. & Wagner, D. 1999: Colony age, neighborhood density and reproductive potential in harvester ants. – Oecologia 109: 556-560.

Gove, A.D., Majer, J.D. & Rico-Gray, V. 2009: Ant assemblages in isolated trees are more sensitive to species loss and replacement than their woodland counterparts. – Basic and Applied Ecology 10: 187-195.

Greenwood, P.J. 1980: Mating systems, philopatry and dispersal in birds and mammals. – Animal Behaviour 28: 1140-1162.

Griesser, M., Drobniak, S.M., Nakagawa, S. & Botero, C.A. 2017: Family living sets the stage for cooperative breeding and ecological resilience in birds. – Public Library of Sciences Biology 15: art. e2000483.

Gros, A., Hovestadt, T. & Poethke, H.J. 2008: Evolution of sex-biased dispersal: the role of sex-specific dispersal costs, demographic stochasticity, and inbreeding. – Ecological Modelling 219: 226-233.

Guénard, B., Shik, J.Z., Booher, D., Lubertazzi, D. & Alpert, G. 2016: Extreme polygyny in the previously unstudied subtropical ant *Temnothorax tuscaloosae* with implications for the biogeographic study of the evolution of polygyny. – Insectes Sociaux 63: 543-551.

Haag-Liautard, C., Vitikainen, E., Keller, L. & Sundström, L. 2009: Fitness and the level of homozygosity in a social insect. – Journal of Evolutionary Biology 22: 134-142.

Hamaguchi, K. & Kinomura, K. 1996: Queen-size dimorphism in the facultatively polygynous ant *Leptothorax spinosior* (Hymenoptera: Formicidae). – Sociobiology 27: 241-251.

Hamilton, W. & May, R. 1977: Dispersal in stable habitats. – Nature 269: 578-581.

Hammond, R.L., Bourke, A.F.G. & Bruford, M.W. 2001: Mating frequency and mating system of the polygynous ant, *Leptothorax acervorum*. – Molecular Ecology 10: 2719-2728.

Hanski, I. 1999: Metapopulation ecology. – Oxford University Press, Oxford, UK, 313 pp.

Härkönen, S.K. & Sorvari, J. 2017: Effect of host species, host nest density and nest size on the occurrence of the shining guest ant *Formicoxenus nitidulus* (Hymenoptera: Formicidae). – Journal of Insect Conservation 21: 477-485.

Harrison, R.G. 1980: Dispersal polymorphisms in insects. – Annual Review of Ecology and Systematics 11: 95-118.

Heinze, J. 2007: The demise of the standard ant (Hymenoptera: Formicidae). – Myrmecological News 11: 9-20.

Heinze, J. & Buschinger, A. 1989: Queen polymorphism in a non-parasitic *Leptothorax* species (Hymenoptera: Formicidae). – Insectes Sociaux 36: 139-155.

Heinze, J., Hölldobler, B. & Yamauchi, K. 1998: Male competition in *Cardiocondyla* ants. – Behavioral Ecology and Sociobiology 42: 239-246.

Heinze, J. & Smith, T.A. 1990: Dominance and fertility in a functionally monogynous ant. – Behavioral Ecology and Sociobiology 27: 1-10.

Heinze, J. & Tsuji, K. 1995: Ant reproductive strategies. – Researches on Population Ecology 37: 135-149.

Helanterä, H. 2016: An organismal perspective on the evolution of insect societies. – Frontiers in Ecology and Evolution 4: art. 6.

Helanterä, H., Strassmann, J.E., Carrillo, J. & Queller, D.C. 2009: Unicolonial ants: Where do they come from, what are they and where are they going? – Trends in Ecology & Evolution 24: 341-349.

Helms IV, J.A. 2018: The flight ecology of ants (Hymenoptera: Formicidae). – Myrmecological News 26: 19-30.

Helms, J.A. & Bridge, E.S. 2017: Range expansion drives the evolution of alternate reproductive strategies in invasive fire ants. – NeoBiota 33: 67-82.

Helms, J.A. & Godfrey, A. 2016: Dispersal polymorphisms in invasive fire ants. – Public Library of Science One 11: art. e0153955.

Helms, J.A. & Kaspari, M. 2014: Found or Fly: Nutrient loading of dispersing ant queens decreases metrics of flight ability (Hymenoptera: Formicidae). – Myrmecological News 19: 85-91.

Helms, J.A. & Kaspari, M. 2015: Reproduction-dispersal tradeoffs in ant queens. – Insectes Sociaux 62: 171-181.

Hölldobler, B. 1981: Foraging and spatiotemporal territories in the honey ant *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae). – Behavioral Ecology and Sociobiology 9: 301-314.

Hölldobler, B. & Bartz, H.S. 1985: Sociobiology of reproduction in ants. In: Hölldobler, B. (Ed.): Experimental behavioral ecology and sociobiology. – Gustav Fischer Verlag, Stuttgart, pp. 237-257.

Hölldobler, B. & Wilson, E.O. 1977: The number of queens: an important trait in ant evolution. – Naturwissenschaften 64: 8-15.

Hölldobler, B. & Wilson, E.O. 1990: The ants. – The Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

Hölldobler, B. & Wilson, E.O. 2008: The super-organism: the beauty, elegance, and strangeness of insect societies. – W.W. Norton & Company, New York, NY, 544 pp.

Holman, L., Dreier, S. & D'Ettorre, P. 2010: Selfish strategies and honest signalling: reproductive conflicts in ant queen associations. – Proceedings of the Royal Society B-Biological Sciences 277: 2007-2015.

Inoue, M.N., Ito, F. & Goka, K. 2015: Queen execution increases relatedness among workers of the invasive Argentine ant, *Linepithema humile*. – Ecology and Evolution 5: 4098-4107.

Johansson, H., Seppä, P., Helanterä, H., Trontti, K. & Sundström, L. 2018: Weak population structure in the ant *Formica fusca*. – PeerJ 6: art. e5024.

Johnson, R.A. 2004: Colony founding by pleometrosis in the semiclaustral seed-harvester ant *Pogonomyrmex californicus* (Hymenoptera: Formicidae). – Animal Behaviour 68: 1189-1200.

Johnson, R.A. 2010: Independent colony founding by ergatoid queens in the ant genus *Pogonomyrmex*: Queen foraging provides an alternative to dependent colony founding. – Insectes Sociaux 57: 169-176.

Kadmon, R. & Allouche, O. 2007: Integrating the effects of area, isolation, and habitat heterogeneity on species diversity: a unification of island biogeography and niche theory. – The American Naturalist 170: 443-454.

Kannowski, P.B. & Johnson, R.L. 1969: Male patrolling behaviour and sex attraction in ants of the genus *Formica*. – Animal Behaviour 17: 425-429.

Kaspari, M., Pickering, J., Longino, J. & Windsor, D. 2001: The phenology of a Neotropical ant assemblage: evidence for continuous and overlapping reproduction. – Behavioral Ecology and Sociobiology 50: 382-390.

Keller, L. 1991: Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera Formicidae). – Ethology Ecology & Evolution 3: 307-316.

Keller, L. 1993: The assessment of reproductive success of queens in ants and other social insects. – Oikos 67: 177-180.

Keller, L. 1995: Social life: The paradox of multiple-queen colonies. – Trends in Ecology & Evolution 10: 355-360.

Keller, L. & Passera, L. 1990: Fecundity of ant queens in relation to their age and the mode of colony founding. – Insectes Sociaux 37: 116-130.

Keller, L., Passera, L. & Suzzoni, J.-P. 1989: Queen execution in the Argentine ant, *Iridomyrmex humilis*. – Physiological Entomology 14: 157-163.

Keller, L. & Ross, K.G. 1993: Phenotypic plasticity and "cultural transmission" of alternative social organizations in the fire ant *Solenopsis invicta*. – Behavioral Ecology and Sociobiology 33: 121-129.

Keller, R.A., Peeters, C. & Beldade, P. 2014: Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. – eLife 3: art. e01539.

Kokko, H. 2003: Are reproductive skew models evolutionarily stable? – Proceedings of the Royal Society B-Biological Sciences 270: 265-270.

Kokko, H. & Lundberg, P. 2001: Dispersal, migration, and offspring retention in saturated habitats. – The American Naturalist 157: 188-202.

Krieger, M.J.B. & Ross, K.G. 2002: Identification of a major gene regulating complex social behavior. – Science 295: 328-332.

Kronauer, D.J.C. 2009: Recent advances in army ant biology (Hymenoptera: Formicidae). – Myrmecological News 12: 51-65.

Kümmerli, R., Gardner, A., West, S. & Griffin, A. 2009: Limited dispersal, budding dispersal, and cooperation: an experimental study. – Evolution 63: 939-949.

Kümmerli, R. & Keller, L. 2007: Reproductive specialization in multiple-queen colonies of the ant *Formica exsecta*. – Behavioral Ecology 18: 375-383.

Lehmann, L., Ravigne, V. & Keller, L. 2008: Population viscosity can promote the evolution of altruistic sterile helpers and eusociality. – Proceedings of the Royal Society B-Biological Sciences 275: 1887-1895.

Lenoir, A., Quérard, L., Pondicq, N. & Berton, F. 1988: Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). – Psyche 95: 21-44.

Lenoir, J.C., Schrempf, A., Lenoir, A., Heinze, J. & Mercier, J.L. 2007: Genetic structure and reproductive strategy of the ant *Cardiocondyla elegans*: strictly monogynous nests invaded by unrelated sexuals. – Molecular Ecology 16: 345-354.

Leppänen, J., Seppä, P., Vepsäläinen, K. & Savolainen, R. 2015: Genetic divergence between the sympatric queen morphs of the ant *Myrmica rubra*. – Molecular Ecology 24: 2463-2476.

Levings, S.C. 1983: Seasonal, annual, and among-site variation in the ground ant community of a deciduous tropical forest: some causes of patchy species distributions. – Ecological Monographs 53: 435-455.

Levings, S.C. & Franks, N.R. 1982: Patterns of nested dispersion in a tropical ground ant community. – Ecology 63: 338-344.

Levings, S.C. & Traniello, J.F.A. 1981: Territoriality, nest dispersion, and community structure in ants. – Psyche 88: 265-319.

Li, X. & Kokko, H. in press: Sex-biased dispersal: a review of the theory. – Biological Reviews; doi: 10.1111/brv.12475.

Libbrecht, R., Oxley, P.R., Kronauer, D.J.C. & Keller, L. 2013: Ant genomics sheds light on the molecular regulation of social organization. – Genome Biology 14: art. 212.

Linksvayer, T.A. 2006: Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. – Evolution 60: 2552-2561.

Linksvayer, T.A. 2015: The molecular and evolutionary genetic implications of being truly social for the social insects. – Advances in Insect Physiology 48: 271-292.

Lowe, W.H. & McPeek, M.A. 2014: Is dispersal neutral? – Trends in Ecology & Evolution 29: 444-450.

MacArthur, R.H. & Wilson, E.O. 1967: The theory of island biogeography. – Princeton University Press, Princeton, NJ, 203 pp.

Manel, S. & Holderegger, R. 2013: Ten years of landscape genetics. – Trends in Ecology & Evolution 28: 614-621.

Manel, S., Schwartz, M.K., Luikart, G. & Taberlet, P. 2003: Landscape genetics: combining landscape ecology and population genetics. – Trends in Ecology & Evolution 18: 189-197.

Marden, J.H. 1987: Maximum lift production during takeoff in flying animals. – Journal of Experimental Biology 130: 235-238.

Marden, J.H. 2000: Variability in the size, composition, and function of insect flight muscles. – Annual Review of Physiology 62: 157-178.

Marshall, D.J. & Morgan, S.G. 2011: Ecological and evolutionary consequences of linked life-history stages in the sea. – Current Biology 21: R718-R725.

Mathias, A., Kisdi, E. & Olivieri, I. 2001: Divergent evolution of dispersal in a heterogeneous landscape. – Evolution 55: 246-259.

McGlynn, T.P. 2012: The ecology of nest movement in social insects. – Annual Review of Entomology 57: 291-308.

Meunier, J., West, S.A. & Chapuisat, M. 2008: Split sex ratios in the social Hymenoptera: A meta-analysis. – Behavioral Ecology 19: 382-390.

Miyakawa, M.O. & Mikheyev, A.S. 2015: QTL mapping of sex determination loci supports an ancient pathway in ants and honey bees. – Public Library of Sciences Genetics 11: art. e1005656.

Mlot, N.J., Tovey, C.A. & Hu, D.L. 2011: Fire ants self-assemble into waterproof rafts to survive floods. – Proceedings of the National Academy of Sciences of the United States of America 108: 7669-7673.

Molet, M., Baalen, M. Van & Peeters, C. 2008: Shift in colonial reproductive strategy associated with a tropical-temperate gradient in Rhytidoponera ants. – The American Naturalist 172: 75-87.

Motro, U. 1983: Optimal rates of dispersal. III. Parent-offspring conflict. – Theoretical Population Biology 23: 159-168.

Motro, U. 1991: Avoiding inbreeding and sibling competition: the evolution of sexual dimorphism for dispersal. – The American Naturalist 137: 108-115.

Nakano, H., Hibino, T., Hara, Y., Oji, T. & Amemiya, S. 2002: The behavior and the morphology of sea lilies with shortened stalks: implications on the evolution of feather stars. – Zoological Science 19: 961-964.

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. & Smouse, P.E. 2008: A movement ecology paradigm for unifying organismal movement research. – Proceedings of the National Academy of Sciences of the United States of America 105: 19052-19059.

Nonancs, P. 1986: Ant reproductive strategies and sex allocation theory. – The Quarterly Review of Biology 61: 1-21.

Nonacs, P. 1992: Queen condition and alate density affect pleometrosis in the ant *Lasius pallitarsis*. – Insectes Sociaux 39: 3-13.

Nonancs, P. & Tobin, J. 1992: Selfish larvae: development and the evolution of parasitic behavior in the Hymenoptera. – Evolution 46: 1605-1620.

Olivieri, I., Michalakis, Y. & Gouyon, P.-H. 1995: Metapopulation genetics and the evolution of dispersal. – The American Naturalist 146: 202-228.

Oster, G.F. & Wilson, E.O. 1978: Caste and ecology in the social insects. – Princeton University Press, Princeton, NJ, 352 pp.

Pamilo, P. 1990: Sex allocation and queen worker conflict in polygynous ants. – Behavioral Ecology and Sociobiology 27: 31-36.

Pamilo, P. 1991a: Evolution of colony characteristics in social insects. I. Sex allocation. – The American Naturalist 137: 83-107.

Pamilo, P. 1991b: Evolution of colony characteristics in social insects. II. Number of reproductive individuals. – The American Naturalist 138: 412-433.

Pamilo, P. 1991c: Life span of queens in the ant *Formica exsecta*. – Insectes Sociaux 38: 111-119.

Pamilo, P. & Rosengren, R. 1983: Sex ratio strategies in Formica ants. –Oikos 40: 24-35.

Pamilo, P., Seppä, P. & Helanterä, H. 2016: Population genetics of wood ants. In: Stockan, J. & Robinson, E. (Eds.): Wood ant ecology and conservation (ecology, biodiversity and conservation). – Cambridge University Press, MA, 304 pp.

Parr, C.L., Dunn, R.R., Sanders, N.J., Weiser, M.D., Photakis, M., Bishop, T.R., Fitzpatrick, M.C., Arnan, X., Baccaro, F., Brandão, C.R.F., Chick, L., Donoso, D.A., Fayle, T.M., Gómez, C., Grossman, B., Munyai, T.C., Pacheco, R., Retana, J., Robinson, A., Sagata, K., Silva, R.R., Tista, M., Vasconcelos, H., Yates, M. & Gibb, H. 2017: GlobalAnts: a new database on the geography of ant traits (Hymenoptera: Formicidae). – Insect Conservation and Diversity 10: 5-20.

Passera, L. & Keller, L. 1992: The period of sexual maturation and the age at mating in *Iridomyrmex humilis*, an ant with intranidal mating. – Journal of Zoology 228: 141-153.

Passera, L. & Keller, L. 1993: Mate availability and male dispersal in the Argentine ant *Linepithema humile* (Mayr) (=Iridomyrmex humilis). – Animal Behaviour 48: 361-369.

Pearcy, M., Goodisman, M.A.D. & Keller, L. 2011: Sib mating without inbreeding in the longhorn crazy ant. – Proceedings of the Royal Society B-Biological Sciences 278: 2677-2681.

Pedersen, J.S. & Boomsma, J.J. 1998: Direct genetic evidence for local mate competition in ants. – Naturwissenschaften 85: 593-595.

Pedersen, J.S., Krieger, M.J.B., Vogel, V., Giraud, T. & Keller, L. 2006: Native supercolonies of unrelated individuals in the invasive argentine ant. – Evolution 60: 782-791.

Peeters, C. 1997: Morphologically "primitive" ants: comparative review of social characters, and the importance of queen-worker dimorphism. In: Choe, J. & Crespi, P. (Eds.): The evolution of social behaviour in insects and arachnids. – Cambridge University Press, Cambridge, MA, 541 pp.

Peeters, C. 2012: Convergent evolution of wingless reproductives across all subfamilies of ants, and sporadic loss of winged queens (Hymenoptera: Formicidae). – Myrmecological News 16: 75-91.

Peeters, C. & Aron, S. 2017: Evolutionary reduction of female dispersal in *Cataglyphis* desert ants. – Biological Journal of the Linnean Society 122: 58-70.

Peeters, C. & Ito, F. 2001: Colony dispersal and the evolution of queen morphology in social Hymenoptera. – Annual Review of Entomology 46: 601-630.

Peeters, C. & Ito, F. 2015: Wingless and dwarf workers underlie the ecological success of ants (Hymenoptera: Formicidae). – Myrmecological News 21: 117-130.

Peeters, C. & Molet, M. 2009: Colonial reproduction and life histories. In: Lach, L., Parr, C. & Abbott, K. (Eds.): Ant ecology. – Oxford University Press, Oxford, UK, pp. 159-176.

Perrin, N. & Goudet, J. 2001: Inbreeding, kinship, and the evolution of natal dispersal. In: Clobert, J., Danchin, E., Dhondt, A.A. & Nichols, J.D. (Eds.): Dispersal. – Oxford University Press, Oxford, UK, 480 pp.

Pfennig, D.W., Wund, M.A., Snell-rood, E.C., Cruickshank, T., Schlichting, C.D. & Moczek, A.P. 2010: Phenotypic plasticity's impacts on diversification and speciation. – Trends in Ecology & Evolution 25: 459-467.

Poethke, H.J. & Hovestadt, T. 2002: Evolution of density-and patch-size-dependent dispersal rates. – Proceedings of the Royal Society B-Biological Sciences 269: 637-645.

Poethke, H.J., Pfenning, B. & Hovestadt, T. 2007: The relative contribution of individual and kin selection to the evolution of density-dependent dispersal rates. – Evolutionary Ecology Research 9: 41-50.

Powers, S.T., Penn, A.S. & Watson, R.A. 2011: The concurrent evolution of cooperation and the population structures that support it. – Evolution 65: 1527-1543.

Pressey, R.L., Cabeza, M., Watts, M.E., Cowling, R.M. & Wilson, K.A. 2007: Conservation planning in a changing world. – Trends in Ecology & Evolution 22: 583-592.

Purcell, J., Brelsford, A., Wurm, Y., Perrin, N. & Chapuisat, M. 2014: Convergent genetic architecture underlies social organization in ants. – Current Biology 24: 2728-2732.

Pusey, A.E. 1987: Sex-biased dispersal and inbreeding avoidance in birds and mammals. – Trends in Ecology & Evolution 2: 295-299.

Ratnieks, F.L.W., Foster, K.R. & Wenseleers, T. 2006: Conflict resolution in insect societies. – Annual Review of Entomology 51: 581-608.

Ratnieks, F.L.W. & Reeve, H.K. 1992: Conflict in single-queen hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. – Journal of Theoretical Biology 158: 33-65.

Rissing, S.W. & Pollock, G.B. 1986: Social interaction among pleometrotic queens of *Veromessor pergandei* (Hymenoptera: Formicidae) during colony foundation. – Animal Behaviour 34: 226-233.

Ronce, O. 2007: How does it feel to be like a rolling stone? Ten questions about dispersal evolution. – Annual Review of Ecology, Evolution, and Systematics 38: 231-253.

Rosengren, R. & Pamilo, P. 1983: The evolution of polygyny and polydomy in mound-building Formica ants. – Acta Entomologica Fennica 42: 65-77.

Rosengren, R., Sundström, L. & Fortelius, W. 1993: Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactic. In: Keller, L. (Ed.): Queen sociality in insects. – Oxford Science Publications, Oxford, UK, pp. 308-333.

Ross, K.G. 1988: Differential reproduction in multiple-queen colonies of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). – Behavioral Ecology and Sociobiology 23: 341-355.

Ross, K.G. & Keller, L. 1998: Genetic control of social organization in an ant. – Proceedings of the National Academy of Sciences of the United States of America 95: 14232-14237.

Ross, K.G., Krieger, M.J.B., Shoemaker, D.D., Vargos, E.L. & Keller, L. 1997: Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. – Genetics 147: 643-655.

Rüppell, O. & Heinze, J. 1999: Alternative reproductive tactics in females: the case of size polymorphism in winged ant queens. – Insectes Sociaux 46: 6-17.

Rüppell, O., Heinze, J. & Hölldobler, B. 2001: Alternative reproductive tactics in the queen-size-dimorphic ant *Leptothorax rugatulus* (Emery) and their consequences for genetic population structure. – Behavioral Ecology and Sociobiology 50: 189-197.

Ryan, P.A., Powers, S.T. & Watson, R.A. 2016: Social niche construction and evolutionary transitions in individuality. – Biology and Philosophy 31: 59-79.

Saastamoinen, M., Bocedi, G., Cote, J., Legrand, D., Guillaume, F., Wheat, C.W., Fronhofer, E.A., Garcia, C., Henry, R., Husby, A., Baguette, M., Bonte, D., Coulon, A., Kokko, H., Matthysen, E., Niitepõld, K., Nonaka, E., Stevens, V.M., Travis, J.M.J., Donohue, K., Bullock, J.M. & Mar Delgado, M. del 2017: Genetics of dispersal. – Biological Reviews 358: 574-599.

Sanetra, M. & Crozier, R.H. 2003: Patterns of population subdivision and gene flow in the ant *Nothomyrmecia macrops* reflected in microsatellite and mitochondrial DNA markers. – Molecular Ecology 12: 2281-2295.

Sanllorente, O., Ruano, F. & Tinaut, A. 2015: Large-scale population genetics of the mountain ant *Proformica longiseta* (Hymenoptera: Formicidae). – Population Ecology 57: 637-648.

Savolainen, R. & Vepsalainen, K. 1988: A competition hierarchy among boreal ants: impact on resource partitioning and community structure. – Oikos 51: 135-155.

Schneirla, T.C. 1971: Army ants, a study in social organization. – W.H. Freeman and Company, San Francisco, CA, 344 pp.

Schrempf, A., Aron, S. & Heinze, J. 2006: Sex determination and inbreeding depression in an ant with regular sib-mating. – Heredity 97: 75-80.

Schrempf, A., Reber, C., Tinaut, A. & Heinze, J. 2005: Inbreeding and local mate competition in the ant *Cardiocondyla batesii*. – Behavioral Ecology and Sociobiology 57: 502-510.

Schultner, E., Oettler, J. & Helanterä, H. 2017: The role of brood in eusocial Hymenoptera. – The Quarterly Review of Biology 92: 39-78.

Seppä, P. 1996: Genetic relatedness and colony structure in polygynous *Myrmica* ants. – Ethology Ecology and Evolution 8: 279-290.

Seppä, P. 2009: Do ants (Hymenoptera: Formicidae) need conservation and does ant conservation need genetics? – Myrmecological News 11: 161-172.

Seppä, P., Fernández-Escudero, I., Gyllenstrand, N. & Pamilo, P. 2006: Obligatory female philopatry affects genetic population structure in the ant *Proformica longiseta*. – Insectes Sociaux 53: 362-368.

Seppä, P. & Pamilo, P. 1995: Gene flow and population viscosity in *Myrmica* ants. – Heredity 74: 200-209.

Shik, J.Z., Donoso, D.A. & Kaspari, M. 2013: The life history continuum hypothesis links traits of male ants with life outside the nest. – Entomologia Experimentalis et Applicata 149: 99-109.

Shik, J.Z., Flatt, D., Kay, A. & Kaspari, M. 2012: A life history continuum in the males of a neotropical ant assemblage: Refuting the sperm vessel hypothesis. – Naturwissenschaften 99: 191-197.

Shik, J.Z. & Kaspari, M. 2009: Lifespan in male ants linked to mating syndrome. – Insectes Sociaux 56: 131-134.

Smallwood, J. 1982: Nest relocations in ants. – Insectes Sociaux 29: 138-147.

Soare, T.W., Kumar, A., Naish, K.A. & O'Donnell, S. 2014: Genetic evidence for landscape effects on dispersal in the army ant *Eciton burchellii*. – Molecular Ecology 23: 96-109.

Sommer, K. & Hölldobler, B. 1995: Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. – Animal Behaviour 50: 287-294.

Starr, C.K. 2006: Steps toward a general theory of the colony cycle in social insects. In: Kipyatkov, V.E. (Ed.): Life cycles in social insects behavior, ecology and evolution. – St. Petersburg University Press, St. Petersburg, pp. 1-20.

Starrfelt, J. & Kokko, H. 2010: Parent-offspring conflict and the evolution of dispersal distance. – The American Naturalist 175: 38-49.

Starrfelt, J. & Kokko, H. 2012a: Bet-hedging-a triple trade-off between means, variances and correlations. – Biological Reviews 87: 742-755.

Starrfelt, J. & Kokko, H. 2012b: The theory of dispersal under multiple influences. In: Clobert, J., Baguette, M., Benton, T. & Bullock, J. (Eds.): Dispersal ecology and evolution. – Oxford University Press, Oxford, UK, pp. 19-28.

Stille, M. 1996: Queen/worker thorax volume ratios and nest-founding strategies in ants. – Oecologia 105: 87-93.

Stuart, R.J. 1987: Lethal fighting among dimorphic males of the ant, *Cardiocondyla wroughtoni*. – Naturwissenschaften 549: 548-549.

Stürup, M., Baer, B. & Boomsma, J.J. 2014: Short independent lives and selection for maximal sperm survival make investment in immune defences unprofitable for leaf-cutting ant males. – Behavioral Ecology and Sociobiology 68: 947-955.

Stürup, M., Boer, S.P.A. den, Nash, D.R., Boomsma, J.J. & Baer, B. 2011: Variation in male body size and reproductive allocation in the leafcutter ant *Atta colombica*: estimating variance components and possible trade-offs. – Insectes Sociaux 58: 47-55.

Sundström, L. 1995: Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. – Behavioral Ecology 6: 132-139.

Sundström, L., Keller, L. & Chapuisat, M. 2003: Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. – Evolution 57: 1552-1561.

Sundström, L., Seppä, P. & Pamilo, P. 2005: Genetic population structure and dispersal patterns in *Formica* ants – a review. – Annales Zoologici Fennici 42: 163-177.

Talbot, M. 1956: Flight activities of the ant *Dolichoderus* (*Hypoclinea*) *mariae* Forel. – Psyche 63: 134-139.

Taylor, P.D. 1992: Altruism in viscous populations – an inclusive fitness model. – Evolutionary Ecology 6: 352-356.

Trivers, R. & Hare, H. 1976: Haplodiploidy and the evolution of the social insects. – Science 191: 249-263.

Trochet, A., Courtois, E.A., Stevens, V.M., Baguette, M., Chaine, A., Schmeller, D.S., Clobert, J., Irschick, D.J. & Wiens, J.J. 2016: Evolution of sex-biased dispersal. – The Quarterly Review of Biology 91: 297-320.

Trunzer, B., Heinze, J. & Hölldobler, B. 1998: Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa*. – Insectes Sociaux 45: 267-276.

Tsuji, K. & Tsuji, N. 1996: Evolution of life history strategies in ants: variation in queen number and mode of colony founding. – Oikos 76: 83-92.

Valen, L. Van 1971: Group selection and the evolution of dispersal. – Evolution 25: 591-598.

Vasconcelos, H.L. 1999: Effects of forest disturbance on the structure of ground-foraging ant communities in central Amazonia. – Biodiversity & Conservation 8: 409-420.

Vepsäläinen, K., Ebse, J.R., Savolainen, R. & Boomsma, J.J. 2009: Mating isolation between the ant *Myrmica rubra* and its microgynous social parasite. – Insectes Sociaux 56: 425-437.

Vepsäläinen, K. & Pisarski, B. 1982: Assembly of island ant communities. – Annales Zoologici Fennici 19: 327-335.

Wandeler, P., Hoeck, P.E.A. & Keller, L.F. 2007: Back to the future: museum specimens in population genetics. – Trends in Ecology & Evolution 22: 634-642.

Wang, J., Wurm, Y., Nipitwattanaphon, M., Riba-Grognuz, O., Huang, Y.-C., Shoemaker, D. & Keller, L. 2013: A Y-like social chromosome causes alternative colony organization in fire ants. – Nature 493: 664-668.

Wenseleers, T. & Oystaeyen, A. Van 2011: Unusual modes of reproduction in social insects: shedding light on the evolutionary paradox of sex. – BioEssays 33: 927-937.

West, S., Pen, I. & Griffin, A. 2002: Cooperation and competition between relatives. – Science 296: 72-75.

Wetterer, J.K., Wild, A.L., Suarez, A.V., Roura-Pascual, N. & Espadaler, X.E. 2009: Worldwide spread of the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). – Myrmecological News 12: 187-194.

Wheeler, D.E. & Buck, N.A. 1995: Storage proteins in ants during development and colony founding. – Journal of Insect Physiology 41: 885-894.

Wheeler, D.E. & Buck, N.A. 1996: Depletion of reserves in ant queens during claustral colony founding. – Insectes Sociaux 43: 297-302.

Wheeler, D.E. & Martínez, T. 1995: Storage proteins in ants (Hymenoptera: Formicidae). – Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 112: 15-19.

Wilson, E.O. 1955: Patchy distributions of ant species in New Guinean rain forests. – Psyche 65: 26-39.

Wilson, E.O. 1958: The beginnings of nomadic and group-predatory behavior in the ponerine ants. – Evolution 12: 24-31.

Wilson, E.O. 1971: Insect societies. – The Belknap Press of Harvard University Press, Cambridge, MA, 562 pp.

Wilson, E.O. & Hunt, G.L. 1966: Habitat selection by the queens of two field-dwelling species of ants. – Ecology 47: 485-487.

Wolf, J.I., Punttila, P. & Seppä, P. 2018: Life-history trait variation in a size- dimorphic ant. – Ecological Entomology 43: 763-773.

Wolff, J.O. 1993: What is the role of adults in mammalian juvenile dispersal? – Oikos 68: 185-190.

Zayed, A. & Packer, L. 2005: Complementary sex determination substantially increases extinction proneness of haplodiploid populations. – Proceedings of the National Academy of Sciences of the United States of America 102: 10742-10746.

Zera, A.J. & Denno, R.F. 1997: Physiology and ecology of dispersal polymorphism in insects. – Annual Review of Entomology 42: 207-230.

Genetic analysis reveals Finnish *Formica fennica* populations do not form a separate genetic entity from *F. exsecta*

Geneettisen analyysin perusteella suomenloviniskan (*Formica fennica*) ja karvaloviniskan (*F. exsecta*) suomalaiset populaatiot eivät eroa toisistaan

II

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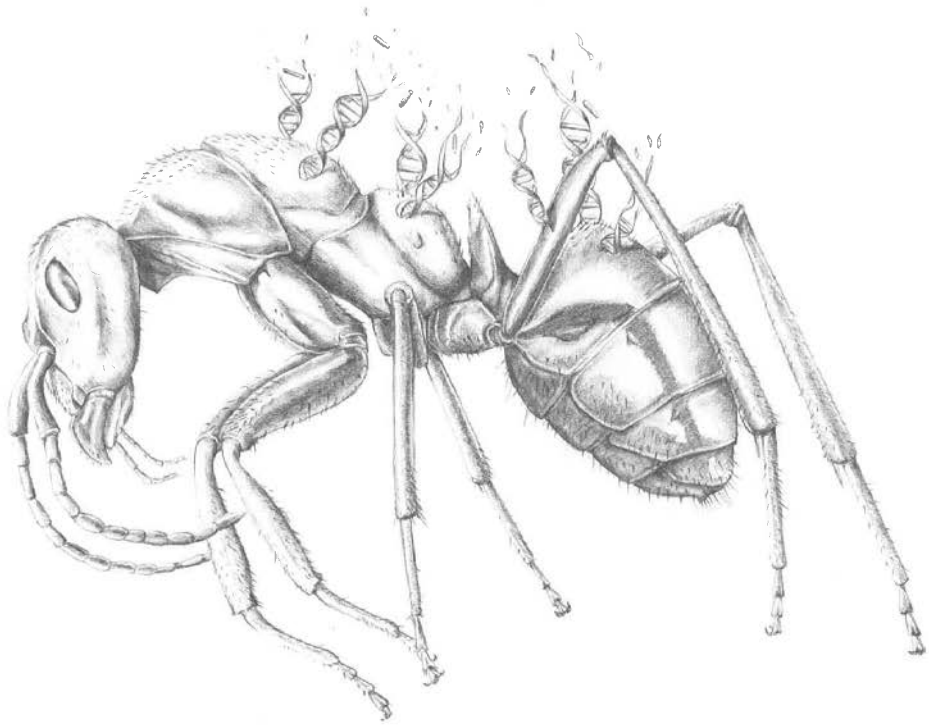
TIIVISTELMÄ

Loviniskamuurahaiset (*Coptoformica* Müller, 1923) on suomumuurahaisten (*Formica* Linnaeus, 1758) alasuku, johon kuuluu noin tusinan verran lajeja. Ne elävät tyypillisesti avoimilla alueilla, ja rakentavat pieniä kekomaisia pesiä heinästä. Alasuvun uusin laji, suomenloviniska (*Formica fennica* Seifert, 2000) kuvattiin morfologisiin tuntomerkkeihin perustuen, mutta lajistatusta ei ole varmistettu molekyylituntomerkeillä. Tässä tutkimuksessa käytämme kolmeatoista DNA-mikrosatelliittijaksoa sekä osittaista mitokondrion COI-geenin sekvenssiä selvittääksemme suomenloviniskan ja kuuden muun pohjoisella havumetsävyöhykkeellä elävän loviniskamuurahaislajin perinnöllistä vaihtelua. Suurin osa tutkimistamme lajeista muodostaa toisistaan erillisiä yksiköitä sekä fylogeneettisissä että spatiaalisissa analyyseissä, ja lajinsisäinen geneettinen vaihtelu on yleensä pientä. Suomenloviniska ryhmittyy kuitenkin yhteen toisen lajin, karvaloviniskan (*F. exsecta* Nylander, 1846), kanssa. Näiden kahden lajin muodostaman ryhmän sisäinen geneettinen vaihtelu on suurta, mutta se heijastelee enemmän maantieteellisiä eroja kuin morfologisia eroavaisuuksia. Suomenloviniskaa ei voida pitää erillisenä lajina, vaan yleisen karvaloviniskan paikallisena muotona.



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Genetic analysis reveals Finnish *Formica fennica* populations do not form a separate genetic entity from *F. exsecta*

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ABSTRACT

Coptoformica Müller, 1923 is a subgenus of *Formica* Linnaeus, 1758 that consists of c. a dozen species of ants that typically inhabit open grassy habitats and build small nest mounds. The most recent addition to the group is *Formica fennica* Seifert, 2000. The description was based on morphological characters, but the species status has not been confirmed by molecular methods. In this study, we use thirteen DNA microsatellite markers and a partial mitochondrial COI gene sequence to assess the species status of *F. fennica*, by comparing the genetic variation among samples identified as *F. fennica* and six other boreal *Formica (Coptoformica)* species. Most of the species studied form separate, discontinuous clusters in phylogenetic and spatial analyses with only little intraspecific genetic variation. However, both nuclear and mitochondrial markers fail to separate the species pair *F. exsecta* Nylander, 1846 and *F. fennica* despite established morphological differences. The genetic variation within the *F. exsecta/fennica* group is extensive, but reflects spatial rather than morphological differences. Finnish *F. fennica* populations studied so far should not be considered a separate species, but merely a morph of *F. exsecta*.

Subjects Ecology, Entomology, Evolutionary Studies
Keywords Species identification, Species delimitation, Hymenoptera, *Coptoformica*, Microsatellites, Barcoding

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Additional Information and Declarations can be found on page 18

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INTRODUCTION

Species is one of the fundamental units in biology, but it is also one that is very hard to define. There are many different and sometimes contrasting species concepts, which can be summarized with an unified species concept: a species is a separately evolving metapopulation lineage (*De Queiroz, 2007*). The practical delimitation of species can depend on several features (*De Queiroz, 2007*), and it can be difficult due to some lineages lacking easily distinguishable features (*Bickford et al., 2007*). In particular, taxa that rely on chemical communication instead of visual cues can be very cryptic to human observers

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(*Mayr, 1963*), which is a plausible explanation for the high amount of cryptic diversity found in ants (*Seifert, 2009*). Also, hybridization between recently diverged lineages is common and further complicates species delimitation (*Abbott et al., 2013*)—also in ants (*Seifert, 1999*). To some extent, inferring the boundaries between species has always been and will always be a matter of agreement and a subject to debate.

Nonetheless, assessing where the species boundaries are is crucial for biologist, and all fields of biology rely on species delimiting done by taxonomists, and the species identification criteria they provide. Using the most up-to-date knowledge is especially important in ecology and population biology, where the behavior or genetics of multiple populations is studied simultaneously. The conclusions of these studies depend on the correct species identifications. *Bortolus (2008)* argues that mistakes in taxonomy in ecological studies can have major cascading errors in our understanding of nature. Ecological studies and descriptions of biodiversity are also the basis on which conservation decisions are made, and thus correct species identification should be a main concern (*Bortolus, 2008*; *Pante et al., 2015*).

Ants in the genus *Formica* Linnaeus, 1758 have been widely studied due to their social behavior and ecological importance. Species delimitation and identification in this taxon is difficult because some of the species are relatively young (*Goropashnaya et al., 2012*). Hybridization is common between closely-related species, and the hybrids are often fertile (*Czechowski, 1993*; *Seifert, 1999*; *Goropashnaya, Fedorov & Pamilo, 2004*; *Korczyńska et al., 2010*; *Kulmuni, Seifert & Pamilo, 2010*). Especially morphological species identification of ants and other social insects has a major practical difficulty that is unique to these taxa (*Ward, 2010*): in many studies species identification is done from worker samples alone, since sampling sexual castes, i.e., the reproductive queens and males, is more difficult. Compared to sexual castes, workers have less morphological variation among species and often more variation within species, which makes species identification with morphological attributes especially difficult (*Ward, 2010*).

Coptoformica Müller, 1923, is a subgenus of *Formica*. Ants in this subgenus live in open habitats and build small nest mounds of grass, typically 20–40 cm high in some of the species, and very low heaps in some of the species (*Seifert, 2000*; *Punttila & Kilpeläinen, 2009*) with a basal area varying greatly (*Sorvari, 2009*). They chop nest material into smaller pieces with their strong mandibles and jaw muscles that extend into the occipital corners of their heads, which gives them their distinctive heart-shaped heads (*Seifert, 2000*). The group includes c. 12 species in the Palaearctic (*Seifert, 2000*; *Schultz & Seifert, 2007*). Since the *Coptoformica* subgenus has several rare species, and their preferred habitats, such as meadows and mires, belong to the most threatened habitat types in Finland (*Kontula & Raunio, 2009*), they are a candidate group for future conservation efforts. According to *National Red Lists (2018)* several species of the group are threatened at varying levels in different European countries, and many of them have declining populations (*Seifert, 2000*). At the moment in Finland, only one of the species, *Formica suecica* Adlerz, 1902 is classified as Near Threatened (IUCN Red List Category NT) due to the overgrowing of their preferred habitats, whereas the rest of the five species occurring in Finland are classified as Least Concern (IUCN Red List Category LC) (*Rassi et al., 2010*).

The most recently described species is *Formica fennica* Seifert, 2000. The description is based on morphological features of samples collected from three locations in Finland and one in the Caucasus, Georgia, with one queen sample from Finland (Kitee) denoted as the holotype (*Seifert, 2000*). Since then, the species has been identified from several other locations in Finland, predominantly from mires in the northern parts of the country (*Punttila & Kilpeläinen, 2009*), from one location in Norway, also from "wet conditions" (*Suvák, 2013*), and from another location in the Caucasus, Azerbaijan (*Schultz & Seifert, 2007*). Based on morphology, *F. fennica* and Central Asian *F. manchu* Wheeler, 1929 are considered to be sister species (*Seifert, 2000*), but locally in Finland, *F. fennica* is both morphologically and ecologically very similar to *F. exsecta* Nylander, 1846. *Formica exsecta* is the most widely distributed species of the subgenus (*Schultz & Seifert, 2007*), and it is very variable both morphologically and ecologically (*Seifert, 2000*). According to *Seifert (2000)*, *F. exsecta* and *F. fennica* are separated from each other by the distribution of standing setae on the gastral terga and clypeus, and by the number of semi-erect setae on the craniad profile of forecoxae. Since there is a lot of variation in each of these characteristics in both species, nest samples with multiple workers are needed to calculate the averages of the characteristics for the separation of the species (*Seifert, 2000*).

The identification of *F. fennica* is further complicated by the existence of a pilosity-reduced form of *F. exsecta*, that was originally described as a separate species called *Formica rubens* Forel, 1874. Not much is currently known of this morph. Based on the original description, *F. rubens* is larger and more brightly and evenly red than *F. exsecta* (*Forel, 1874*). According to current understanding, intraspecific color morphs are very common in ants, as is size variation, and usually these kinds of characters are not adequate for species identification (*Seifert, 2009*). *Formica rubens* was recently synonymized with *F. exsecta* based on the examination of four individuals of the type series collected from Switzerland, because all morphological characters measured were within the range of *F. exsecta* (*Seifert, 2000*). However, after the synonymization, *Ødegaard (2013)* stated: "*F. (C.) rubens* is interpreted as a mutant conspecific with *F. (C.) exsecta* (Bernhard Seifert in litt.), but it is not impossible that *F. rubens* may turn out to be a good species in the future." *Ødegaard (2013)* recommended using extreme caution when identifying *F. fennica*. In *Ødegaard*'s (*2013*) data from *Coptoformica* colonies from mires in Hedmark, Norway, several colony samples fit the description of *F. fennica* based "on the presence of microhairs on the eyes combined with lack of setae on T1 and T2 and partly T3, and 0–3 setae on the front of the fore coxae", but these were identified to represent the setae-reduced *rubens* mutant of *F. exsecta*, even though the details of the identification are not mentioned. Similarly, the Finnish mire samples of *Formica fennica* studied by *Punttila & Kilpeläinen (2009)* were identified using the same key (*Seifert, 2000*), but it cannot be ruled out that they represent another case of the *rubens* morph.

The close resemblance to *F. exsecta* and *F. fennica*, and the lack of molecular verification of the species status of *F. fennica* created the need for this study. Using only morphological methods for species identification is risky in the presence of intermediate individuals, and makes further ecological studies on these two species very difficult. Molecular methods have proven to be especially valuable when working with morphologically conserved

species groups: the number of described cryptic species has increased exponentially after the introduction of polymerase chain reaction (PCR) and the molecular methods it enables (*Bickford et al., 2007*). Conversely, also two morphs of a same species can be erroneously described as two separate species, which are later synonymized after more thorough examination. This is not a trivial phenomenon: A Web of Science search (January 2018) with the search query ''synonymiz* OR synonymis*'' yields 3,658 articles labelled with some ecological field (zoology, entomology, plant sciences, evolutionary biology, biodiversity conservation, marine freshwater biology, ecology, mycology, parasitology, microbiology, limnology, ornithology). Thus, confirming the taxonomy of studied taxa with molecular methods is very advisable.

In this work we evaluate the existence of *F. fennica* as a separate species using molecular methods, and investigate its position in *Formica* phylogeny (*Goropashnaya et al., 2012*). The goal of this study is to test whether *F. fennica* is a separately evolving lineage in the same extent as the other species of the subgenus. Given that we use genetic data as our sole line of evidence, our approach is consistent with the biological species concept that emphasizes reproductive isolation and the lack of gene flow as the most important species delimiting properties (*Mayr, 1942*). The hypothesis is that all seven morphologically identified *Coptoformica* species included in this study are recovered as separate lineages also in analyses based on molecular methods. Further, we test the hypothesis of *F. fennica* being a sister species of *F. manchu* (*Seifert, 2000*) among the limited number of species used in this study. The aim of the sampling scheme is to investigate the species status of *F. fennica* in Finland, in the currently known core area of its distribution, leaving other biogeographical areas outside the scope of this study.

MATERIALS AND METHODS

Sampling and species identification

The bulk of samples used in this study were collected during the 10th Finnish National Forest Inventory (NFI) carried out by the Natural Resources Institute Finland (earlier Finnish Forest Research Institute) during the years 2005–2008, also used by *Punttila & Kilpeläinen (2009)*. This dataset was supplemented with additional samples from various areas of Finland, collected during the years 2008–2015 (Fig. 1; Table S1). The study area covers over 1,100 kilometers in south-north direction, reaching from the hemiboreal zone to the northern border of the northern boreal zone. Samples of two additional species from eastern Siberia, *Formica pisarskii* Dlussky, 1964 and *F. manchu* were also included. These samples were originally collected for another study (*Goropashnaya et al., 2012*) and the original morphological identifications were done by B. Seifert (P. Pamilo in litt.).

The samples collected in Finland included usually 15–20 individuals per nest, but in rare cases—when the amount of active workers was low due to bad weather or to the weakened condition of the nest population—this amount was not achieved. The ants were identified with the key of *Seifert (2000)* using sample averages of the critical characteristics based on five or more worker individuals. When identifying *F. fennica* from less hairy samples of *F. exsecta*, 10–20 workers were inspected. When the sample contained less workers, all

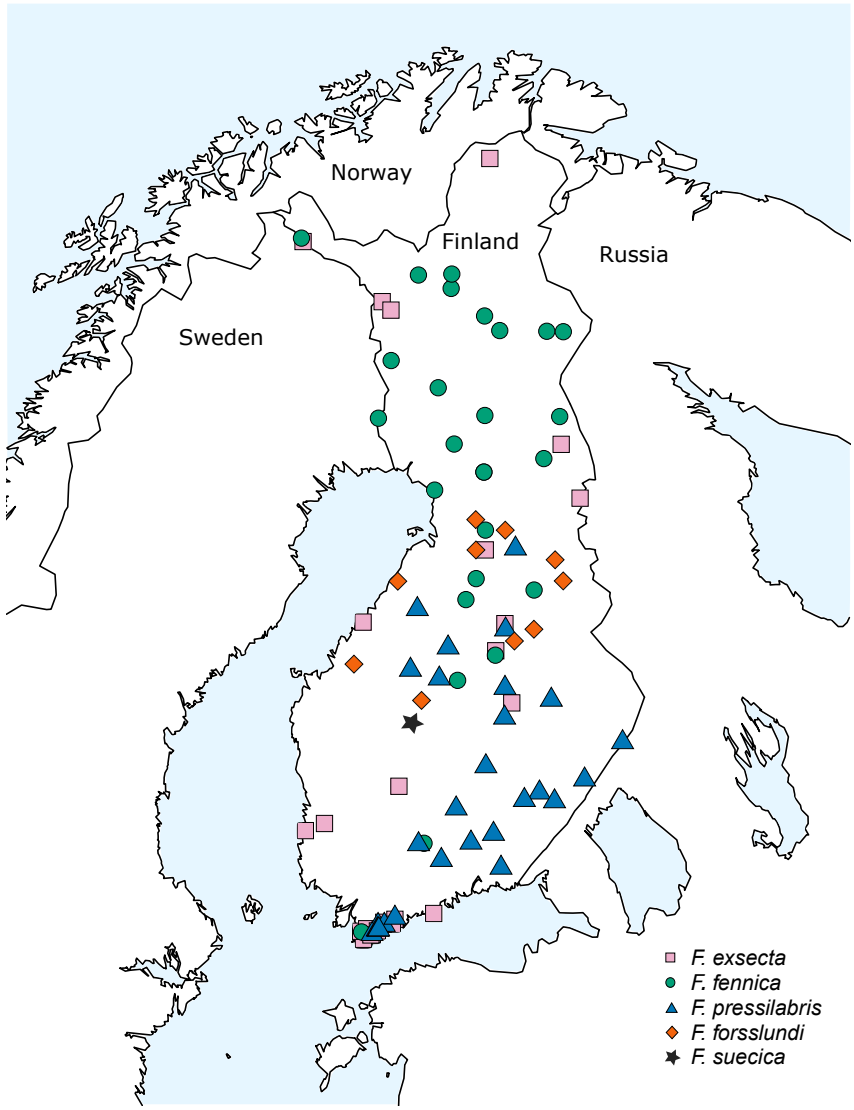

Figure 1 **Sampling locations in Finland.**

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the available individuals were checked. All individuals identified as *F. fennica* using this key are hereafter referred to with this name, with the understanding that identifications as the *rubens* morph of *F. exsecta* might in some cases be more accurate (*Ødegaard, 2013*). In total, the Finnish dataset includes all the five *Coptoformica* species known to occur in

Table 1 Geographic distributions of the study species in the Palaearctic Region, reproduced from *Schultz & Seifert (2007)*.

Species	Distribution in Europe	Distribution in Asia
F. exsecta	Temperate to boreal, planar to subalpine and submeridional-subalpine	Oreo-Turanian and Tibetan to boreal, montane to subalpine
F. fennica	South boreal and Caucasian-montane	?
F. forsslundi	Temperate to boreal, planar to submontane	Tibetan to Central-Siberian-Daurian
F. manchu	–	Tibetan to Central-Siberian-Daurian
F. pisarskii	–	Mongolian to Central-Siberian-Daurian
F. pressilabris	Temperate to south boreal, planar to subalpine	Tibetan to Central-Siberian-Daurian and East Manshurian, montane to subalpine
F. suecica	North temperate to boreal, in the Alps montane to subalpine	–/?

Notes.
–not present
?not known

Finland: *F. fennica* (33 nests from 26 locations), *F. exsecta* (38/27), *F. pressilabris* Nylander, 1846 (42/29), *F. forsslundi* Lohmander, 1949 (13/10), *F. suecica* (2/1). The geographic distributions of the seven study species are given in Table 1.

One *F. fennica* population included in this study (samples: FF_178 —FF_181) was one of the three Finnish populations used in the original species description (*Seifert, 2000*) and sampled by the same researcher (J. Sorvari) 12 years after the original sampling. Between these two sampling times the continuity of the population had been monitored yearly by J. Sorvari.

Molecular methods and data analysis

DNA of two individuals per nest was extracted using Chelex© (Biorad, Hercules, CA, USA) extraction protocol or NucleoSpin® Tissue Kit by Macherey-Nagel. Same individuals were used for both microsatellite genotyping and DNA barcoding. As NFI samples were not originally collected for a genetic study, their storage conditions had not been optimal, resulting often in poor quality DNA, and several samples could not be sequenced successfully. Most of the poor quality samples were *F. fennica* samples, likely because they had previously been most intensively handled for the morphological identification. However, shorter microsatellite fragments could be amplified for almost all of these samples too. The *F. fennica* samples from the population originally used for species description are of good quality and were sequenced without problems. Detailed protocols for both DNA microsatellite methods and mtDNA sequencing together with a table of primer information are given in Table S2.

DNA microsatellite genotyping

Two individuals from each nest were genotyped to assess nuclear genetic variation within and between species, and to confirm the morphology-based species delimitations and identifications. Thirteen DNA microsatellite markers (*Chapuisat, 1996*; *Gyllenstrand, Gertsch & Pamilo, 2002*; *Trontti, Tay & Sundström, 2003*; *Hasegawa & Imai, 2004*) were used in four multiplexes with the Type-it Microsatellite PCR Kit (QIAGEN, Valencia, CA,

USA) according to the manufacturer's instructions. PCR products were analyzed with a 3730 ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA) and alleles were scored using GeneMapper 5.0. (Applied Biosystems). DNA microsatellite data were analyzed with Genalex 6.502 (*Peakall & Smouse, 2006*; *Peakall & Smouse, 2012*), and R packages Hierfstat (*Goudet, 2005*) and Adegenet (*Jombart, 2008*). Six samples with more than 50% missing microsatellite data were omitted from further analyses.

Genetic variation at DNA microsatellite loci was described for all species with more than two sampling localities. For these species, pairwise F_{ST} values from the allelic distance matrix, and Nei's standard genetic distances D (*Nei, 1972*) were calculated to assess genetic differentiation between species. When the genetic differentiation between *F. exsecta* and *F. fennica* was found to be minor, the correlation of linear genetic distances and log-transformed geographical distances within *F. exsecta/fennica* subset was investigated with a maximum-likelihood population-effects (MLPE) model with Residual maximum likelihood (REML) estimation (*Clarke, Rothery & Raybould, 2002*; *Van Strien, Keller & Holderegger, 2012*) with the R package 'lme4' (*Bates et al., 2015*).

Separation of the species based on nuclear genetic variation was analyzed at the individual level with mixture analysis using model-based Bayesian clustering with software Baps 6.0 (*Corander, Waldmann & Sillanpää, 2003*; *Corander, Marttinen & Mäntyniemi, 2006*). Only one individual per nest was used to eliminate the possible effect of nest structure in the analysis, as previously done by *Seppä et al. (2011)*. The software was allowed to find the most probable number of clusters with repeated runs using different upper limits for the cluster number (first 5 times K7—K20, and thereafter 20 times K11—K16). The hypothesis was that each morphologically identified species would form separate clusters. After the initial analysis revealed that several *F. exsecta* and *F. fennica* samples cluster together, the same procedure was repeated using only *F. exsecta* and *F. fennica* samples, to assess if there is finer scale clustering within this group (first 5 times K2—K25, and thereafter 20 times K18—K24).

Similar analyses were run also with software Structure (*Pritchard, Stephens & Donnelly, 2000*). However, the mathematical model used by Structure does not deal well with unbalanced sampling and low sample sizes (*Kalinowski, 2011*; *Puechmaille, 2016*), which is apparent in our data. Thus, although the overall results for the focal species are similar with both Baps and Structure, Baps was deemed to be more suitable with our sampling patterns. Therefore only the Baps results are discussed further.

Discriminant analysis of principal components, DAPC (*Jombart et al., 2010*) was done for the whole microsatellite dataset to assess if morphologically different samples would also form discontinuous clusters based on nuclear genetic variation. Cross-validation for the optimal number of principal components (PCs) was carried out as instructed by the developers, and based on the highest mean predictive success and lowest root mean squared error, 24 principal components (of the total 126) were included in the final DAPC. Missing data were substituted with the mean allele frequencies. The analysis was repeated with only *F. exsecta* and *F. fennica* samples in order to check how well the optimal model for this subset of data is able to separate the two species. Based on cross validation, 63 PCs

(of the total 123) should be included for the analysis to achieve the highest accuracy and lowest error.

DNA barcoding

Part of the gene Cytochrome c oxidase subunit I (COI) was amplified for 85 samples from different nests to assess mitochondrial variation among the subgenus. All *F. fennica* samples that could be sequenced successfully were included in the analysis (24 samples), together with a geographically representative subset of samples from other species (leaving out samples collected from the same or nearby locations): *F. exsecta* (29), *F. pressilabris* (18), *F. forsslundi* (10), *F. suecica* (2), *F. manchu* (1), *F. pisarskii* (1) (details given in S1). PCR primers designed by *Seppä et al. (2011)* were used with the Phusion PCR kit (Finnzymes) according to manufacturer's instructions. Amplification products were purified and sequenced in the Institute of Biotechnology of the University of Helsinki using the aforementioned primers.

The obtained 525 base-pair sequences were assembled and aligned with Geneious 8.1.7 (Biomatters) with Muscle alignment (*Edgar, 2004*). Sequence divergences as numbers and percentages of differing nucleotides were calculated for all pairs of haplotypes. Maximum likelihood analyses of the aligned barcode regions were performed using the program RAxML v8 (*Stamatakis, 2014*). The analyses were run in CIPRES (*Miller, Pfeiffer & Schwartz, 2010*) with the GTR model, and partitioned by codon position. Bootstrap support values were evaluated with 1,000 bootstrap replicates of the data and plotted onto the best scoring tree with *Figtree (2018)*. Of the 85 *Coptoformica* sequences, 13 representing all the different haplotypes were included in the ingroup. An additional sequence of *F. exsecta* collected in Finland was obtained from GenBank (AB103364.1). The analyses included three species as outgroup: *Formica (Serviformica) lemani* Bondroit, 1917, *Formica (Formica s. str.) truncorum* Fabricius, 1804, and *Formica (Formica s. str.) pratensis* Retzius, 1783. The molecular data for these taxa were obtained from GenBank (AB019425.1, AB010929.1 and AB103363.1, respectively).

Six of the 24 *F. fennica* samples (290, 294, 296, 304, 310, 312) in the ingroup representing three different haplotypes were excluded from the final analysis because they placed with species in other subgenenera in the phylogeny. The risk of this result being due to contamination or an error in the sequencing of poor quality DNA, or due to nuclear copies of mitochondrial DNA, was considered too high. However, these samples did not stand out from other samples in the microsatellite dataset, and were therefore not excluded from microsatellite analyses, although two did have too much missing data and were excluded for this reason. The full phylogeny and haplotype distance table with all 85 sequences is presented in S4. The phylogeny of the 14 ingroup taxa representing the haplotypes of the remaining 79 sequences and the additional *F. exsecta* obtained from GenBank is hereafter presented and discussed.

Table 2 Genetic differentiation between species. Below diagonal: pairwise FST values ($p < 0.001$ for all). Above diagonal: pairwise Nei's genetic distance (D).

	Fe	Ff	Ffo	Fp
Fe		0.110	0.872	0.799
Ff	0.025		0.788	0.748
Ffo	0.204	0.182		0.719
Fp	0.161	0.146	0.190	

Notes.
Fe, *F. exsecta*; Ff, *F. fennica*; Ffo, *F. forsslundi*; Fp, *F. pressilabris*.

RESULTS

The variation in the microsatellite markers is described in Table S3 . The amount of missing data is 2.16% for the whole microsatellite dataset of two individuals per nest, and 1.26% for the dataset of a single individual per nest.

The pairwise F_{ST} values (Table 2) between different species are generally much higher (0.15–0.20), than the values between *F. exsecta* and *F. fennica* (0.03). All F_{ST} values are significant ($p < 0.001$). Nei's D (Table 2) show the same pattern with higher values between other species pairs (0.72–0.87) and lower values between *F. exsecta* and *F. fennica* (0.11). Among *F. exsecta/fennica* samples, the pairwise genetic distances are explained by geographical distance (MLPE: $\beta = 0.34$, $SE = 0.02$, $P < 0.0001$).

In Bayesian clustering (Fig. 2A), the optimal number of genetic clusters for the whole dataset of one individual per nest was $K = 14$ (Posterior probability= 0.45), but other cluster numbers also gain large support ($K = 13$, $P = 0.37$; $K = 12$, $P = 0.12$; $K = 15$, $P = 0.06$). In the most optimal partition, *F. exsecta* and *F. fennica* share one major cluster, with additional smaller clusters, and the number of these additional *F. exsecta /fennica* clusters is the only difference between the other most optimal cluster numbers. Morphologically defined species represent all other clusters, and each species has only one cluster except *F. manchu* with its two samples clustering separately. When Bayesian clustering is repeated using only *F. exsecta* and *F. fennica* samples (Fig. 2B), the structure is broken down into several clusters of only few individuals, the most probable number of clusters being $K = 22$ ($P = 0.38$), $K = 21$ ($P = 0.29$), $K = 20$ (0.17) and $K = 23$ (0.14). In the most optimal partition, there are seven clusters shared between *F. exsecta* and *F. fennica* samples.

In DAPC of the whole microsatellite dataset (Fig. 3), *F. exsecta* and *F. fennica* cluster together. Other morphologically defined species form more distinct clusters, clearly separated from other species, with the exception of the two individuals of *F. pisarskii* and four individuals of *F. manchu* grouping loosely together. The model's ability to reassign individuals to their morphologically defined species is 100% for the other groups, but only 86.3% for *F. exsecta* and 86.4% for *F. fennica*, and in the cases when the assignment does not succeed according to morphology, *F. fennica* samples are always assigned to be *F. exsecta*, and vice versa. The consistency of DAPC classification with the morphological species identifications of *F. exsecta* and *F. fennica* samples is visualized in Fig. 4. When the model is fit for the subset of *F. exsecta* and *F. fennica* only, it is unable to reliably assign the samples to two groups with reasonably small number of PCs. The best possible fit

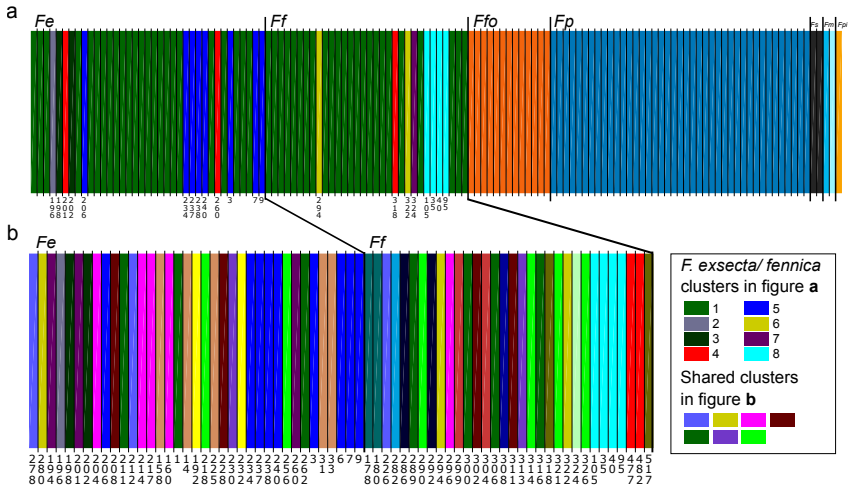

Figure 2 Bayesian clustering of seven *Coptoformica* species obtained with the software Baps. Abbreviations: Fe, *F. exsecta*; Ff, *F. fennica*; Ffo, *F. forsslundi*; Fp. *F. pressilabris*; Fs, *F. suecica*; Fm, *F. manchu*; Fpi, *F. pisarskii*. (A) Clustering for the whole dataset (the optimal $K = 14$). (B) Clustering for *F. exsecta* and *F. fennica* samples (the optimal $K = 22$).

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is achieved with 63 PCs, over half of the total number of PCs, which makes the model overfitted. This means that the explanatory power of the model with additional samples would be very poor, as it already uses individual level characteristics instead of group level characteristics to separate the two groups. The overfitted model can assign all samples of *F. exsecta* correctly, but only 97% of *F. fennica* samples.

In mitochondrial DNA barcoding, most studied species have species-specific haplotypes. *Formica pressilabris* has two haplotypes (diverging from each other by 6 nucleotides/1.14%), and *F. suecica* and *F. forsslundi* both only one. Also the single samples of *F. manchu* and *F. pisarskii* have unique haplotypes. *Formica exsecta* and *F. fennica* share the most common haplotype, which is also the only haplotype for *F. exsecta*. The *F. exsecta* haplotype from GenBank (AB103364.1) is different from the one obtained in this study. Apart from the shared *F. exsecta/fennica* haplotype, there are three additional haplotypes in *F. fennica* samples. The divergence among different *F. fennica* haplotypes varies between 1–18 nucleotides (0.19%–3.43%). The haplotype divergences between different species vary between 1–21 nucleotides (0.19%–4.00%). Table 3 shows all haplotype divergences measured in this study. The best scoring phylogenetic tree is presented in Fig. 5. The included *Coptoformica* samples form a clade. Two lineages composed of *F. fennica* and *F. exsecta* samples are recovered basal to the other *Coptoformica* species. *Formica fennica* and *F. exsecta* samples do not form monophyletic groups. In this taxon sampling *F. manchu* and F. *fennica* are not sister groups.

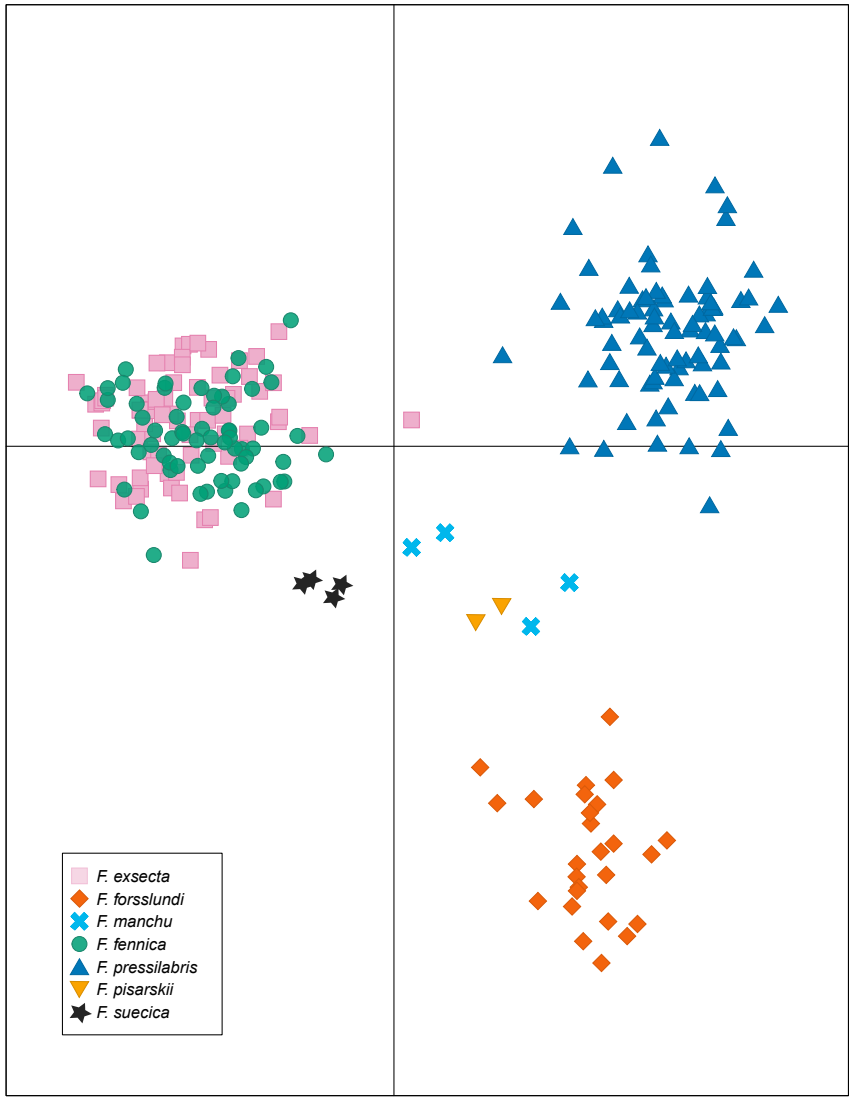

Figure 3 Discriminant analysis of principal components (DAPC) of the microsatellite data of seven *Coptoformica* species with 24 principal components included.

Full-size DOI: 10.7717/peerj.6013/fig-3

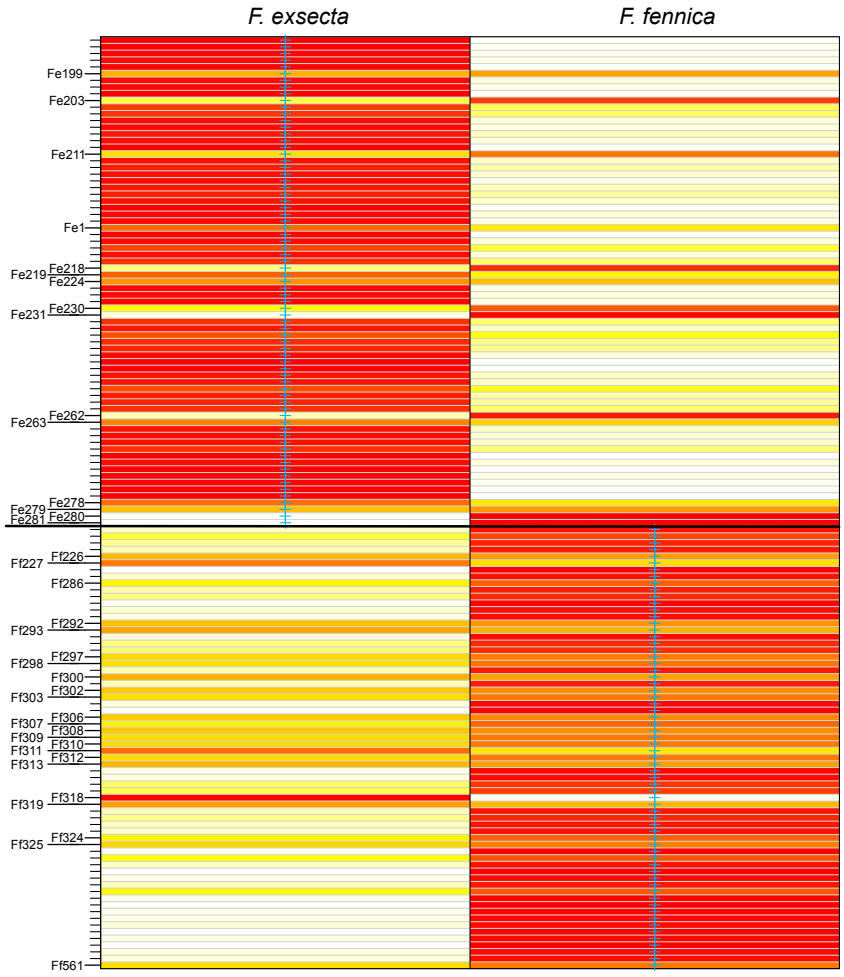

Figure 4 Assignment plot from discriminant analysis of principal components (DAPC). Blue cross marks the prior morphological species identity. Individuals are reassigned to these groups based on the DAPC model with 24 principal components. The color gradient represents membership probabilities (Red = 1 White = 0). Individuals that are assigned into the wrong group with >10% probability are named. Overall, the assignment of individuals to their morphological groups succeeds with the accuracy of 86.3% for *F. exsecta* and 86.4% for *F. fennica.*

Full-size DOI: 10.7717/peerj.6013/fig-4

Table 3 Divergences between the COI barcode haplotypes found in this study, and one reference haplotype from Genbank (5). Below diagonal: number of differing nucleotides. Above diagonal: percentages of differing nucleotides.

Haplotypes	*n*	1	2	3	4	5	6	7	8	9	10	11
1 *F_fennica* _306	1		3.09	3.24	0.19	4.00	3.24	3.81	3.62	3.43	3.43	3.62
2 *F_fennica* _314	1	16		0.19	3.24	1.14	3.24	3.43	3.62	3.43	3.62	3.24
3 *F_fennica* _288	1	17	1		3.43	0.95	3.43	3.62	3.81	3.62	3.62	3.24
4 *F_exsecta/fennica* _234	44	1	17	18		3.81	3.43	4.00	3.81	3.62	3.62	3.81
5 F_*exsecta* _AB103364.1	–	21	6	5	20		4.00	3.81	4.00	3.81	3.81	3.81
6 *F_suecica* _400	2	17	17	18	18	21		2.48	2.29	3.24	3.24	3.62
7 *F_pisarskii* _25	1	20	18	19	21	20	13		0.19	2.48	2.48	2.86
8 *F_manchu* _27	1	19	19	20	20	21	12	1		2.29	2.29	2.67
9 *F_pressilabris* _264	17	18	18	19	19	20	17	13	12		1.14	1.14
10 *F_pressilabris* _390	1	18	19	19	19	20	17	13	12	6		0.38
11 *F_forsslundi* _342	10	19	17	17	20	20	19	15	14	6	2	

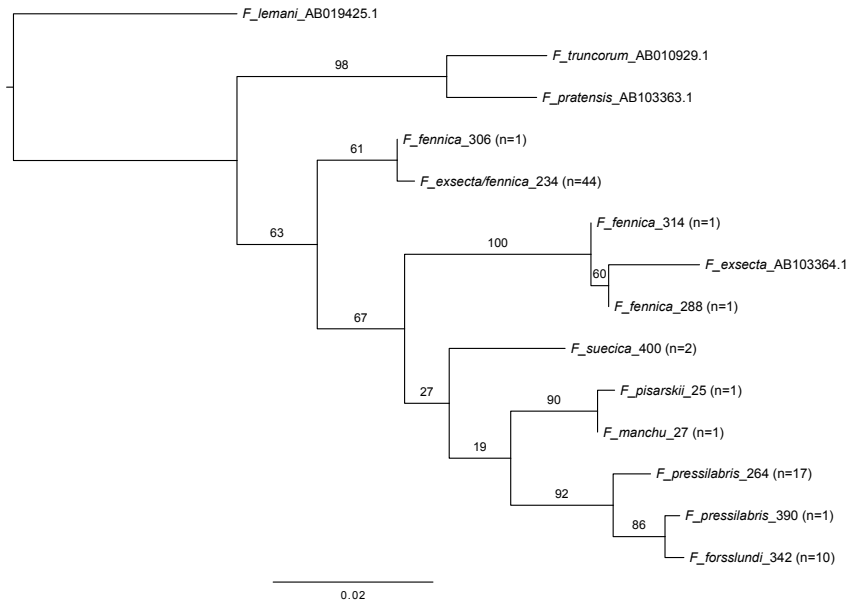

Figure 5 Maximum likelihood tree of COI barcodes of seven *Coptoformica* species and three additional *Formica* species. The additional samples obtained from GenBank. Bootstrap values shown next to the nodes. Note that some of the branches do not have sufficient bootstrap support and the tree should not be used to interpret the phylogeny.

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The geographical distribution of all individuals diverging from the main group of *F. exsecta/fennica* in either the nuclear or the mitochondrial dataset was mapped, but no clear patterns appear: the genetic differences are distributed throughout the sampling area (Fig. 6).

DISCUSSION

The morphological identifications for most of the *Coptoformica* species match the genetic patterns revealed in this study. Our results support the species identities of these species. In contrast, the morphological identifications of *F. exsecta* and *F. fennica* do not match the genetic patterns in any of the analyses. Both mitochondrial sequences and nuclear microsatellite genotypes reveal mixed patterns, with most of the samples of these two species clustering together regardless of morphology. One of the *F. fennica* populations sampled for this study was used in the original description of the species (*Seifert, 2000*). Also the samples from this population cluster together with *F. exsecta* samples in all analyses with both mitochondrial and nuclear markers. Thus, the hypothesis that the morphologically identified species *F. fennica* is also genetically differentiated, is not supported.

Genetic differentiation among individuals of *F. exsecta/fennica* group is distributed throughout the geographic range with no obviously distinct populations or areas. There is minor differentiation between *F. fennica* and *F. exsecta* samples overall, seen in the low but significant pairwise F_{ST} value calculated from microsatellite data. However, the significant effect of spatial distance, combined with the uneven sampling pattern, with more samples identified as *F. fennica* collected in northern areas of Finland and more samples identified as *F. exsecta* from southern areas, suggest this differentiation reflects isolation by distance rather than a species difference. Furthermore, the F_{ST} and Nei's D values between samples of *F. exsecta* and *F. fennica* are overall drastically lower than the same values calculated between other pairs of species. This indicates an ongoing gene flow between morphologically identified *F. exsecta* and *F. fennica*. This is contrasted clearly with the other *Coptoformica* species that are more distinct genetic entities based on the F_{ST} values.

Reflecting the F_{ST} values, Bayesian clustering of the microsatellite data reveals clear structuring at the species level in *Coptoformica*, except within the *F. exsecta/fennica* group. Since the genetic differentiation between *F. exsecta* and *F. fennica* is minor, Baps analysis does not find stable partitions for this part of the data. The analysis does reveal some structuring, separating few individuals from both of these species into separate clusters. But since these individuals do not separate from the main group with other analysis methods, and the clusters correspond to geographical locations even when the geographic information was not used as informative prior, the structuring is most likely due to minor differentiation between local populations, and does not reflect species-level differences. The weakness of the structuring is shown in the low probability scores for the most optimal partitions, and in the way the structuring almost completely breaks down to the location level, when *F. exsecta/fennica* data are analyzed without the other species. It is also notable that in the analysis with only *F. exsecta* and *F. fennica,* several of the small clusters are shared

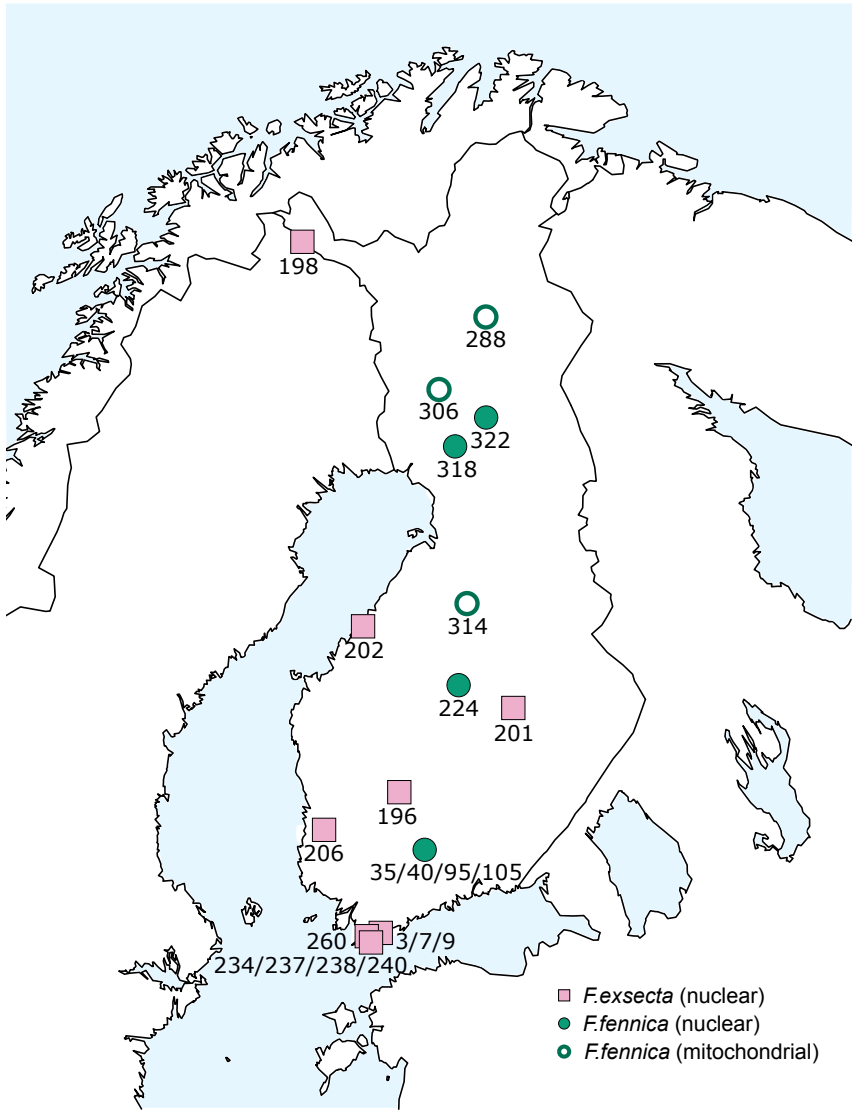

Figure 6 Geographical distribution of diverging *F. exsecta* and *F. fennica* samples. Individuals/populations that diverge from the main combined clusters of *F. exsecta/fennica* in nuclear or mitochondrial markers are shown. Nuclear clusters were determined with Baps from DNA microsatellite data and mitochondrial clusters as haplotypes of the partial COI barcode.

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between the two species, further affirming the mismatch between morphological species identification and molecular analysis. The other species cluster perfectly by morphological identifications.

F. exsecta and *F. fennica* cluster together also in discriminant analysis of principal components (DAPC) of the whole microsatellite dataset, and the model is unable to separate these groups reliably, although it does show minor genetic differentiation between some of the samples of *F. exsecta* and *F. fennica*. Since the analysis does cluster all of the other species with 100% accuracy, this result shows signs of admixture and misclassified individuals between *F. exsecta* and *F. fennica*. When *F. exsecta* and *F. fennica* samples are analyzed alone, the model with the highest accuracy and lowest error has to use over half of the total number of PCs, making it overfitted. Strikingly, even the overfitted model is unable to assign all of the samples of *F. fennica* correctly, which clearly shows that the species is not separated from *F. exsecta* but instead has individuals that are genetically extremely close to *F. exsecta*. Based on these results, we conclude that even though there is some differentiation between some of the samples of *F. exsecta* and *F. fennica*, the separation between these two species is substantially smaller than the separation between other species of the subgenus.

Also the mitochondrial data are clearly supporting the results of the nuclear data: most of *F. exsecta* and *F. fennica* samples share the same mitochondrial haplotype, whereas the other species have species-specific haplotypes. However, some *F. fennica* samples do diverge from this main haplotype. The pairwise sequence divergences between different *F. fennica* haplotypes vary from 0.19% to 3.43%. The average interspecific sequence divergence in *Coptoformica* has previously been reported to be 3.61% (*Goropashnaya et al., 2012*), although this number is based on longer sequences, a different mitochondrial gene and a larger geographic range that we used. Usually intraspecific diversity in COI barcodes is quite low, and sequence divergences of 2% or 3% have been suggested as suitable cut-off values for separating different species (*Hebert et al., 2003*; *Smith, Fisher & Hebert, 2005*). In a barcoding study of 51 ant species in Mauritius, a threshold of 2% sequence divergence was suitable (*Smith & Fisher, 2009*). However, *Jansen, Savolainen & Vepsäläinen (2009)* report intraspecific divergences up to 5.54% in Palearctic *Myrmica* species when sampling covers large geographic areas, and interspecific values as low as 0–0.96%. The latter is in line with the low values reported in this study between *F. pisarskii* and *F. manchu* (0.19%) and *F. pressilabris* and *F. forsslundi* (0.38%). Our data show that no arbitrary cut-off value should be trusted. Given the above, the mitochondrial sequence divergence in *F. fennica* samples in this study is within the bounds of intraspecific sequence divergence, but it is still on the high end of the scale, suggesting this group has genetic diversity worth additional studies.

Strikingly, when differences from the main haplotype occur in mitochondrial sequences of some *F. fennica* individuals, similar differentiation is not present in the nuclear DNA of these same individuals. Even though there is large variation in the *F. exsecta/fennica* group in both mitochondrial and nuclear datasets, no distinct sub-groups appear. Overall, the observed variation in *F. exsecta/fennica* group shows different patterns in mitochondrial and nuclear datasets, so that individuals divergent in one marker type belong to the major

cluster in the other, with no geographical patterns to be seen (Fig. 6). Based on this result, all *F. exsecta/fennica* samples included in this study can be considered to be part of the same gene pool.

Mitochondrial DNA can differ from nuclear DNA due to various reasons, most notably incomplete lineage sorting, unrecognized paralogy, and hybridization resulting in introgression (*Funk & Omland, 2003*; *Toews & Brelsford, 2012*; *Wallis et al., 2017*). Similar types of patterns of mito-nuclear discordance are shown in hybrids of *Formica aquilonia* Yarrow, 1955 and *Formica polyctena* Förster, 1850 (*Beresford et al., 2017*). According to *Funk & Omland (2003)*, if hybridization happened long ago, the persisting introgressed alleles are more likely to be phylogenetically basal and less likely to be geographically associated with the parental lineages. A historical hybridization of *F. exsecta* and a species not found in present-day Finland is a possible explanation for the observed non-monophyly and mito-nuclear discordance of *F. fennica* samples. In order to thoroughly investigate the observed nuclear and mitochondrial genetic variation in *F. exsecta/fennica* group, more extensive sampling at the population level would be needed.

The phylogenetic analysis presented in this study is based solely on partial COI data and a limited taxon sampling, which explains the low bootstrap support and the differences compared to the previously published partial phylogeny of *Coptoformica* species (*Goropashnaya et al., 2012*). Since the earlier phylogeny is based on substantially longer sequences and a better geographical coverage than used here, it should be considered more trustworthy. The main structure of the previous phylogeny with *F. exsecta* branching basally to the other *Coptoformica* species, is well supported also in the phylogeny presented here. The hypothesis that *F. fennica* samples form a distinct branch as a sister group with *F. manchu* was not supported. Although the sampling of this study is geographically restricted, and the data should not be used for full species delimitation nor for interpreting the exact phylogenetic relationships among these species, the result of *F. fennica* and *F. exsecta* grouping mostly together and branching basally to the other *Coptoformica* species is clear, and supports the results of nuclear data.

Our results show that the studied Finnish *F. fennica* populations should not be considered as a separate genetic entity from *F. exsecta*. None of the *F. fennica* populations were genetically differentiated from *F. exsecta* strongly enough to be considered a different species, including one of the populations used in the original description of *F. fennica* (*Seifert, 2000*). According to an earlier study, some of the samples that matched the species description of *F. fennica* actually belonged to the *rubens* morph of *F. exsecta* (*Ødegaard, 2013*). Based on this study, all Finnish *F. fennica* populations may also belong to the *rubens* (or some other) morph of *F. exsecta*. Since the sampling in this study does not cover the whole distribution area of *F. fennica*, the samples from other areas (i.e., Caucasus *Seifert, 2000*; *Schultz & Seifert, 2007*) and Norway (*Suvák, 2013*) should be re-analyzed in the light of these results for more accurate species delimitation.

Should some of the *F. exsecta* morphs represent different stages of a speciation continuum, it would be advisable to use an integrative approach combining both modern morphological and genetic methods, and possibly also other methods such as biochemical analyses (e.g.. cuticular hydrocarbons) and ethological and ecological analyses (*Dayrat,*

2005; Seifert, 2009; Schlick-Steiner et al., 2010). This would result in a fuller understanding of the observed diversity in *F. exsecta* and a more reliable species delimitation. Especially for conservation planning, it would be important to consider if some of the morphs of *F. exsecta* form evolutionarily significant units. Based on the data presented in this study, it is not possible to separate clear genetically distinct lineages, which has been an important criterion in many definitions of evolutionarily significant units (Fraser & Bernatchez, 2001). It is still worthwhile to consider whether the high morphological and genetic variation found in *F. exsecta* would be worth conserving. This study highlights the importance of taxonomic studies as reference for ecologists and conservation biologists.

CONCLUSIONS

Both nuclear and mitochondrial markers fail to separate the species pair *F. exsecta* and *F. fennica* despite established, although not clear cut, morphological differences. The genetic variation within the *F. exsecta/fennica* group is extensive, but does not reflect the proposed morphological differences. It is impossible to divide these samples into two separate species based on our molecular data. The geographically restricted sampling of this study does not allow full species delimitation, but the result concerning the status of *F. fennica* is clear. Finnish *F. fennica* populations studied so far should not be considered a separate species, but merely a morph of *F. exsecta*.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Sanja Maria Hakala conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Perttu Seppä and Heikki Helanterä conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Maria Heikkilä analyzed the data, prepared figures and/or tables, approved the final draft.
- Pekka Punttila performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Jouni Sorvari performed the experiments, contributed reagents/materials/analysis tools, approved the final draft.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The COI barcoding sequences were submitted to GenBank (and also included as a Supplemental File). GenBank accession numbers:

BankIt2134696 F_fennica_306 MH649366,
BankIt2134696 F_exsecta_fennica_234 MH649367.
BankIt2134696 F_pressilabris_264 MH649368.
BankIt2134696 F_pressilabris_390 MH649369.
BankIt2134696 F_manchu_27 MH649370.
BankIt2134696 F_pisarskii_25 MH649371.
BankIt2134696 F_forsslundi_342 MH649372.
BankIt2134696 F_suecica_400 MH649373.
BankIt2134696 F_fennica_314 MH649374.
BankIt2134696 F_fennica_288 MH649375.

Data Availability

The following information was supplied regarding data availability:

The sequence and microsatellite data are uploaded as Supplemental Files.

Supplemental Information

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REFERENCES

Abbott R, Albach D, Ansell S, Arntzen JW, Baird SJE, Bierne N, Boughman J, Brelsford A, Buerkle CA, Buggs R, Butlin RK, Dieckmann U, Eroukhmanoff F, Grill A, Cahan SH, Hermansen JS, Hewitt G, Hudson AG, Jiggins C, Jones J, Keller B, Marczewski T, Mallet J, Martinez-Rodriguez P, Möst M, Mullen S, Nichols R, Nolte AW, Parisod C, Pfennig K, Rice AM, Ritchie MG, Seifert B, Smadja CM, Stelkens R, Szymura JM, Väinölä R, Wolf JB, Zinner D. 2013. Hybridization and speciation. *Journal of Evolutionary Biology* **26**:229–246 DOI 10.1111/j.1420-9101.2012.02599.x.

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**:1–48.

Beresford J, Elias M, Pluckrose L, Sundström L, Butlin RK, Pamillo P, Kulmuni J. 2017. Widespread hybridization within mound-building wood ants in Southern Finland results in cytonuclear mismatches and potential for sex-specific hybrid breakdown. *Molecular Ecology* **26(15)**:4013–4026 DOI 10.1111/mec.14183.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22(3)**:148–155 DOI 10.1016/j.tree.2006.11.004.

Bortolus A. 2008. Error cascades in the biological sciences: the unwanted consequences of using bad taxonomy in ecology. *Ambio* **37**:114–118 DOI 10.1579/0044-7447(2008)37[114:ECITBS]2.0.CO;2.

Chapuisat M. 1996. Characterization of microsatellite loci in *Formica lugubris* B and their variability in other ant species. *Molecular Ecology* **5**:599–601 DOI 10.1111/j.1365-294X.1996.tb00354.x.

Clarke RT, Rothery P, Raybould AF. 2002. Confidence limits for regression relationships between distance matrices: estimating gene flow with distance. *Journal of Agricultural, Biological, and Environmental Statistics* **7**:361–372 DOI 10.1198/108571102320.

Corander J, Marttinen P, Mäntyniemi S. 2006. Bayesian identification of stock mixtures from molecular marker data. *Fishery Bulletin* **104**:550–558.

Corander J, Waldmann P, Sillanpää MJ. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* **163**:367–374.

Czechowski W. 1993. Hybrids in red wood ants. *Annales Zoologici* **44**:43–53.

Dayrat B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**:407–415 DOI 10.1111/j.1095-8312.2005.00503.x.

De Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**:879–886 DOI 10.1080/10635150701701083.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**:1792–1797 DOI 10.1093/nar/gkh340.

Figtree. 2018. Figtree graphical viewer of phylogenetic trees. *Available at http://tree.bio.ed.ac.uk/software/figtree/* (accessed on January 2018).

Forel A. 1874. *Les Fourmis de la Suisse*. Zürich: Société Helvétique des Sciences Naturelles.

Fraser DJ, Bernatchez L. 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology* **10**:2741–2752 DOI 10.1046/j.1365-294X.2001.t01-1-01411.x.

Funk DJ, Omland KE. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics* **34**:397–423 DOI 10.1146/annurev.ecolsys.34.011802.132421.

Goropashnaya AV, Fedorov VB, Pamilo P. 2004. Recent speciation in the Formica rufa group ants (Hymenoptera , Formicidae): inference from mitochondrial DNA phylogeny. *Molecular Phylogenetics and Evolution* **32**:198–206.

Goropashnaya AV, Fedorov VB, Seifert B, Pamilo P. 2012. Phylogenetic relationships of Palaearctic *Formica* species (Hymenoptera, Formicidae) based on mitochondrial cytochrome B sequences. *PLOS ONE* **7**:e41697 DOI 10.1371/journal.pone.0041697.

Goudet J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F - statistics. *Molecular Ecology Notes* **5**:184–186 DOI 10.1111/j.1471-8286.2004.00828.x.

Gyllenstrand N, Gertsch PJ, Pamilo P. 2002. Polymorphic microsatellite DNA markers in the ant *Formica exsecta*. *Molecular Ecology Notes* **2**:67–69 DOI 10.1046/j.1471-8286.2002.00152.x.

Hasegawa E, Imai S. 2004. Characterization of microsatellite loci in red wood ants *Formica* (s. str.) spp. and the related genus *Polyergus*. *Molecular Ecology Notes* **4**:200–203 DOI 10.1111/j.1471-8286.2004.00614.x.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**:313–321 DOI 10.1098/rspb.2002.2218.

Jansen G, Savolainen R, Vepsäläinen K. 2009. DNA barcoding as a heuristic tool for classifying undescribed Nearctic *Myrmica* ants (Hymenoptera: Formicidae). *Zoologica Scripta* **38**:527–536 DOI 10.1111/j.1463-6409.2009.00386.x.

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**:1403–1405 DOI 10.1093/bioinformatics/btn129.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**:94.

Kalinowski ST. 2011. The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. *Heredity* **106**:625–632.

Kontula T, Raunio A. 2009. New method and criteria for national assessments of threatened habitat types. *Biodiversity and Conservation* **18**:3861–3876 DOI 10.1007/s10531-009-9684-5.

Korczyńska J, Gajewska M, Pilot M, Czechowski W, Radchenko A. 2010. Genetic polymorphism in mixed colonies of wood ants (Hymenoptera: Formicidae) in southern Finland and its possible origin. *European Journal of Entomology* **107**:157–167 DOI 10.14411/eje.2010.021.

Kulmuni J, Seifert B, Pamilo P. 2010. Segregation distortion causes large-scale differences between male and female genomes in hybrid ants. *Proceedings of the National Academy of Sciences of the United States of America* **107**:7371–7376 DOI 10.1073/pnas.0912409107.

Mayr E. 1942. *Systematics and the origin of species, from the viewpoint of a zoologist*. New York: Columbia University Press, 334.

Mayr E. 1963. *Animal species and evolution*. Cambridge: Harvard University Press.

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments workshop (GCE)*. Piscataway: IEEE, 1–8.

National Red Lists. 2018. National Red List database. *Available at http://www.nationalredlist.org/* (accessed on January 2018).

Nei M. 1972. Genetic distance between populations. *The American Naturalist* **106**:283–292 DOI 10.1086/282771.

Ødegaard F. 2013. New and little known ants (Hymenoptera, Formicidae) in Norway. *Norwegian Journal of Entomology* **60**:172–175.

Pante E, Puillandre N, Viricel A, Arnaud-Haond S, Aurelle D, Castelin M, Chenuil A, Destombe C, Forcioli D, Valero M, Viard F, Samadi S. 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Molecular Ecology* **24**:525–544 DOI 10.1111/mec.13048.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**:288–295 DOI 10.1111/j.1471-8286.2005.01155.x.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**:2537–2539 DOI 10.1093/bioinformatics/bts460.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**:945–959.

Puechmaille SJ. 2016. The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* **16**:608–627.

Punttila P, Kilpeläinen J. 2009. Distribution of mound-building ant species (Formica spp. Hymenoptera) in Finland: preliminary results of a national survey. *Annales Zoologici Fennici* **46**:1–15 DOI 10.5735/086.046.0101.

Rassi P, Hyvärinen E, Juslén A, Mannerkoski I. 2010. *Suomen lajien uhanalaisuus—Punainen kirja 2010, The 2010 Red List of Finnish Species*. Helsinki: Ympäristöministeriö & Suomen ympäristökeskus.

Schlick-Steiner BC, Steiner FM, Seifert B, Stauffer C, Christian E, Crozier RH. 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. *Annual Review of Entomology* **55**:421–438 DOI 10.1146/annurev-ento-112408-085432.

Schultz R, Seifert B. 2007. The distribution of the subgenus *Coptoformica* Müller, 1923 (Hymenoptera: Formicidae) in the Palaearctic Region. *Myrmecological News* **10**:11–18.

Seifert B. 1999. Interspecific hybridisations in natural populations of ants by example of a regional fauna (Hymenoptera, Formicidae). *Insectes Sociaux* **46**:45–52 DOI 10.1007/s000400050111.

Seifert B. 2000. A taxonomic revision of the ant subgenus *Coptoformica* Mueller, 1923 (Hymenoptera, Formicidae). *Zoosystema* **22**:517–568.

Seifert B. 2009. Cryptic species in ants (Hymenoptera: Formicidae) revisited: we need a change in the alpha-taxonomic approach. *Myrmecological News* **12**:149–166.

Seppä P, Helanterä H, Trontti K, Punttila P, Chernenko A, Martin SJ, Sundström L. 2011. The many ways to delimit species: hairs, genes and surface chemistry. *Myrmecological News* **15**:31–41.

Smith MA, Fisher BL. 2009. Invasions, DNA barcodes, and rapid biodiversity assessment using ants of Mauritius. *Frontiers in Zoology* **6**:31 DOI 10.1186/1742-9994-6-31.

Smith MA, Fisher BL, Hebert PDN. 2005. DNA barcoding for effective biodiversity assessment of a hyperdiverse arthropod group: the ants of Madagascar. *Philosophical Transactions of the Royal Society B* **360**:1825–1834.

Sorvari J. 2009. Foraging distances and potentiality in forest pest insect control: an example with two candidate ants (Hymenoptera: Formicidae). *Myrmecological News* **12**:211–215.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313 DOI 10.1093/bioinformatics/btu033.

Suvák M. 2013. First record of *Formica fennica* Seifert, 2000 (Hymenoptera, Formicidae) in Norway. *Norvegian Journal of Entomology* **60**:73–80.

Toews DPL, Brelsford A. 2012. The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology* **21**:3907–3930 DOI 10.1111/j.1365-294X.2012.05664.x.

Trontti K, Tay WT, Sundström L. 2003. Polymorphic microsatellite markers for the ant *Plagiolepis pygmaea*. *Molecular Ecology Notes* **3**:575–577 DOI 10.1046/j.1471-8286.2003.00516.x.

Van Strien MJ, Keller D, Holderegger R. 2012. A new analytical approach to landscape genetic modelling: least-cost transect analysis and linear mixed models. *Molecular Ecology* **21**:4010–4023 DOI 10.1111/j.1365-294X.2012.05687.x.

Wallis GP, Cameron-Christie SR, Kennedy HL, Palmer G, Sanders TR, Winter DJ. 2017. Interspecific hybridization causes long-term phylogenetic discordance between nuclear and mitochondrial genomes in freshwater fishes. *Molecular Ecology* **26**:3116–3127 DOI 10.1111/mec.14096.

Ward PS. 2010. Taxonomy, phylogenetics, and evolution. In: Lach L, Parr CL, Abbott KL, eds. *Ant ecology*. Oxford: Oxford University Press, 3–17.

Social polymorphism in *Formica* ants is a result of dispersal behavior rather than dispersal morphology

Formica-muurahaisten sosiaalinen monimuotoisuus johtuu levittäytymiskäyttäytymisestä, ei levittäytymismorfologiasta

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III

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TIIVISTELMÄ

Levittäytymisen evoluutio ja sosiaalisuuden evoluutio ovat yhteydessä toisiinsa. Levittäytyminen on tarpeellista sukulaisten välisen kilpailun ja sisäsiitoksen välttämiseksi, mutta toisaalta levittäytymisen rajoittaminen mahdollistaa sukulaisten välisen hyödyllisen sosiaalisen vuorovaikutuksen. Muurahaisten monimutkaiset yhteiskunnat, joissa suuri osa tytärkuningattarista jää kotipesään, ovat yhteydessä heikkoon levittäytymiseen, mutta syy-seuraussuhteet eivät ole kunnolla tiedossa. Tässä tutkimuksessa tarkastelimme kuuden lähisukuisen *Formica*-lajin levittäytymiskykyä. Lajien yhteiskuntien kuningatarmäärät ja tyypilliset yhteiskunnan perustamistavat vaihtelevat, ja lajit ovat kolmesta eri alasuvusta. Tutkimalla näiden lajien nuorten tytärkuningatarten ja koiraiden ruumiiseen varastoituja resursseja pystyimme analysoimaan levittäytymiskyvyn ja sosiaalisen rakenteen evoluutiota. Mittasimme kaikenkaikkiaan 1954:n yksilön ruumiin mittasuhteita, kuten lentolihasten suhteellisen koon; glykogeenin, triglyseridin ja proteiinin määrät kolorimetrisillä menetelmillä; sekä lentolihasten mikroskooppisen rakenteen transmissioelektronimikroskopialla.

Tulostemme mukaan yksilöiden kunto ei ennusta sitä, millainen levittäytymisstrategia lajilla on. Tämä on jossain määrin vastoin aiemman hyönteisillä ja muurahaisilla tehdyn tutkimuksen mukaisia odotuksia. Sosiaalisella rakenteella on kuitenkin pieni yhteys koiraiden lentolihasten suhteelliseen kokoon. Tämä on mielenkiintoinen esimerkki sukupuolten yhteisevoluutiosta: todennäköisesti kuningatarten kotiin jääminen heijastuu vastaavana käytöksenä koirailla, ja lopulta myös niiden rakenteessa. Kaiken kaikkiaan koiraiden levittäytymiskyky on parempi kuin kuningatarten, mikä tukee hypoteesia muurahaisten levittäytymisen sukupuolittuneisuudesta.

Superkoloniaalisten *Formica*-kuningatarten lisääntynyt kotiin jääminen näyttää olevan käyttäytymispiirre eikä seuraus muuttuneesta levittäytymisresurssien jaosta. Kuningattaret voivat jättää levittäytymisen väliin, vaikka yhteiskunta antaa niille siihen resurssit. Tämä herättää uusia kysymyksiä levittäytymiseen liittyvistä ristiriidoista monikuningattarisilla *Formica*-lajeilla.

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Social polymorphism in *Formica* ants is a result of dispersal behavior rather than dispersal morphology

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ABSTRACT:
Dispersal evolution and social evolution are interlinked. Dispersal is necessary for avoiding kin competition and inbreeding, but limited dispersal also allows beneficial social interactions with kin. In ants, a correlation between poor dispersal and complex societies, where a big proportion of queens are philopatric, is well documented, but the underlying causal mechanisms are not clear. In this study we investigate the dispersal ability of six closely related *Formica* species that vary in colony queen numbers and typical nest founding modes, from three different subgenera. Investigating resource allocation in the bodies of young queens and males allows us to analyze the evolution of dispersal ability and social organization. We measured the body ratios including wing muscle ratio; glycogen, triglyceride and protein resources with colorimetric assays; and microscopic wing muscle structures with transmission electron microscopy, with an overall sample size of 1594 individual males and queens.

Our results suggest that the physical condition of individuals does not strongly correlate with the dispersal patterns of the species, somewhat contrary to assumptions based on earlier studies in both ants and other insects. There was still a minor effect of social organization on male flight muscle ratio, which is an interesting case of sexual coevolution of dispersal traits: it is likely that the queen philopatric behavior is reflected in male behavior and also in male morphology. Overall, according to our morphological proxies, males had better dispersal ability than queens, supporting the hypothesis that ant dispersal is sex biased regardless of social organization. In supercolonial *Formica* queens, increased philopatry seems to be more a behavioral trait rather than a consequence of resource allocation into dispersal ability. The queens may choose not to disperse even when the society provides them with the resources for it. This raises new questions about conflicts over dispersal in the highly polygynous *Formica* species.

INTRODUCTION

In natural populations, local resource competition and potential habitat destruction require that organisms move to new locations in order to survive on evolutionary time scales (Van Valen 1971). Resource competition is especially harmful with relatives, as it decreases inclusive fitness, which reinforces selection for dispersal – even in social animals that simultaneously also benefit from interactions with their relatives (Hamilton and May 1977, West et al. 2002). Dispersal also allows individuals to avoid breeding with their kin, which could lead to inbreeding depression (Bengtsson 1978). Due to these reasons, all organisms have evolved strategies to disperse away from their original habitats and away from their kin. Even sessile organisms, such as plants and many marine invertebrates, have mobile stages at some points of their life cycles.

Social insect colonies can be seen as superorganisms that are bound to their sedentary nesting sites and often only disperse through their mobile sexual castes, the young males and queens (Hölldobler and Wilson 2008, Helanterä 2016). For most social insects, dispersal by wing is an inseparable element of their reproductive strategy (Boomsma et al. 2005), and dispersal shapes the evolution of the sexual castes. For example in most ant species, the young queens and males leave their natal colonies for mating flights, after which the males die and the queens found new colonies (Hölldobler and Wilson 1990, Bourke and Franks 1995). Although the queens spend most of their adult lives within their colonies, the strong selection pressures during dispersal are a major driver of their evolution (Helms 2018). Flight-related trade-offs and resource allocation affect the evolution of the queens (Helms and Kaspari 2015), and thus the evolution of the colonies they found. The adult males, who are not directly involved in the colony social life, have an indirect effect through mating and dispersal strategies that coevolve with the queen strategies (Heinze and Tsuji 1995, Peeters and Aron 2017, Hakala et al. 2019)

In some ant species all young daughter queens disperse and found new independent colonies, whereas in others a proportion of them stays in their natal colonies as extra queens resulting in (secondarily) polygynous colonies. In such colonies queens reproduce cooperatively and share resources, including the worker force (Boomsma et al. 2014). In many ant taxa, polygyny is frequently accompanied with polydomous colony organization, where a society (colony) occupies several interconnected nests instead of a single one. Such a strategy allows more effective resource acquisition and often also a yet higher number of queens and thus an even larger colony size (Debout et al. 2007). In some taxa, polygynous and polydomous societies may develop into extremely large colonies with thousands – or even more – of egg-laying queens, when they are referred to as supercolonies (Helanterä et al. 2009). This kind of continuum from simple (monogynous with one queen and/or monodomous with one nest) to more complex societies (polygynous and polydomous) that can often be observed even among closely related species (Hölldobler and Wilson 1977), offers an informative study system for inferring the connection of social organization and dispersal, and their evolution.

The correlation between limited dispersal and complex societies in ants is well documented with gene flow analyses in several taxa (e.g. Seppä and Pamilo 1995; Sundström et al. 2005). Ant community analyses also support this: species with polydomous social organization seem to be poorer at reaching isolated areas (e.g. Mabelis 1994; Sorvari 2017). However, the exact evolutionary reasons and causal mechanisms for this correlation have not been assessed in detail. It is obvious that dispersal behavior differs between species with monodomous and highly polydomous social organization – philopatric queens are an inbuilt characteristic of the polydomous and supercolonial ant societies. One of the main unanswered questions is, whether the observed difference in dispersal is due to difference in the dispersal ability and morphology or whether it is an active behavioral choice.

The dispersal ability of insects is a sum of several physiological and morphological traits. As dispersal happens by wing, size of the flight muscles is one of the most important factors – in ants more precisely the size of the indirect flight muscles they use for contracting the thorax and thus moving the wings (Dudley 1999). In several insects, the thorax mass / body mass ratio (hereafter thorax ratio) has been identified as a key predictor for flight ability and dispersal (e.g. Srygley and Chai 1990; Thomas et al. 1998; Berwaerts et al. 2002; Steyn et al. 2016), including ants (Helms and Kaspari 2014, Helms and Godfrey 2016). The thorax is assumed to be mostly filled with flight muscles in most insects. However, ant females also have strong neck muscles inside their thoraxes, and they take different amounts of space inside the thorax in different species (Keller et al. 2014). Additionally, ant queens can histolyse their flight muscles (Janet 1907, Jones 1979, Hölldobler and Wilson 1990), which means that their presence in the thorax cannot be guaranteed without direct observation. Thus, the thorax ratio cannot be assumed to represent the flight ability in a straightforward way across different ant species. Measuring the muscle size directly by using the wing muscle mass/ body mass ratio (hereafter muscle ratio) is a better option. This measure correlates well with flight performance across a wide range of animal phyla (Marden 1987; 1989).

In addition to muscle size, also the structure of the muscles affects flight performance (Marden 2000). The

myofibril and mitochondria densities correlate with flight performance (Sohal et al. 1972, Fernandes et al. 1991, Rauhamäki et al. 2014). The mitochondrial inner membranes host the cytochrome c oxidase enzyme that catalyzes the reduction of oxygen to water in cellular respiration, and affects directly the metabolic rate and thus the flight performance of the individuals (Niitepõld et al. 2009, Rauhamäki et al. 2014). In ants, detailed studies on the microscopic muscle structures are rare, but a study in the socially polymorphic *Formica truncorum* showed that queens from polydomous colonies have slightly fewer mitochondria in their muscles than queens from monodomous colonies - suggesting poorer flight ability (Johnson et al. 2005). Another aspect of muscle structures that is especially important for ants, is the possibility of flight muscle histolysis. It is usually thought to happen only in queens during nest founding after the dispersal flight (Hölldobler and Wilson 1990), but this has not been widely tested. In many other insects, flight muscles can be histolyzed also before flight, and damage from histolysis may render the muscles useless even if they are still present (Fairbairn and Desranleau 1987).

In ants, as in many other insects, the main fuel for flight is glucose stored in the flight muscles and fat body as glycogen (Peakin 1964, Toom et al. 1976, Passera et al. 1990, Wegener 1996). Strong muscles may be enough to lift the ant in the air, but only sufficient glycogen reserves ensure a proper onset of flight. In many insects, also fatty acids are used as a biochemical fuel for prolonged flight (Chino and Downer 1982, Wegener 1996), but in ants they seem to be only used by queens as an energy source during the nest founding period (Keller and Passera 1989, Wheeler and Buck 1996). Males have been reported to have negligible amounts of stored fat (Boomsma and Isaaks 1985). Along with fatty acids, queens also store proteins to be used as energy source during nest founding, and these storage proteins form a substantial proportion of their total protein amount (Wheeler and Buck 1995, 1996, Wheeler and Martínez 1995). Several studies show that these biochemical traits correlate with flight and nest founding strategies in ants, both within-species and among-species: Queens participating in the mating flights and founding their nests independently have higher amounts of glycogen, fat and storage proteins, compared to queens not participating in the mating flights but instead founding their nests dependently with workers from their natal nest (Keller and Passera 1989, Passera and Keller 1990, Sundström 1995, Hahn et al. 2004). Similar results have been shown for the male glycogen amounts, although there are substantially less studies done on them (Passera and Keller 1990, Sundström 1995).

Due to energetic and physical constraints, there are trade-offs between the different dispersal traits. Especially queens face a strong trade-off between resources invested into flight ability and abdominal resource loading for nest founding (the found or fly hypothesis: Helms and Kaspari 2014, Helms and Kaspari 2015). Queens that stay in their natal colonies do not need resources for flight or nest founding, and therefore they could allocate more of their resources directly to reproduction. Ant males do not need resources for colony founding, but may face a similar trade-off with flight and other resources, especially testes size and sperm amount, as shown with other insect males (Saglam et al. 2008). Ant males rarely produce sperm after sexual maturation, and need to carry their whole supply of sperm during dispersal (Hölldobler and Bartz 1985, Boomsma et al. 2005, Baer and Boomsma 2006), which makes the amount of sperm a possible restriction for their flight ability.

Ant males are traditionally studied much less than the two female castes, and our knowledge on ant male dispersal is rather limited (Boomsma et al. 2005, Shik et al. 2013, Hakala et al. 2019). This is unfortunate, as ant dispersal is mating dispersal and thus strongly coevolves with mating strategies and mate localization. To fully understand ant dispersal evolution, it is crucial to study the strategies of both sexes, i.e. the full dispersal strategies of the species. It seems likely that ant dispersal is generally male biased due to higher evolutionary restrictions and trade-offs affecting the queens, but a study bias towards species with more complex social organization prevents concluding whether this is a general rule (Hakala et al. 2019). In *Formica* ants, for instance, dispersal seems to be especially strongly male biased in polygynous and polydomous species (Sundström et al. 2005), but a recent study shows that this pattern does not necessarily hold in a monodomous and only facultatively polygynous species, *F. fusca* (Johansson et al. 2018). Studying a wide range of species with varying social organizations is required in order to

assess whether the male biased dispersal in ants is a general rule.

In this study, we investigate the dispersal ability of three species pairs of *Formica* ants, from three different subgenera. The contrast of monodomous social organization and high levels of polygyny and polydomy in each of the three species pairs provides an excellent model for studying the evolution of dispersal ability and social organization. The queens of all of these species have wings, but it is unclear if the ability to fly and disperse is weakened in the polydomous species, where the queens regularly do not fly. Our main hypothesis is that philopatric queen behavior is reflected in their morphology, so that the polydomous species that disperse less have clearly smaller allocation to dispersal traits. Additionally, we expect male dispersal traits to be stronger overall than the queen traits. However, the hypotheses for male dispersal traits in connection to social organization are difficult to formulate due to lack of studies on ant male dispersal. It is unclear, whether and how the change in social organization affects mating competition or the costs of inbreeding for the haploid males and diploid queens, and how this in turn affects the coevolving dispersal strategies of the two sexes. We present two opposing hypotheses: 1. The selection pressures for both sexes are similar, and thus also male traits are weaker in polydomous than in monodomous species. 2. When female dispersal strategy shifts towards philopatry in polydomous societies, selection for male dispersal gets stronger, which leads to polydomous males evolving stronger dispersal traits.

MATERIALS AND METHODS

Species and populations:

We sampled six *Formica* species from three different subgenera: *F. pratensis* and *F. aquilonia* from the *F. rufa* group (red wood ants), *F. exsecta* and *F. pressilabris* from *Coptoformica*, and *F. fusca* and *F. cinerea* from *Serviformica*, forming three species pairs, each with a species with simple monodomous societies and a species with complex, highly polygynous and polydomous societies (Table 1). In *Formica* ants, polydomous colonies can generally grow very big, into supercolonies consisting of even thousands of nests (Markó et al. 2012). The two Coptoformica species have both types of

Table 1: The sampled *Formica* species and the social organization of the populations studied.

Taxonomic group	Species	Population	Coordinates		Social organization**				References
			x	y	mg	pg	pd	s	
Serviformica	*F. fusca*	Prästkullantie	59.98011	23.3492	x	x			Hannonen et al. 2002; Bargum et al. 2007; Ozan et al. 2013
		Hankoniementie	59.91213	23.25797	x	x			
		Joskär	59.8453	23.25593	x	x			
	F. cinerea	Råudden-Stenudden	59.82635	23.02146				x	Goropashnaya, et al. 2001
F. rufa group	*F. pratensis*	Koverhar	59.89058	23.19769	x				Helanterä et al. 2016
	F. aquilonia	Myggfors	59.98952	23.22813				x	Schultner et al. 2016
		Långstrand	59.94771	23.17163				x	
Coptoformica	*F. pressilabris*	Prästkullantie	59.98011	23.3492	x	x			Kennedy et al. 2014
		Särkkilen	59.95325	23.7011				x	Schultner et al. 2014)
	F. exsecta	Prästkullantie	59.98011	23.3492	x / x				Kennedy et al. 2014
		Joskär	59.8453	23.25593	x				Sundström et al. 2003
		Tvärminne	59.84441	23.24846	x / x				SH pers obs
		Öby	59.93966	23.19824				x	Hakala et al. 2018

** The social organization: mg = monogyny (one queen in a colony, monodomous = a single nest in a colony), pg = polygyny (several queens in a colony, monodomous), pd = polydomy (several nests in a colony), s = supercoloniality (huge polygynous and polydomous colonies). The social organization of these populations is analyzed in the referenced previous studies.

societies in our study area, and we sampled both for intraspecific analyses. For the main analyses including all of the species, only the typical monodomous populations of *F. exsecta* were included, leaving out the supercolony atypical for the area. Similarly, only the well-established large polydomous population of *F. pressilabris* is included in the among species analysis, leaving out the monodomous population inhabiting a clear-cut forest area and consisting of small nests only. All of the study populations are located in the vicinity of Tvärminne Zoological Station in southern Finland.

Field sampling:
We monitored natural field colonies of the six *Formica* species for maturation of sexual brood by gently excavating the mounds. The *F. rufa* group species were sampled solely from the colonies in the field to ensure uninterrupted development. We sampled young males and virgin daughter queens when they emerged on top of the nest. No individuals were sampled from within the nest, as their age and maturity could not be assessed without this behavioral signal. *Serviformica* species have underground colonies that cannot be monitored for exact timing of emergence of the sexuals without serious disruption of the nest structures. Thus for these species, nest material, workers and brood were sampled from the field colonies when the brood was in late pupal stage, and brought to the laboratory for maturation. The sexual individuals were subsequently sampled from the laboratory nests when they emerged on top of the nest, as described above. *Coptoformica* individuals were mostly sampled from the field colonies, but the sample set was supplemented with laboratory-grown individuals to ensure high enough sample size. *F. pressilabris* queens were never observed on top of their nests in the field colonies, and therefore they are all laboratory-grown. Sampling was mostly done in 2016, but the data for *F. fusca* and *F. cinerea* was supplemented in 2017 to ensure high enough sample sizes. The spermathecae of c. 20 daughter queens of each of the study species were dissected to ensure that they were unmated.

The laboratory nests were plastic boxes coated with fluon to prevent ants climbing out, and covered with a cloth to prevent males and queens flying out. The nests were watered daily and maintained with Bhatkar & Whitcomb diet (1970) in room temperature and natural light cycle. The laboratory rearing took place in Tvärminne Zoological Station (for 2016 samples) or in the Viikki Plant Growth Facilities in Helsinki (for 2017 samples).

Specimen handling and flight muscle ratio:
After sampling, the individuals were kept on ice to minimize water loss and usage of metabolic resources during the transfer. Each individual's wet mass was measured in three parts separately: head, thorax (with legs but without the wings), and abdomen. After weighing, specimens were snap frozen in liquid nitrogen and stored in -80 °C for muscle size and metabolic analyzes. As the size of these species varies considerably, thorax mass and abdomen mass ratios were calculated and used for further analyses instead of the absolute mass.

Flight muscle ratios were measured for a total of 650 specimens by dissection as described by Marden (1987). Individual specimens were weighed alone, except for the smallest *F. pressilabris* and *F. exsecta* males that were pooled within the nests in order to improve the repeatability of weighing. For these specimens, the final muscle sizes are nest averages.

Transmission electron microscopy
For each species and both sexes, nine individuals from three different nests were further prepared for transmission electron microscopy analysis of the flight muscles (total n=108). After weighing the individuals, the thorax was dissected to allow the fixation buffer to penetrate the muscle tissues. Legs and leg and neck muscle tissues were removed, and the petiole was cut off. The thorax was ventrally opened, but flight muscle tissue was kept intact inside the thorax. The specimen was submerged in fixation buffer containing 2% glutaraldehyde, 100mM Na-cacodylate buffer pH 7.4, 150mM saccharose, first for 2h at room temperature and subsequently for 22h at +4C. After fixation, samples were stored 1-2 months in a solution containing 2% formaldehyde, 100mM Na-cacodylate buffer pH 7.4 and 150mM saccharose.

Further sample handling was done in the Electron Microscopy Unit in the Institute of Biotechnology, University of Helsinki. The samples were post-fixed using 2% non-reduced OsO4 (EMS, Hatfield, PA) in H2O for 2h at room temperature; dehydrated in ethanol gradient (50%, 70%, 96%, 100%, 100%,

15 min. each) and 99.5% acetone (Sigma) for 30 min; infiltrated into low-viscosity resin (TAAB, LV Medium) using the following resin: acetone ratios: 1:1 (2h), 2:1 (2h), 1:0 (o/n), 1:0 (4h). The resin blocks were cured in 60^{o}C overnight. Mounting orientation of the final sample blocks was adjusted for observing cross sections of the myofibrils, verified by roundness of cross-sections of myofilaments.

For morphometric analyses for each specimen, systematic random sampling was used to obtain 10-14 images from different cells at the magnification of 2500x (single image area = 69.2 μm^2, n=1 240) with Jeol JEM-1400 transmission electron microscope, equipped with Gatan Orius SC 1000B bottom mounted CCD-camera. The collected 16-bit TEM images were stored in DM4 format, and processed using the software Microscopy Image Browser (MIB) (Belevich et al. 2016). The contrast was normalized such that the mean intensity for each image was set to 24000 and standard deviation of the intensity histogram to 3000 counts. After that the images were converted to the 8-bit format using intensity 12000 as a black point and 42000 as white point. To assess differences in mitochondrial and myofibril size and density, their profiles were manually traced using MIB and an interactive pen display. The mitochondria profiles were analyzed into two categories: 1) complete profiles and 2) the profiles clipped at the edges of the images. To assess the total mitochondria and myofibril areas (μm^2) per image, we used Stereology tool of MIB with the sampling grid size of 50 x 50 nm.

The analysis of individual cell organelles was done using a custom made plugin of MIB. For each image the following properties were extracted for each complete myofibril profile (n=9 757): CentroidX, CentroidY, TotalArea, minDiameterAverage (calculated by using ultimate erosion to find the middle line of the organelle profile; measuring the distance to the closest edge for each point of the middle line; converting the collected values to the diameter by multiplying them by 2 plus pixel size; and finally averaging the measurements); and from each complete mitochondrion profile (n=27 633): CentroidX, CentroidY, TotalArea, ThresholdedArea, RatioOfAreas, minDiameterAverage. For estimation of degradation of mitochondria we used an empirical threshold value of 140 and calculated the ratio of thresholded vs. original area for each mitochondrion.

To analyze the organization of myofibrils in our images, distances among the centroids of the closest complete myofibrils in our images were calculated by Delaunay triangulation ('delaunay' function in Matlab), excluding the distances on the outer border (freeBoundary option). The bordering distances do not represent true distances to the closest organelles as the neighboring organelles were clipped with the image edges. To get a measure independent from the size of the myofibrils, we analyze the relative standard deviation (=coefficient of variation) of their distances for each image. The smaller the CV value, the more evenly organized the myofibrils are.

To assess the shape of mitochondria in our images, a value for mitochondrion shape was calculated as follows:

$$Shape = 1 - \frac{\text{minDiameterAverage}}{2 * \sqrt{\text{TotalArea}/\pi}}$$

Thus, a completely round mitochondrion profile shape gets a value of 0, and the closer the value is to 1, the longer the profile shape is. Long mitochondria profile shapes (Shape > 0.5) indicate the existence of elongated, tubular mitochondria, although their amount in the cross-section images will be low in all cases due to the way elongated mitochondria align with the myofibrils (Yu et al. 2003).

Colorimetric assays for glycogen, triglyceride and protein analysis

Glycogen, triglyceride and protein concentrations were measured for 470 individuals from the same nests that were used for the muscle size measurements. All protocols were adjusted from Tennessen et al. (2014) to allow for measuring all concentrations from the same individuals. 384 well plate format was used to allow for small sample volumes. Individual ants were measured for most species, with the exception of *F. pressilabris* males and females, and small *F. exsecta* males that were analyzed pooled with the other individuals collected from the same nest to ensure reliable results. Body weight of 10mg was used as a cut-off for pooling the samples. The sample used in these measurements contained the thorax and abdomen. The head was not used as eye pigments may interfere with the measurement, and the head does not contain storage resources.

The deep frozen samples were homogenized with Qiagen Tissuelyser II machine and metal beads prior to adding buffer. To allow comparing the results of different sized individuals, the samples were standardized by their wet weight by using 10ul BPST buffer/ 1 mg of mass of the whole individual. Glycocen concentrations were analyzed by breaking the glycogen to glucose with amyloglucosidase and comparing the obtained total glucose concentration to the free glucose concentration of each sample as a paired assay with Sigma Aldrich Glucose (GO) Assay Kit. The triglyceride concentrations were analyzed with Sigma Aldrich Serum Triglyceride Determination Kit. Protein concentrations were analyzed with Sigma Aldrich Bradford Reagent. The absorbances were measured with the Enspire multimode plate reader.

Statistical analyses

The raw data were analyzed in R (R Core Team 2013) with the package lme4 (Bates et al. 2014), and estimates for p-values were obtained with the package lmerTest (Kuznetsova et al. 2017). Linear mixed models (LMM) were used for the body proportion and biochemical data. In all of these analyses, the measured variable was used as a response variable, and the social organization (monodomous/ polydomous), sex, and their interaction were used as fixed effects, except for the triglyceride measurements where there are data only for the queens and thus only the social organization is used as fixed effect. The species and nest were used as nested random effects. The equality of variances between males and females, as well as between the two social types, were tested with an F-test. As the variance of these data differ between the inspected classes in some cases, the reliability of LMM results was checked with robust LMM models that give more weight to samples with values closer to the mean, with the package robustlmm (Koller 2016).

Similar LMM analyses on body proportions and biochemical measurements were repeated for *F. exsecta* and *F. pressilabris* with additional samples from the divergent social organization (polydomous for *F. exsecta*, and monodomous for *F. pressilabris*) to analyze within-species variation. In these analyses the nest was used as a random effect. The polydomous *F. exsecta* population produces an extremely male-biased sex ratio, and thus our sample size for polydomous *F. exsecta* queens is very small (N=7). All of them were used for the muscle size analysis, and thus the effect of social organization on the biochemical resources was not analyzed for *F. exsecta* queens.

The microscopical muscle structure data were analyzed similarly with either LMMs or with generalized linear mixed models (GLMM) with gamma distribution and an identity link function. As most mitochondria profiles get small values of degradation with our initial thresholding, the initial values of degradation are not especially informative. Thus, the presence of high values of degradation (>10% of mitochondria profile area) was analyzed with a GLMM with binomial distribution and a logit link function. In all data for individual cell organelles the nested random effect structure was as follows: species/nest/individual/ image. For the image level measurements (total mitochondria/myofibril area and the CV of distances among myofibrils) the random effect structure was species/nest/individual.

All models were validated as instructed by (Zuur 2016). Benjamini-Hochberg procedure (1995) was used to correct p-values of all of the LMMs and GLMMs to decrease the false discovery rate arising from high number of measurements and corresponding statistical tests. False discovery rate of 0.05 was used in the correction.

The microscopic muscle structure data were combined into a single data set by calculating image means for the measured variables. Correlations among the measured variables for both sexes separately were visualized with the R package corrplot (Wei and Simko 2017). The associations between different measurements were inspected with Pearson's product moment correlation coefficient with the significance level $p = 0.01$. The dataset was centered, scaled and transformed (Box and Cox 1964), and linear discriminant analyses (LDA) were performed with the R package MASS (Venables and Ripley 2002) for males and queens separately to investigate whether the six species or the two social types differ overall by their dispersal traits. As none off the measured microscopical traits were too strongly intercorrelated (cut-off correlation coefficient 0.7) they were all kept in the analyses. The LDAs were repeated for 60% of the data, and the remaining 40% was used to validate the model accuracy in classifying the samples into the correct groups.

The body ratio and biochemical data were similarly combined into a single data set by calculating nest means for the measured variables. Correlations were analyzed and LDA performed as above. For males the LDA included the total body weight, abdomen ratio, muscle ratio, protein concentration and glycogen concentration. Thorax ratio was not included due to a strong correlation with abdomen ratio (correlation coefficient -0.86). For the queens the LDAs included abdomen ratio, muscle ratio, protein concentration, glycogen concentration and triglyceride concentration. Total body weight and thorax ratio were not included due to a strong correlation with abdomen ratio (correlation coefficients 0.71 and -0.81, respectively).

RESULTS

All results for all of the measured variables for each species, social organization and sex are summarized in Appendix Tables 1 and 2.

Body proportions and flight muscle ratio

The muscle ratios are higher in males (species averages vary between 0.23 - 0.31) than in queens (species averages 0.15 - 0.25) (Figure 1, Table 2). There is a trend of male muscle ratios being smaller in polydomous species than monodomous species, with no effect in queens, seen in the significant interaction in our models (Figure 1, Table 2). Polydomous species

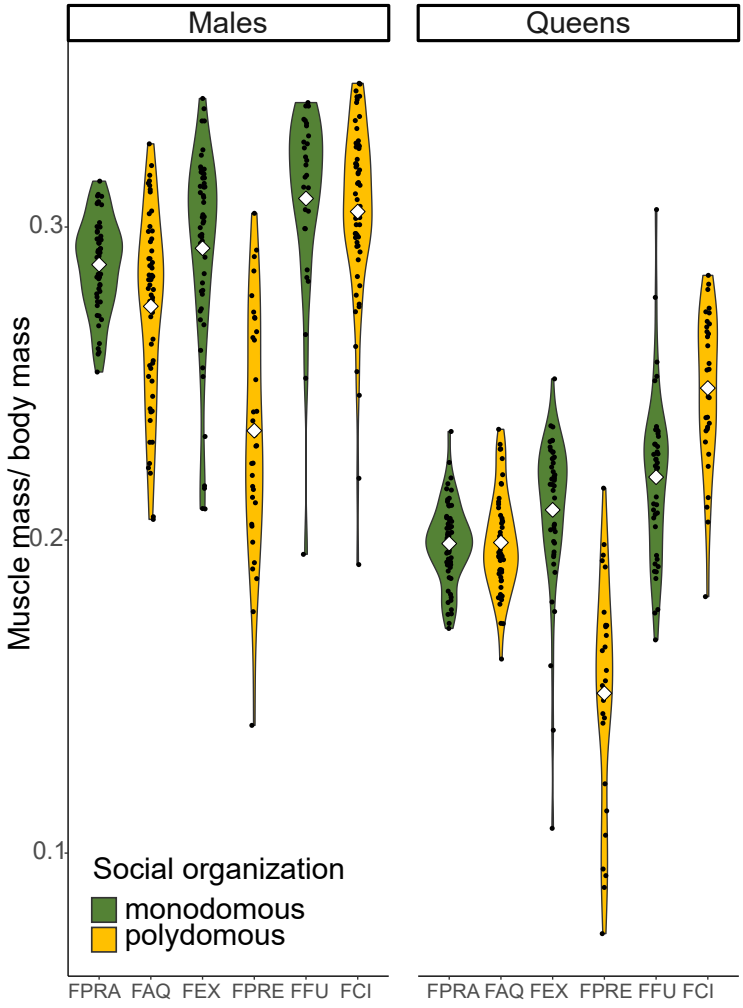

Figure 1. Flight muscle mass to body mass ratio by species and by sex. All data are visualized as data points and density plots, the mean is visualized with a diamond shape. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*. The males have significantly larger muscle ratios than the queens, and the monodomous males have larger muscle ratios than the polydomous males (Table 2).

Table 2: Analyses of the body proportions and biochemical measurements. In all of the analyses, the social organization (monodomous/polydomous), sex, and their interaction were used as fixed effects, except for the triglyceride measurements where there are data only for queens and thus only the social organization is used as fixed effect. Species and nest were used as random effects. The Benjamini-Hochberg procedure (BF, 1995) was used to correct the parameter p-values to decrease the false discovery rate. Tests where the p-value is smaller than the BH critical value are considered significant. Non-significant results are presented in grey.

Response / Parameter names	robust LMM			LMM				
Total body mass	**β**	**SE**	**t-value**	**β**	**SE**	**t-value**	**p**	**BH**
Intercept (Monodomous males)	0.0185080	0.0072089	2.567	1.820e-02	6.096e-03	2.985	0.0405	
Polydomous	-0.0060243	0.0101945	-0.591	-5.783e-03	8.621e-03	-0.671	0.5390	0.040
Queens	0.0056916	0.0002665	21.355	6.493e-03	3.210e-04	20.225	<2e-16	<0.001
Interaction	0.0004248	0.0003604	1.179	-5.105e-04	4.355e-04	-1.172	0.2414	0.027
Abdomen ratio								
Intercept (Monodomous males)	0.405258	0.029833	13.584	4.079e-01	2.569e-02	15.875	8.79e-05	
Polydomous	0.002636	0.042174	0.063	9.032e-04	3.631e-02	0.025	0.981344	0.05
Queens	0.012979	0.003493	3.716	1.482e-02	3.978e-03	3.726	0.000205	0.01
Interaction	0.007757	0.004741	1.636	4.371e-03	5.406e-03	0.809	0.418969	0.036
Thorax ratio								
Intercept (Monodomous males)	0.5231822	0.0120647	43.36	0.521735	0.010692	48.795	6.81e-07	
Polydomous	-0.0166650	0.0170384	-0.98	-0.016098	0.015088	-1.067	0.344	0.032
Queens	-0.0740592	0.0029078	-25.47	-0.075294	0.003251	-23.161	<2e-16	0.001
Interaction	0.0009972	0.0039677	0.25	0.003730	0.004438	0.840	0.401	0.036
Muscle ratio								
Intercept (Monodomous males)	0.298065	0.018023	16.538	0.295129	0.017316	17.044	5.91e-05	
Polydomous	-0.026567	0.025471	-1.043	-0.025746	0.024465	-1.052	0.350971	0.032
Queens	-0.087161	0.003240	-26.902	-0.085334	0.003814	-22.374	< 2e-16	0.002
Interaction	0.019357	0.004629	4.182	0.018642	0.005444	3.424	0.000692	0.01
Protein concentration								
Intercept (Monodomous males)	3.06562	0.21579	14.207	3.19579	0.20451	15.626	5.02e-08	
Polydomous	0.08502	0.30396	0.280	0.13086	0.28900	0.453	0.661	0.042
Queens	-1.46493	0.21629	-6.773	-1.47031	0.25704	-5.720	4.95e-08	0.007
Interaction	0.23503	0.29881	0.787	0.08137	0.35822	0.227	0.821	0.047
Glycogen concentration								
Intercept (Monodomous males)	1.15765	0.19927	5.809	1.2132	0.2094	5.793	0.00173	
Polydomous	-0.59650	0.27995	-2.131	-0.5851	0.2938	-1.991	0.10105	0.022
Queens	0.09018	0.13470	0.669	0.2220	0.1769	1.255	0.21057	0.025
Interaction	0.61968	0.17933	3.456	0.5486	0.2384	2.302	0.02197	0.014
Triglyceride concentration (queens)								
Intercept (Monodomous)	0.5550	0.1768	3.139	0.5430	0.1728	3.142	0.0371	
Polydomous	-0.2549	0.2490	-1.024	-0.2327	0.2433	-0.956	0.3963	0.034

also have larger variance in the mean muscle ratio than the monodomous species (Appendix Table 3).

The queens are overall larger than males of the same species (Table 2, Appendix Figure 1). There is a lot of size variation among the species, but it is not consistently explained by the social organization (Table 2, Appendix Figure 1). The body ratios are opposite in males and queens: the queens have larger abdomen ratios than males, whereas males have larger thorax ratios than queens (Table 2). *Coptoformica* queens have larger heads than other *Formica* queens, and their other body ratios are accordingly affected: especially the abdomens are smaller than in other *Formica* (Appendix Figure 1).

Microscopic muscle structure

Overall, the muscle structure analysis reveals that all species have well-structured flight muscles with rather evenly-organized myofibrils (average CV of distances per image for each species and sex varies between 0.218-0.257, Appendix Table 1). All of the studied species have large quantities of mitochondria almost filling the spaces among the myofibrils (on average, 40.0% of the total cross section area), though individual mitochondrium profile areas are small (Figure 2). There is large variation within species, but only small variation among species (Appendix Figures 2 and 3). There are signs of minor degradation of mitochondria, but it is not extensive (Appendix Figures 4 and 5). None of the differences in muscle structures can be explained by social organization or sex (Appendix Figures 2 and 3 and Table 4).

For the muscle structure data, the sex-specific LDA models assigned the samples to two groups based on social organization with 66% and 76% accuracy for queens and males, respectively. Even though the single measurements do not show differentiation, this suggests there is minor differentiation in overall muscle structures between the two social organizations. The LDA models were not able to find species-level differentiation based on muscle structures, with model accuracies of 37.23% for the queens and 49.16% for the males. (Figure 3)

Glycogen, triglyceride and protein analysis

There are species-specific differences in the amounts of metabolic resources, but none of this variation is consistently explained by the social organization (Table 2). Glycogen concentrations in males are similar in closely related species, whereas queens have large variation among the species regardless of the phylogeny (Figure 4a). The queens also have greater overall variation in glycogen concentration (Appendix Table 3).

The mean soluble protein concentrations are similar in the six species, with no effects of the social organization (Figure 4b, Table 2). Males have more

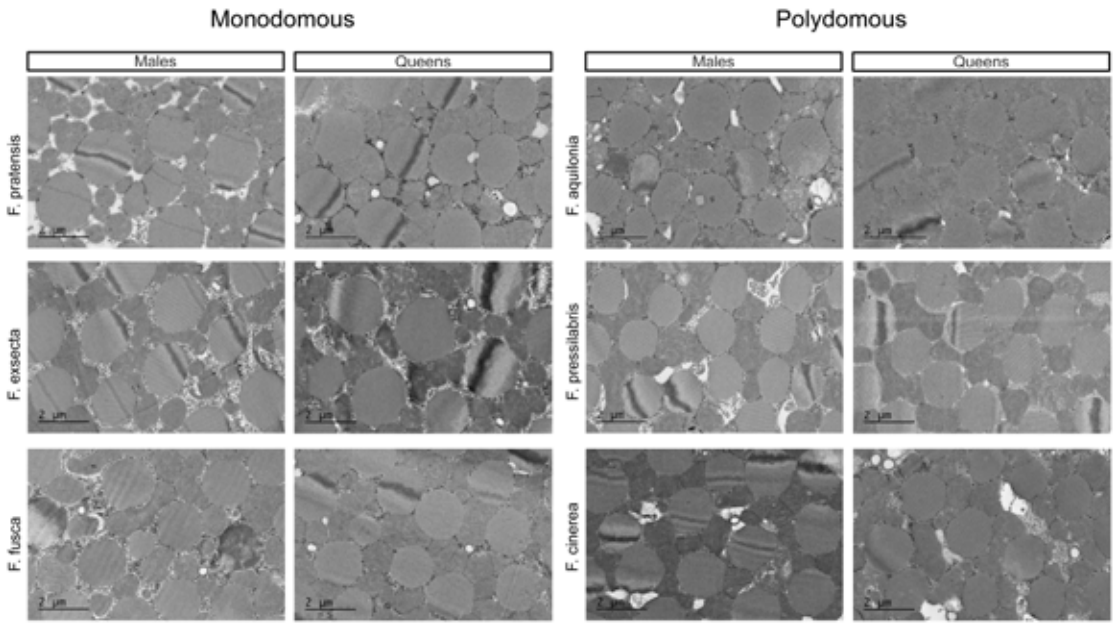

Figure 2. Typical transmission electron microscopy images of the flight muscle tissue for all of the sampled *Formica* species and both sexes. All species have functioning muscles, and there are no big differences in the mitochondria and myofibril sizes or structures among the species. Myofibril cross sections are visible as round, equally distributed shapes in the images. Mitochondria are the unevenly distributed oval shapes, with cristae visible inside. The black granules between the cell organelles are glycogen.

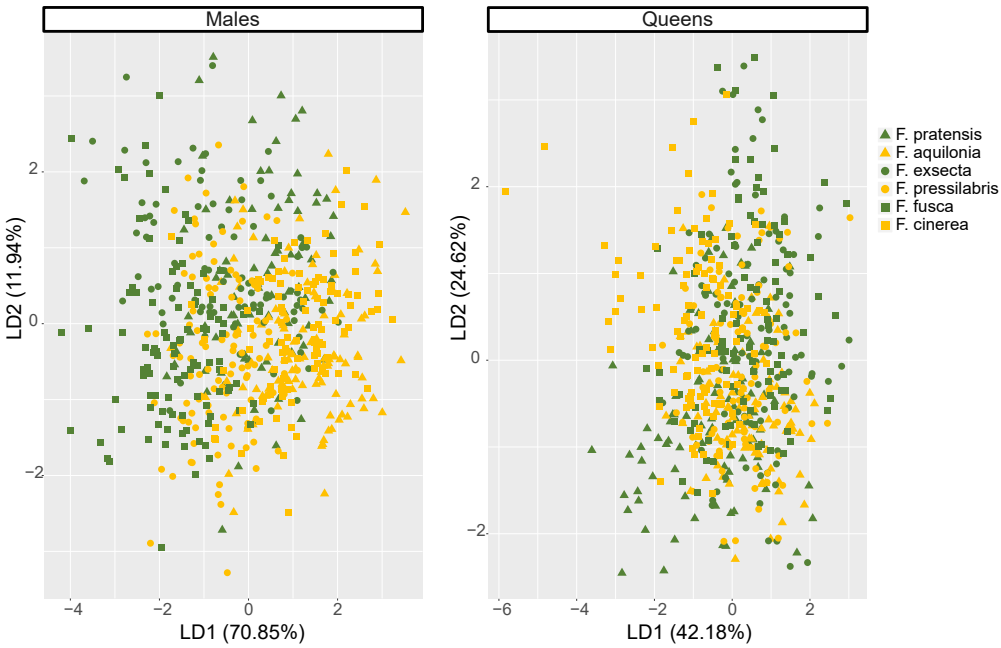

Figure 3: Linear discriminant analyses for each species by microscopical muscle structure data. The symbol denotes the subgenus: triangle = *Formica rufa* –group, circle= *Coptoformica*, rectangle: *Serviformica*. The color denotes social organization: dark green = monodomous, yellow = polydomous.

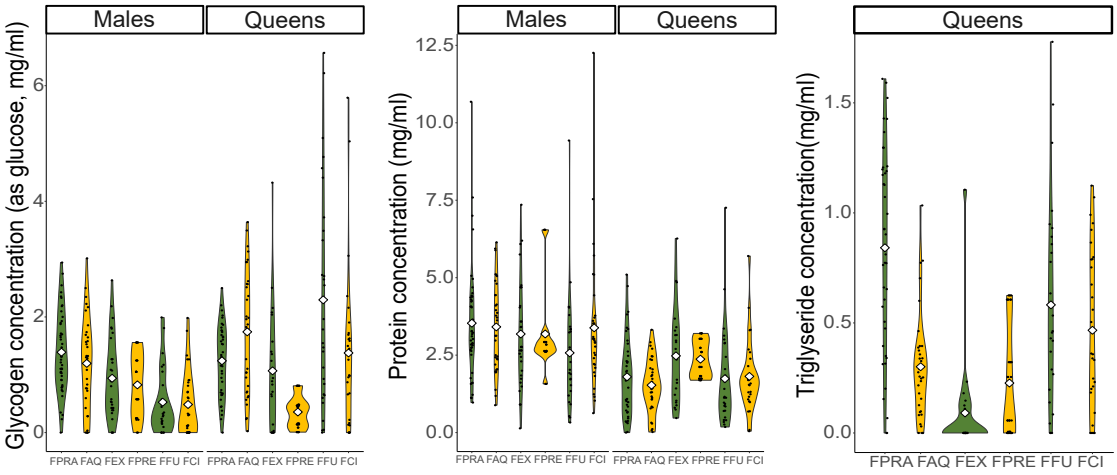

Figure 4: Biochemical measurements by species and by sex. All data are visualized as data points and density plots, the mean is visualized with a diamond shape. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*. The social organization of each species is highlighted with color: dark green = monodomous, yellow = polydomous. Social organization does not explain any of the variation (Table 2).
a) Glycogen concentration, b) protein concentration, c) triglyceride concentration for the queens.

protein than queens and the variation is larger in males than in queens (Figure 4b, Table 2, Appendix Table 3).

Males do not have measurable amounts of stored triglyceride – only 15.3% of the males in our dataset had values higher than zero, and some of these were among the pooled *F. pressilabris* individuals, making the actual number possibly even smaller. As it is likely that these rare positive values reflect their last meal rather than stored resources, the male triglyceride measurements were not analyzed further. Also some of the *Coptoformica* queens had such low amounts of triglyceride that it could not be measured with our assay. Overall the queen triglyceride concentration varies greatly among species (Figure 4c).

Overall differentiation

In both sexes, there is a correlation between individual size and muscle ratio: larger individuals also have proportionally larger muscles. The thorax ratio and abdomen ratio are strongly negatively correlated in both sexes. In queens, the body proportion and biochemical measurements correlate overall more strongly than in males, who don't have significant correlation among most of these measures. However, the thorax ratio and muscle ratio do not correlate in queens, but do correlate positively in males. (Appendix Figure 7)

For the body ratio and biochemical data, the sex-specific LDA models assigned the samples to groups based on social organization with 53% and 68% accuracy for queens and males, respectively, and to groups based on species with 53% and 74% accuracy for queens and males, respectively. Thus, the models fail to separate the queens, but function slightly better for the male data. For the queens the observed clustering reflects more the subgenus than the social organization or species, as the *Coptoformica* queens cluster separately from the other species with the first linear discriminant (Figure 5).

Within-species variation

F. exsecta has smaller individuals in the polydomous than in the monodomous populations, whereas *F. pressilabris* has no size difference between the social organizations – the individuals of this species are always very small. In *F. pressilabris*, the queens are slightly smaller than the males, which differs from the general pattern of in *Formica* where queens are usually the largest caste.

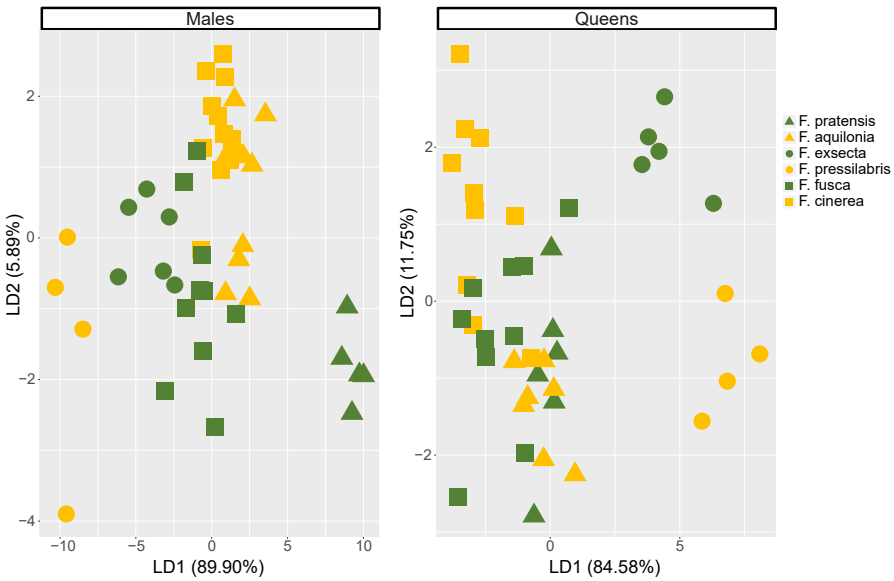

Figure 5: Linear discriminant analyses for each species by morphological and biochemical data. The symbol denotes the subgenus: triangle = *Formica rufa* –group, circle = *Coptoformica*, rectangle = *Serviformica*. The color denotes social organization: dark green = monodomous, yellow = polydomous.

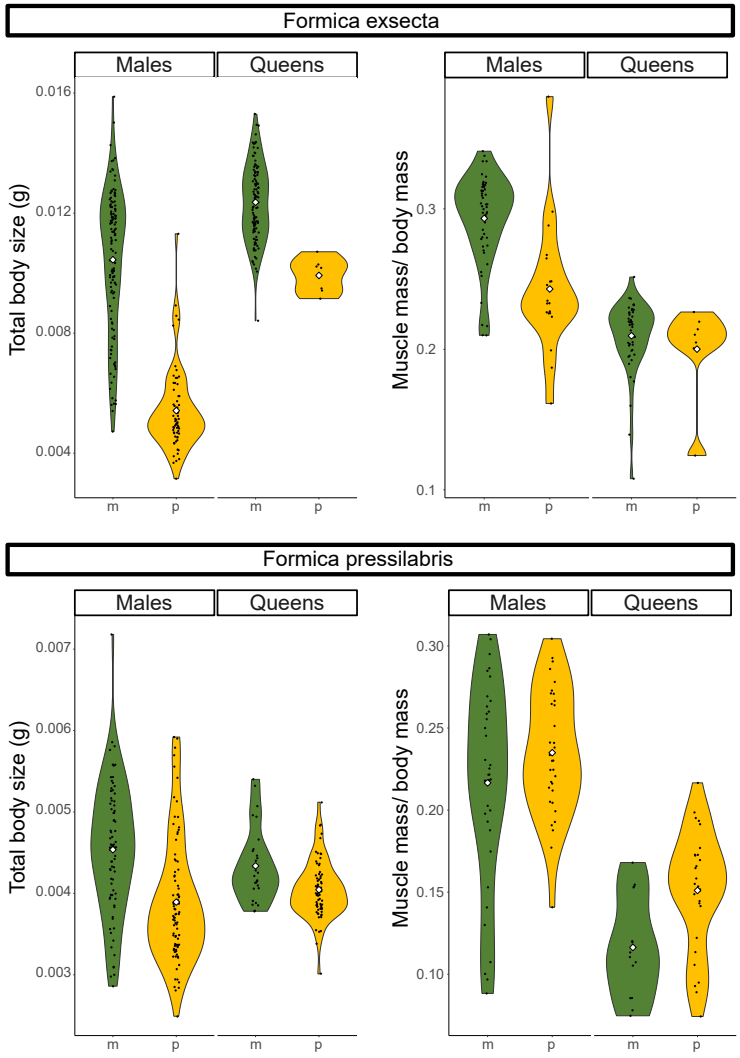

Figure 6: Total body size and muscle mass ratio by social organization and by sex for *F. exsecta* and *F. pressilabris*. All data are visualized as density plots (violin plots) and all data points are shown, the mean is visualized with a diamond shape. The color denotes social organization: dark green = monodomous (m), yellow = polydomous (p). The differences between the two social organizations are significant if *F. exsecta* but not in *F. pressilabris* (Appendix 2: Table 2).

In *F. exsecta*, both males and queens have smaller muscle ratio in the polydomous population than in the monodomous populations, whereas in *F. pressilabris* there is no effect of social organization on the muscle ratio. There are no intraspecific differences in the biochemical measurements between the two social organizations. (Appendix Tables 2 and 5).

DISCUSSION

Our hypothesis that the queens of the polydomous species would have smaller resource allocation to dispersal traits than queens of the monodomous species was not met. Thus, social polymorphism in *Formica* ants seems to be a result of queen behavior rather than queen dispersal morphology. However, male muscle ratios are smaller in the polydomous species, showing that their morphology is slightly differentiated between the two social organizations. The hypothesis for sex-biased dispersal ability was met, as males have larger flight muscle ratios than the queens across the genus.

Formica flight ability

In general, *Formica* muscle sizes are adequate for flight in all the studied species. Marden (1987) specifies a threshold of 12-16% of minimum muscle ratio for successful take off, and all of our species exceed this. The only exception is a proportion of *F. pressilabris* queens that have smaller muscle ratios than this,

suggesting that in this species the queen flight ability is partially compromised. *Formica pressilabris* seems to be overall the poorest flyer in our dataset, with by far the smallest muscle ratios in both sexes, and the lowest glycogen concentrations in the queens.

In addition to muscle ratios, also the muscle structures indicate functional muscles in all the species and both sexes. The total mitochondrial area, 40% of the muscle cross sections, compares well to other flying insects. *Libellula* dragonflies, good examples of strong flyers because they spend about a third of their time in flight (Marden et al. 1996), have only slightly larger mitochondria areas (46%, Marden 1989). Even *F. pressilabris*, with its smallest muscles, has good muscle structures, and is likely able to fly adequately when the muscles are large enough. When comparing the ant muscle structures to actively flying insects, there is one minor difference: flying insects often have very large and fused mitochondria that tightly envelope the myofibrils (Edwards and Ruska 1955, Sohal and Allison 1971, Sohal et al. 1972), whereas most of the mitochondria in our samples are singular and more or less round-shaped.

Of our study species, the dispersal of *F. exsecta* has been shown to happen mostly over short distances (Sundström et al. 2003, Vitikainen et al. 2015). *Formica exsecta* has rather average dispersal traits when compared to the other species in our dataset, suggesting that none of the species are exceptionally good dispersers. This is in line with studies on other ants. For example *Solenopsis invicta* queens seem to have resources for an average flight of only 45 minutes, which means they disperse long distances only passively by wind (Vogt et al. 2000).

Sex biased flight ability

Overall, the muscle ratios are larger in males, indicating that *Formica* males are stronger flyers than *Formica* queens, regardless of the social organization, concurring with the male-biased dispersal shown in gene flow analyses (Sundström et al. 2005). Interestingly, the glycogen concentration does not have any overall sex differences. It is possible that the queen and male flight has different energetic needs because of their different body shapes and larger abdomen drag in queens (Helms 2018). In *S. invicta*, queens increase their metabolic rate more than males during flight (Vogt et al. 2000). This means that the glycogen resources cannot be directly compared between the sexes. The sexes may also have different wing shapes, which can affect their flight energetics and dispersal ability, as has been shown in several other insects (e.g. Berwaerts et al. 2002; Hoffmann et al. 2007; Yao and Katagiri 2011).

In *F. fusca*, gene flow was recently shown to be unbiased between the sexes, or even slightly female biased (Johansson et al. 2018), in contrast to the general male bias in *Formica* ants (Sundström et al. 2005). In our data the males of this species still have larger muscles than the queens. However, the glycogen reserves of *F. fusca* males are low compared to other *Formica* males, and it seems possible that this limits their flight duration. If the two sexes disperse similar distances, or queens disperse only after mating, the unbiased gene flow patterns are easily explained (Johansson et al. 2018). Unfortunately observational studies on dispersal and mating behavior in natural populations have not been done for *F. fusca*.

The effect of social organization

Against our main hypothesis, monodomous queens do not have clearly higher allocation to dispersal traits than the polydomous queens. Dispersal abilities vary among species, showing the importance of species specific ecological and evolutionary constraints in dispersal evolution, but there is no overall pattern due to the social organization. Thus, queen philopatry in polydomous societies is likely a behavioral decision and not caused by the queen morphology. It is possible that we have missed the philopatric queens in our sampling, if they are rare or do not come to the nest surface even for mating – but this seems unlikely. For example in *F. pressilabris*, also the queens with so small flight muscle ratios that they are likely unable to fly, still appeared on the nest surface where we sampled them.

In our male data, the muscle ratios are overall slightly larger in monodomous than in polydomous males, indicating that flight ability is lower in polydomous males. Although this effect is small, it is biologically meaningful and indicates evolution on dispersal ability in connection to social organization. Muscle ratio is the most important trait measured in this study, as it straightforwardly affects the flight ability of the individuals (Marden 1987). Additionally, social organization affects the variance of the body proportions, including muscle ratio that varies more

in the polydomous species. This indicates that selection on mating and breeding strategies may be disruptive in polydomous societies and, as a result, they have more variation in dispersal traits compared to monodomous societies. If there is simultaneous selection for dispersal and philopatry in the queens, it is possible that the same is true for the males: if local mating with the philopatric queens is possible, some males may be selected not to allocate their resources to flight muscles. Due to low relatedness in polydomous societies, the risk of inbreeding is a smaller selection pressure in them than in monodomous societies, which makes evolution of increased philopatry more likely (Vitikainen et al. 2015, Hakala et al. 2019).

The combined microscopic muscle structures are slightly differentiated between the two social organizations based on LDA, but there is no such separation by species. It is not clear how this is reflected in the muscle function, but the result supports our conclusion that although the evolutionary changes in dispersal traits in connection to social organization are small in *Formica* ants, they do exist.

Coevolution of dispersal and mating

When considering the possibility of increased philopatry in *Formica* males, we need to consider their mating system – of which we have surprisingly scarce information apart from a general notion of it being complex. *Formica* queens seem to use pheromones to attract males, and males follow these cues to find them in the field. The males can also visually locate male aggregations that form when several males locate the same queens. Instead of mating away from their natal colony, queens can use their pheromones to attract males towards their natal colony, and likely do so in all of the polygynous and polydomous species where daughter queens are recruited back to their natal colony. However, the amount of studies analyzing mating behavior in *Formica* is very small and with a small number of species – from our study species only *F. aquilonia* and *F. exsecta* have been studied in this respect. (Kannowski and Johnson 1969, Cherix et al. 1991, 1993, Walter et al. 1993, Fortelius 2005, Martin et al. 2014).

In polydomous ants, there may be plenty of opportunities for local mating, and evolution of philopatric males seems possible in the light of our data on their dispersal morphology. In supercolonial *F. paralugubris* mating within the nest is very common based on genetic data (Chapuisat et al. 1997, Chapuisat and Keller 1999). Although there is no data on male behavior for all our study species, we hypothesize that it has changed in the polydomous species due to selection for local mating. As an extreme example, simultaneous selection for both local mating and dispersal explains the evolution of separate morphs in wing-dimorphic insects, due to a trade-off between sperm amount and flight (Zera and Denno 1997, Saglam et al. 2008). In ants such male dimorphism is very rare, but does exist in *Cardiocondyla* ants (Kinomura and Yamauchi 1987, Heinze and Hölldobler 1993). We do not have data on male sperm amounts, but we did measure their total protein amount that can be considered to somewhat represent the amount of seminal fluid, which contains large amounts of proteins (Avila et al. 2011). The concentrations vary substantially within species, showing that there are possibilities for selection. Despite the possibility, there is no correlation between muscle size and protein concentration in males, and no differences between the two social organizations, revealing no clear division to two different male types in general. However, in *F. exsecta*, there are two male size morphs (Fortelius et al. 1987) that we will discuss in a later section.

Proper analysis of both sperm quantity and mating behavior across the different social organizations would be interesting. Polydomous *Formica* is substantially easier to induce to mate in the laboratory (Fortelius 1987, but see also Martin et al. 2014), which indicates that in polydomous species there indeed has been evolution towards local mating, possibly through decoupling their mating propensity from flight.

Differing ecologies of the study species

Males have fewer significant correlations among the measured dispersal traits than the queens, and they can also be more accurately assigned to both species and social organization than queens with the LDA based on combined muscle ratio, body proportion and biochemical data. These results indicate that there may be slightly more diversification of male dispersal traits, possibly because males are under smaller evolutionary constraints and trade-offs than the queens and may thus be more free to evolve (Helms and Kaspari 2015, Hakala et al. 2019). One such evolutionary constraint for the queens is the distinct head shape of *Coptoformica*: they have long heads with strong jaw muscles because

workers chop grass into nest-building material, and the queens share this morphological feature with them (Seifert 2000). This likely restricts their other body proportions and thus their dispersal evolution – resulting in smaller abdomen ratios and smaller triglyceride amounts, compared to other *Formica* queens. These differences explain why they separate from other species by the first linear discriminant in our LDA.

As previously mentioned, such ecological and evolutionary constraints strongly affect dispersal. The most obvious ecological difference in our chosen study species is the mode of nest founding: Only the two *Serviformica* species are capable of independent nest founding, and of them only *F. fusca* is an obligate independent founder whereas *F. cinerea* can also use dependent founding (Collingwood 1979, Goropashnaya et al. 2001). The other study species are dependently founding or temporary social parasites that sneak into the nests of other *Formica* ants instead of founding their own nests (Collingwood 1979, Buschinger 2009). Following the logic of the found or fly hypothesis, which states that there is a trade-off between flight and nest founding abilities (Helms and Kaspari 2014, 2015), an obligatory independent founder should have higher nest founding resources and thus poorer flight resources compared to species that have other options for nest founding. This seems to be the case in fire ants where the leaner queens with proportionally larger muscles are able to fly longer, but act like parasites due to smaller nest founding resources (Helms and Godfrey 2016).

Based on our results in *Formica*, the found or fly trade-off does not play out as expected: the socially parasitic queens do not have larger muscle ratios or consistently poorer biochemical resources. On the contrary, the highest queen muscle ratios are in *Serviformica* species, and the highest triglyceride concentrations are in one of the social parasites, *F. pratensis*. The only obligate independent nest founder, *F. fusca*, has better queen resources for both flying and nest founding than average *Formica*. The pioneer ecology of *F. fusca* (Hannonen et al. 2004, Johansson et al. 2018) is likely to create strong selection pressure for both good dispersal ability and good nest founding ability, whereas the parasitic species may be under completely different selection pressures arising from their parasitic lifestyles (Brandt et al. 2005, Hakala et al. 2019).

Our study species are very differently sized, and thus we mostly discuss their dispersal resources as size-corrected measurements: ratios and concentrations. However, the relationship of actual body size, dispersal ability and colony resource allocation is very complex. Because metabolism scales with body size in such a way that larger individuals have relatively smaller metabolic rates, the energetic costs of all activities, including flight and maintenance of flight muscles, also depend on the body size (Chown and Nicolson 2004, Suarez et al. 2004, Chown et al. 2007). Thus, absolute body size also plays a role in dispersal ability. According to our results, the body size correlates positively with several of the size-corrected measurements, which indicates that larger individuals are overall more able to have good dispersal resources. Direct measurements of dispersal physiology and metabolic rates would aid in confirming this hypothesis. These kind of measurements might also reveal additional variation in the dispersal ability, which is not visible in the morphological proxies used in this study.

Smaller queens have long been associated with more complex ant societies, a phenomenon connected to the so-called polygyny syndrome, and ultimately limited dispersal (Rosengren and Pamilo 1983, Keller 1993). It is important to note, however, that this syndrome could also be called the polydomy syndrome, as Rosengren and Pamilo (1983) and Keller (1993), among others, mostly investigated species that are polygynous and polydomous instead of purely polygynous. Producing smaller sexuals is a sign of smaller colony level resource allocation in them. According to our results the body size is not consistently explained by social organization at the species level: the *F. rufa* group species and *Coptoformica* species follow this pattern, whereas the *Serviformica* species do not. It is possible that the socially polymorphic *F. cinerea* (Goropashnaya et al. 2001) has transitioned to polydomous colony structure evolutionarily so recently that it does not show the expected size difference when compared to its relative *F. fusca*.

Within-species variation

F. exsecta males are size dimorphic: tiny males are found in predominantly polygynous populations, and large males in monogynous populations (Fortelius et al. 1987). Monogynous populations have rather large male size variation (Vitikainen et al. 2015), and it is easy to hypothesize that all populations have the

potential to produce small males. However, for some reason small males are selected for in the polydomous populations, leading to reduction or loss of big males in them. Previously smaller males have been considered better dispersers (Fortelius et al. 1987), but in this work the small male size correlates with substantially lower muscle mass / body mass ratios and thus likely with poorer dispersal at least through active flight. More work on dispersal behavior of *F. exsecta* males is needed to resolve this contradiction. In addition to the males, also *F. exsecta* queens are smaller in the polydomous population compared to monodomous populations. The evolution of decreased size of sexuals in polydomous *F. exsecta* might reflect the same selection pressures that have led to the evolution of tiny sexual castes in the closely related *F. pressilabris*.

Interestingly, although both *F. exsecta* and *F. pressilabris* are socially polymorphic, only *F. exsecta* has a size difference between the two social organizations. Additionally, in *F. exsecta* but not in *F. pressilabris*, muscle ratios are smaller in the polydomous population in both sexes. *Formica exsecta* has monodomous background populations, from which polydomous subpopulations occasionally arise (Seppä et al. 2004). Thus, it may have more evolutionary flexibility than *Formica pressilabris*, whose populations are more commonly polydomous (Czechowski 1975, Collingwood 1979, Seifert 2000). *Formica pressilabris* may have lost its variability of dispersal traits and exhibit only the more derived polydomous traits.

Variation of traits is the currency evolution uses – not just genetic variation but to some extent also plastic variation (Pfennig et al. 2010, Moczek et al. 2011). In our study, the variation of most of the measured traits is large. Such variation could lead to condition dependent dispersal decisions at the individual level (Bowler and Benton 2004), as it seems to do in *F. truncorum* (Sundström 1995). The switch from monodomous to polydomous social organization likely starts as a plastic response to environmental pressure and individual condition. The polydomous populations may subsequently lose some of their original plasticity and variation, leading to more fixed traits. This could explain the evolution of small sexuals in commonly polydomous and supercolonial *F. pressilabris*, although the exact selection pressures should be further analyzed.

Possibility of dispersal conflict

As the supercolonial *Formica* queens are able to fly but are still philopatric, the societies seem to waste substantial amounts of resources when producing them. The societies provide the young queens with functional flight muscles but many of the young queens do not use them. We suggest the high levels of philopatry could be interpreted as a selfish behavior of the young queens, who avoid the risks of dispersal and are able to start reproducing sooner. Selfish behavior is hypothesized to increase in supercolonial ant societies (Helanterä et al. 2009) due to low relatedness among nestmates. It indeed seems that the supercolonial *Formica* societies have more queens than they need, even hundreds per nest and thousands per polydomous colony (Stockan and Robinson 2016). If excessive amounts of queens stay in their natal nest, the behavior of supernumerary queens can be seen as almost parasitic (Rosengren et al. 1993a).

We further suggest, that increased selfish philopatry of the queens may lead to conflict over dispersal in the supercolonial societies, where the other members of the society would prefer the young queens to disperse more than they are willing to (Motro 1983, Starrfelt and Kokko 2010, Hakala et al. 2019). According to previous theory, the polygynous (and polydomous) populations of *Formica* have density dependent selection for both dispersing and philopatric queens (Rosengren et al. 1993). However, to our knowledge this theory has not been rigorously tested, or even precisely analyzed for complex haplodiploid societies, and no density dependence in their dispersal has been shown. Instead, it is possible that in the extremely polygynous *Formica* populations there is a feedback loop between philopatry leading to low relatedness that leads to more selfish queens that are even more philopatric (Hakala et al. 2019).

CONCLUSIONS

All *Formica* species in our study have adequate dispersal resources, even though queens in half of our study species are highly philopatric. It seems that overall dispersal does not correlate well with morphological proxies for dispersal ability at the species level, but it is more of a behavioral trait. A recent study showed that in the Mediterranean fruit fly a measure of flight performance (vertical flight force output) did not differ between individuals classified as dispersers

vs. sedentary, whereas the number and duration of voluntary flight bursts did (Steyn et al. 2016). Together with our results, this suggests that the ability to disperse may not be the main driver of the actual dispersal events in nature, but behavior has a large role. This is in line with the notion that evolution can be driven by behavior, followed by morphological changes (Diogo 2017).

Thus, evolution of polydomous and supercolonial societies in *Formica* is indeed a result of a change in queen dispersal behavior, as suggested already by Rosengren et al. (1993). There is also some evolution of male dispersal morphology that we assume to be related to increased behavioral philopatry in males, too. Male morphology may have changed more freely than the queen morphology because it is evolutionarily less restricted (Hakala et al. 2019). This would be a novel example of sexual coevolution in ants that are usually studied for their social female traits alone.

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REFERENCES

Avila, F. W., L. K. Sirot, B. A. LaFlamme, C. D. Rubinstein, and M. F. Wolfner. 2011. Insect seminal fluid proteins: identification and function. Annual Review of Entomology 56:21–40.

Baer, B., and J. J. Boomsma. 2006. Mating biology of the leaf-cutting ants *Atta colombica* and *A. cephalotes*. Journal of Morphology 267:1165–1171.

Bargum, K., H. Helanterä, and L. Sundström. 2007. Genetic population structure, queen supersedure and social polymorphism in a social Hymenoptera. Journal of Evolutionary Biology 20:1351–1360.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2014. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1–48.

Belevich, I., M. Joensuu, D. Kumar, H. Vihinen, and E. Jokitalo. 2016. Microscopy Image Browser: A Platform for segmentation and analysis of multidimensional datasets. PLoS Biology 14:1–13.

Bengtsson, B. O. 1978. Avoiding inbreeding: at what cost? Journal of Theoretical Biology 73:439–444.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate : a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological) 57:289–300.

Berwaerts, K., H. Van Dyck, and P. Aerts. 2002. Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. Functional Ecology 16:484–491.

Bhatkar, A., and W. H. Whitcomb. 1970. Artificial diet for rearing various species of ants. The Florida Entomologist 53:229.

Boomsma, J. J., B. Baer, and J. Heinze. 2005. The evolution of male traits in social insects. Annual Review of Entomology 50:395–420.

Boomsma, J. J., D. B. Huszár, and J. S. Pedersen. 2014. The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. Animal Behaviour 92:241–252.

Boomsma, J. J., and J. A Isaaks. 1985. Energy investment and respiration in queens and males of *Lasius niger* (hymenoptera, formicidae). Behavioral Ecology and Sociobiology 18:19–27.

Bourke, A. F. G., and N. R. Franks. 1995. Social Evolution in Ants. Princeton University Press, Princeton, UK.

Bowler, D. E., and T. G. Benton. 2004. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80:205–225.

Brandt, M., S. Foitzik, B. Fischer-Blass, and J. Heinze. 2005. The coevolutionary dynamics of obligate ant social parasite systems - Between prudence and antagonism. Biological Reviews of the Cambridge Philosophical Society 80:251–267.

Buschinger, A. 2009. Social parasitism among ants: a review (Hymenoptera: Formicidae). Myrmecological News 12:219–235.

Chapuisat, M., J. Goudet, and L. Keller. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. Evolution 51:475–482.

Chapuisat, M., and L. Keller. 1999. Extended family structure in the ant *Formica paralugubris*: the role of the breeding system. Behavioral Ecology and Sociobiology, 46(6), 405-412.

Cherix, D., D. Chautems, D. J. C. Fletcher, W. Fortelius, G. Gris, L. Keller, L. Passera, R. Rosengren, E. L. Vargo, and F. Walter. 1991. Alternative reproductive strategies in *Formica lugubris* Zett (Hymenoptera, Formicidae). Ethology Ecology & Evolution. 3:61–66.

Cherix, D., D. J. C. Fletcher, D. Chautems, W. Fortelius, G. Gris, L. Keller, R. Rosengren, E. L. Vargo, and F. Walter. 1993. Attraction of the sexes in *Formica lugubris* Zett (Hymenoptera: Formicidae). Insectes Sociaux 40:319–324.

Chino, H., and R. G. H. Downer. 1982. Insect hemolymph lipophorin: A mechanism of lipid transport in insects. Advances in Biophysics 15:67–92.

Chown, S. L., E. Marais, J. S. Terblanche, C. J. Klok, J. R. B. Lighton, and T. M. Blackburn. 2007. Scaling of insect metabolic rate is inconsistent with the nutrient supply network model. Functional Ecology 21:282–290.

Chown, S. L., and S. W. Nicolson. 2004. Insect physiological ecology. mechanisms and patterns. Oxford University Press, Oxford.

Collingwood, C. 1979. The Formicidae (Hymenoptera) of Fennoscandia and Denmark. Fauna Entomolgica Scandinavica 8:64.

Czechowski, W. 1975. Bionomics of *Formica (Coptoformica) pressilabris* Nyl. (Hymenoptera, Formicidae). Annales Zoologici 33:103–125.

Debout, G., B. Schatz, M. Elias, and D. Mckey. 2007. Polydomy in ants: what we know, what we think we know, and what remains to be done. Biological Journal of the Linnean Society 90:319–348.

Diogo, R. 2017. Evolution driven by organismal behavior - a unifying view of life, function, form, mismatches and trends. Springer International Publishing.

Dudley, R. 1999. The biomechanics of insect flight: form, function, evolution. Princeton University Press, Princeton.

Edwards, G., and H. Ruska. 1955. The function and metabolism of certain insect muscles in relation to their structure. Journal of Cell Science s3-96:151–159.

Fairbairn, D. J., and L. Desranleau. 1987. Flight threshold, wing muscle histolysis, and alary polymorphism: correlated traits for dispersal tendency in the *Gerridae*. Ecological Entomology 12:13–24.

Fernandes, J., M. Bate, and K. Vijayraghavan. 1991. Development of the indirect flight muscles of *Drosophila*. Development 113:67–77.

Fortelius, W. 1987. Different patterns of female behaviour in mono- and polydomous *Formica* populations. in Chemistry and biology in social insects. J. Peperny.

Fortelius, W. 2005. Mating behaviour in the polygynous/polydomous wood ant *Formica aquilonia*. Annales Zoologici Fennici 42:213–224.

Fortelius, W., P. Pamilo, R. Rosengren, and L. Sundström. 1987. Male size dimorphism and alternative reproductive tactics in *Formica exsecta* ants (Hymenoptera, Formicidae). Annales Zoologici Fennici 24:45-54.

Goropashnaya, A. V., P. Seppä, and P. Pamilo. 2001. Social and genetic characteristics of geographically isolated populations in the ant *Formica cinerea*. Molecular Ecology 10:2807–2818.

Hahn, D., R. Johnson, N. Buck, and D. Wheeler. 2004. Storage protein content as a functional marker for colony-founding strategies: a comparative study within the harvester ant genus *Pogonomyrmex*. Physiological and biochemical zoology. 77:100–108.

Hakala, S. M., P. Seppä, M. Heikkilä, P. Punttila, J. Sorvari, and H. Helanterä. 2018. Genetic analysis reveals Finnish *Formica fennica* populations do not form a separate genetic entity from *F. exsecta*. PeerJ 6:e6013.

Hakala, S. M., P. Seppä, and Helanterä. 2019. Evolution of dispersal in ants (Hymenoptera: Formicidae): a review on the dispersal strategies of sessile superorganisms. Myrmecological News 29:35–55.

Hamilton, W., and R. May. 1977. Dispersal in stable habitats. Nature 269:578–581.

Hannonen, M., H. Helanterä, and L. Sundström. 2004. Habitat age, breeding system and kinship in the ant *Formica fusca*. Molecular Ecology 13:1579–1588.

Hannonen, M., M. Sledge, S. Turillazzi, and L. Sundström. 2002. Queen reproduction, chemical signalling and worker behaviour in polygyne colonies of the ant *Formica fusca*. Animal Behaviour 64: 477-485.

Heinze, J., and B. Hölldobler. 1993. Fighting for a harem of queens: physiology of reproduction in *Cardiocondyla* male ants. Proceedings of the National Academy of Sciences of the United States of America 90:8412–8414.

Heinze, J., and K. Tsuji. 1995. Ant reproductive strategies. Researches on Population Ecology 37:135–149.

Helanterä, H. 2016. An Organismal perspective on the evolution of insect societies. Frontiers in Ecology and Evolution 4:1–12.

Helanterä, H., J. Kulmuni, and P. Pamilo. 2016. Sex allocation conflict between queens and workers in *Formica pratensis* wood ants predicts seasonal sex ratio variation. Evolution 70:2387–2394.

Helanterä, H., J. E. Strassmann, J. Carrillo, and D. C. Queller. 2009. Unicolonial ants: where do they come from, what are they and where are they going? Trends in Ecology & Evolution 24:341–9.

Helms, J. 2018. The flight ecology of ants (Hymenoptera: Formicidae). Myrmecological News 26:19–30.

Helms, J. A., and A. Godfrey. 2016. Dispersal polymorphisms in invasive fire ants. PLoS ONE 11:1–20.

Helms, J. A., and M. Kaspari. 2014. Found or fly: Nutrient loading of dispersing ant queens decreases metrics of flight ability (Hymenoptera: Formicidae). Myrmecological News 19:85–91.

Helms, J. A., and M. Kaspari. 2015. Reproduction-dispersal tradeoffs in ant queens. Insectes Sociaux 62:171–181.

Hoffmann, A. A., E. Ratna, C. M. Sgrò, M. Barton, M. Blacket, R. Hallas, S. De Garis, and A. R. Weeks. 2007. Antagonistic selection between adult thorax and wing size in field released *Drosophila melanogaster* independent of thermal conditions. Journal of Evolutionary Biology 20:2219–2227.

Hölldobler, B., and H. S. Bartz. 1985. Sociobiology of reproduction in ants. Pages 237–257 in Experimental Behavioral Ecology and Sociobiology (editors Hölldobler, B. and Lindauer, M.). Gustav Fischer Verlag, Stuttgart.

Hölldobler, B., and E. O. Wilson. 1977. The number of queens: An important trait in ant evolution. Naturwissenschaften 64:8–15.

Hölldobler, B., and E. O. Wilson. 2008. The Superorganism. WW Norton & Company Inc.

Hölldobler, B., and O. E. Wilson. 1990. The ants. Harvard University Press, Cambridge.

Janet, C. 1907. Anatomie du corselet et histolyse des muscles vibrateurs, apres le vol nuptial, chez la reine de la fourmi (*Lasius niger*). Limoges, Imprimerie Ducourtieux et Gout:1–149.

Johansson, H., P. Seppä, H. Helanterä, K. Trontti, and L. Sundström. 2018. Weak population structure in the ant *Formica fusca*. PeerJ 6:e5024.

Johnson, C. A., L. Sundström, and J. Billen. 2005. Development of alary muscles in single- and multiple- queen populations of the wood ant *Formica truncorum*. Annales Zoologici Fennici 42:225–234.

Jones, R. 1979. The structure, development and degeneration of the flight muscles in the imported fire ant *Solenopsis invicta* an ultrastructural investigation. Ph.D. Dissertation. Texas A&M University, College Station, Texas, USA.

Kannowski, P. B., and R. L. Johnson. 1969. Male patrolling behaviour and sex attraction in ants of the genus *Formica*. Animal Behaviour 17:425–429.

Keller, L. 1993. Queen number and sociality in insects. Oxford University Press, Oxford.

Keller, L., and L. Passera. 1989. Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). Oecologia 80:236–240.

Keller, R. A., C. Peeters, and P. Beldade. 2014. Evolution of thorax architecture in ant castes highlights trade-off between flight and ground behaviors. eLife 3: e01539.

Kennedy, P., T. Uller, and H. Helanterä. 2014. Are ant supercolonies crucibles of a new major transition in evolution? Journal of Evolutionary Biology 27:1784–96.

Kinomura, K., and K. Yamauchi. 1987. Fighting and mating behaviors of dimorphic males in the ant - *Cardiocondyla wroughtoni*. Journal of Ethology 5:75–81.

Koller, M. 2016. "robustlmm: An R package for robust estimation of linear mixed-effects models." Journal of Statistical Software 75:1–24.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest package: tests in linear mixed effects models. Journal of Statistical Software 82(13).

Mabelis, A. 1994. Flying as a survival strategy for wood ants in a fragmented landscape (Hymenoptera, Formicidae). Memorabilia Zoologica 48:147–170.

Marden, J. H. 1987. Maximum lift production during takeoff in flying animals. Journal of Experimental Biology 130:235–238.

Marden, J. H. 1989. Bodybuilding dragonflies: costs and benefits of maximizing flight muscle. Physiological Zoology 62:505–521.

Marden, J. H. 2000. Variability in the size, composition, and function of insect flight muscles. Annual Review of Physiology 62:157–178.

Marden, J. H., M. G. Kramer, and J. Frisch. 1996. Age-related variation in body temperature, thermoregulation and activity in a thermally polymorphic dragonfly. Journal of Experimental Biology 199:529–535.

Markó, B., Z. Czekes, K. Eros, E. Csata, and A. M. Szász-Len. 2012. The largest polydomous system of *Formica* ants (Hymenoptera: Formicidae) in Europe discovered thus far in Romania. North-Western Journal of Zoology 8:287–291.

Martin, S. J., S. Shemilt, and K. Trontti. 2014. Nest-mate recognition cues are not used during or influenced by mating in the ant *Formica exsecta*. Ethology Ecology & Evolution 26:40–48.

Moczek, A. P., S. Sultan, S. Foster, C. Ledón-Rettig, I. Dworkin, H. F. Nijhout, E. Abouheif, and D. W. Pfennig. 2011. The role of developmental plasticity in evolutionary innovation. Proceedings of the Royal Society B: Biological Sciences 278:2705–13.

Motro, U. 1983. Optimal rates of dispersal. III. Parent-offspring conflict. Theoretical Population Biology 23:159–168.

Niitepõld, K., A. D. Smith, J. L. Osborne, D. R. Reynolds, L. Norman, A. P. Martin, J. H. Marden, O. Ovaskainen, I. Hanski, J. L. Osborne, J. H. Marden, and P. Martin. 2009.

Flight metabolic rate and pgi genotype influence butterfly dispersal rate in the field. Ecology 90:2223–2232.

Ozan, M., H. Helanterä, and L. Sundström. 2013. The value of oviposition timing, queen presence and kinship in a social insect. Proceedings of the Royal Society B: Biological Sciences 280:10–15.

Passera, L., and L. Keller. 1990. Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). Journal of Comparative Physiology B 160:207–211.

Passera, L., L. Keller, A. Grimal, D. Chautems, D. Cherix, D. J. C. Fletcher, W. Fortelius, R. Rosengren, and E. L. Vargo. 1990. Carbohydrates as energy source during the flight of sexuals of the ant *Formica lugubris* (Hymenoptera: Formicidae). Entomologia Generalis 15:25–32.

Peakin, G. 1964. Food reserves in the reproductive castes of *Lasius flavus* Fab. (Hymenoptera). in Proceedings of the XIIth International Congress of Entomology, London (Vol. 3030).

Peeters, C., and S. Aron. 2017. Evolutionary reduction of female dispersal in *Cataglyphis* desert ants. Biological Journal of the Linnean Society 122:58–70.

Pfennig, D. W., M. A. Wund, E. C. Snell-rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution 25:459–467.

R Core Team. 2013. A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rauhamäki, V., J. Wolfram, E. Jokitalo, I. Hanski, and E. P. Dahlhoff. 2014. Differences in the aerobic capacity of flight muscles between butterfly populations and species with dissimilar flight abilities. PLoS ONE 9:1–8.

Rosengren, R., and P. Pamilo. 1983. The evolution of polygyny and polydomy in mound-building *Formica* ants. Acta Entomologica Fennica 42:65–77.

Rosengren, R., L. Sundström, and W. Fortelius. 1993a. Monogyny and polygyny in *Formica* ants: the result of alternative dispersal tactic. In Queen number and sociality in insects (editor Keller, L.). Oxford Science publications, Oxford.

Saglam, I. K., D. A. Roff, and D. J. Fairbairn. 2008. Male sand crickets trade-off flight capability for reproductive potential. Journal of Evolutionary Biology 21:997–1004.

Schultner, E., A. Gardner, M. Karhunen, and H. Helanterä. 2014. Ant larvae as players in social conflict: relatedness and individual identity mediate cannibalism intensity. The American Naturalist 184:E161–E174.

Schultner, E., J. Saramäki, and H. Helanterä. 2016. Genetic structure of native ant supercolonies varies in space and time. Molecular Ecology 25:6196–6213.

Seifert, B. 2000. A taxonomic revision of the ant subgenus *Coptoformica* Mueller, 1923 (Hymenoptera, Formicidae). Zoosystema 22:517–568.

Seppä, P., N. Gyllenstrand, J. Corander, and P. Pamilo. 2004. Coexistence of the social types: genetic population structure in the ant *Formica exsecta*. Evolution 58:2462–71.

Seppä, P., and P. Pamilo. 1995. Gene flow and population viscosity in *Myrmica* ants. Heredity 74:200–209.

Shik, J. Z., D. A. Donoso, and M. Kaspari. 2013. The life history continuum hypothesis links traits of male ants with life outside the nest. Entomologia Experimentalis et Applicata 149:99–109.

Sohal, R. S., and V. F. Allison. 1971. Age-related changes in the finestructure of the flight muscle in the house fly. Experimental Gerontology 6:167–172.

Sohal, R. S., J. L. McCarthy, and V. F. Allison. 1972. The formation of "giant" mitochondria in the fibrillar flight muscles of the house fly, *Musca domestica* L. Journal of Ultrasructure Research 39:484–495.

Sorvari, J. 2017. Wood ant assemblages of *Formica rufa* group on lake islands and in mainland woodland in Central Finland. Entomologica Fennica, 29(1), 21-29.

Srygley, R. B., and P. Chai. 1990. Flight morphology of neotropical butterflies: palatability and distribution of mass to the thorax and abdomen. Oecologia 84:491–499.

Starrfelt, J., and H. Kokko. 2010. Parent-offspring conflict and the evolution of dispersal distance. The American Naturalist 175:38–49.

Steyn, V. M., K. A. Mitchell, and J. S. Terblanche. 2016. Dispersal propensity, but not flight performance, explains variation in dispersal ability. Proceedings of the Royal Society B: Biological Sciences, 283(1836), 20160905.

Stockan, J. A., and E. J. H. Robinson, editors. 2016. Wood ant ecology and conservation. Cambridge University Press, Cambridge.

Suarez, R. K., C. A. Darveau, and J. J. Childress. 2004. Metabolic scaling: A many-splendoured thing. Comparative Biochemistry and Physiology - B Biochemistry and Molecular Biology 139:531–541.

Sundström, L. 1995. Dispersal polymorphism and physiological condition of males and females in the ant, *Formica truncorum*. Behavioral Ecology 6:132–139.

Sundström, L., L. Keller, and M. Chapuisat. 2003. Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. Evolution 57:1552–1561.

Sundström, L., P. Seppä, and P. Pamilo. 2005. Genetic population structure and dispersal patterns in *Formica* ants- a review. Annales Zoologici Fennici 42:163–177.

Tennessen, J. M., W. E. Barry, J. Cox, and C. S. Thummel. 2014. Methods for studying metabolism in *Drosophila*. Methods 68:105–115.

Thomas, C. D., J. K. Hill, and O. T. Lewis. 1998. Evolutionary consequences of habitat fragmentation in a localized butterfly. Journal of Animal Ecology 67:485–497.

Toom, P., C. Johnson, and E. Cupp. 1976. Utilization of body reserves during preovisposition activity by *Solenopsis invicta*. Annals of the Entomological Society of America 69:145–148.

Van Valen, L. 1971. Group selection and the evolution of dispersal. Evolution 25:591–598.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S-PLUS. Fourth Edition. Springer, New York.

Vitikainen, E. I. K., C. Haag-Liautard, and L. Sundström. 2015. Natal dispersal, mating patterns, and inbreeding in the ant *Formica exsecta*. The American Naturalist 186:716– 727.

Vogt, J., A. Appel, and S West M. 2000. Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren. Journal of insect physiology 46:697–707.

Walter, F., D. J. C. Fletcher, D. Chautems, D. Cherix, L. Keller, W. Francke, W. Fortelius, R. Rosengren, and E. L. Vargo. 1993. Identification of the sex pheromone of an ant, *Formica lugubris* (Hymenoptera, Formicidae). Naturwissenschaften 80:30–34.

Wegener, G. 1996. Flying insects: Model systems in exercise physiology. Experientia 52:404–412.

Wei, T., and V. Simko. 2017. R package "corrplot": Visualization of a Correlation Matrix (Version 0.84).

West, S., I. Pen, and A. Griffin. 2002. Cooperation and competition between relatives. Science 296:72–75.

Wheeler, D. E., and N. A. Buck. 1995. Storage proteins in ants during development and colony founding. Journal of Insect Physiology 41:885–894.

Wheeler, D. E., and N. A. Buck. 1996. Depletion of reserves in ant queens during claustral colony founding. Insectes Sociaux 43:297–302.

Wheeler, D. E., and T. Martínez. 1995. Storage proteins in ants (Hymenoptera: Formicidae). Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 112:15–19.

Yao, I., and C. Katagiri. 2011. Comparing wing loading, flight muscle and lipid content in ant-attended and non-attended *Tuberculatus* aphid species. Physiological Entomology 36:327–334.

Yu, L.-Y., E. Jokitalo, Y.-F. Sun, P. Mehlen, D. Lindholm, M. Saarma, and U. Arumäe. 2003. GDNF-deprived sympathetic neurons die via a novel nonmitochondrial pathway. The Journal of Cell Biology 163:987–997.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42:207–230.

Zuur, A. F. 2016. A protocol for conducting and presenting results of regression-type analyses. Methods in Ecology and Evolution 7:636–645.

Appendix for Chapter III

Appendix Table 1 (next page): Results and sample sizes for all of the measured variables for each species and both sexes. For *F. exsecta* and *F. pressilabris*, the numbers presented here are calculated from their main populations. For measurements from the additional populations with differing social organization, see Table 2. The biochemical resources are presented as mass-corrected concentrations in TBE buffer (10 ul/ 1 mg) to allow comparisons among the differently sized species. The total mitochondria and myofibril areas are measured from TEM images with a total area of 69.2 µm2. The male triglyceride measurements are shown in grey, as they are based on a very few individuals while most males had zero values.

		F. pratensis Monodomous		*F. aquilonia* Polydomous		*F. exsecta* Monodomous		*F. pressilabris* Polydomous		*F. fusca* Monodomous		*F. cinerea* Polydomous	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	N ind	138	134	135	142	125	112	87	71	69	93	127	74
	N nest	15	14	11	13	13	10	12	8	13	15	29	28
Weight (g)	Mean	0.0288	0.0390	0.0184	0.0255	0.0104	0.0124	0.0039	0.0040	0.0144	0.0225	0.0164	0.0232
	SD	0.0032	0.0044	0.0022	0.0019	0.0023	0.0013	0.0008	0.0004	0.0017	0.0044	0.0018	0.0026
Abdomen ratio	Mean	0.435	0.459	0.440	0.473	0.374	0.347	0.373	0.350	0.378	0.485	0.420	0.444
	SD	0.018	0.023	0.041	0.024	0.024	0.032	0.025	0.038	0.028	0.060	0.024	0.037
Thorax ratio	Mean	0.502	0.427	0.496	0.415	0.540	0.489	0.509	0.443	0.548	0.411	0.514	0.447
	SD	0.016	0.019	0.038	0.022	0.023	0.024	0.025	0.028	0.028	0.048	0.023	0.031
	N ind	52	62	58	50	54	47	35	29	30	42	60	32
	N nest	11	14	11	10	13	10	10	7	13	15	26	19
Muscle ratio	Mean	0.29	0.20	0.27	0.20	0.29	0.21	0.23	0.15	0.31	0.22	0.30	0.25
	SD	0.01	0.01	0.03	0.02	0.03	0.03	0.04	0.04	0.03	0.03	0.03	0.02
	N ind	46	43	35	36	32	22	24	32	26	29	34	30
	N nest	10	10	9	9	6	6	6	7	10	10	14	14
Protein (mg/ml)	Mean	3.531	1.800	3.411	1.530	3.182	2.476	3.184	2.370	2.573	1.741	3.381	1.814
	SD	1.812	1.253	1.421	0.829	1.700	1.558	1.359	0.564	1.865	1.536	2.170	1.150
Glycogen (mg/ml)	Mean	1.386	1.236	1.190	1.742	0.920	1.005	0.825	0.356	0.478	2.281	0.445	1.339
	SD	0.703	0.620	0.804	1.030	0.707	1.154	0.544	0.260	0.628	1.922	0.586	1.398
Free glucose (mg/ml)	Mean	1.234	0.717	1.506	0.656	1.361	0.650	1.103	0.233	0.503	0.404	0.445	0.984
	SD	0.564	0.499	0.566	0.301	0.819	0.434	0.588	0.186	0.549	0.272	0.532	0.724
Triglyceride (mg/ml)	Mean	0.000	0.842	0.001	0.301	0.016	0.091	0.058	0.227	0.000	0.582	0.002	0.467
	SD	0.000	0.453	0.007	0.238	0.089	0.238	0.093	0.253	0.000	0.452	0.012	0.369
	N ind	9	9	9	9	9	9	9	9	9	9	9	9
	N nest	3	3	3	3	3	3	3	3	3	3	3	3
Total mitochondria area (um2/)	Mean	26.906	27.426	27.974	29.980	26.167	28.037	27.704	28.040	30.168	27.545	25.487	27.253
	SD	3.989	3.123	3.609	2.547	3.354	4.486	3.706	3.058	3.793	3.786	3.302	4.280
Mitochondrion profile area (um2)	Mean	0.887	0.754	0.694	0.992	0.957	0.997	1.028	0.925	1.319	0.876	0.736	0.715
	SD	0.532	0.480	0.463	0.584	0.686	0.649	0.635	0.585	0.844	0.608	0.470	0.505
Mitochondrium profile degraded area (um2)	Mean	0.020	0.014	0.033	0.023	0.031	0.046	0.068	0.042	0.063	0.022	0.022	0.036
	SD	0.078	0.051	0.095	0.067	0.097	0.152	0.205	0.157	0.188	0.095	0.089	0.144
Mitochondrium profile shape	Mean	0.272	0.305	0.283	0.298	0.311	0.320	0.294	0.302	0.337	0.337	0.281	0.273
	SD	0.145	0.154	0.149	0.149	0.157	0.159	0.158	0.153	0.164	0.157	0.144	0.142
Total myofibril area (um2)	Mean	30.322	33.325	32.645	31.118	28.771	30.174	28.424	31.607	26.770	30.922	33.321	30.180
	SD	3.208	3.268	3.108	2.564	4.101	4.737	3.558	4.347	3.047	3.950	3.160	4.665
Myofibril profile diameter (um)	Mean	1.660	1.741	1.619	1.505	1.649	1.549	1.459	1.559	1.444	1.529	1.631	1.571
	SD	0.302	0.316	0.256	0.231	0.279	0.324	0.250	0.257	0.289	0.269	0.268	0.241
CV of distances among myofibrils	Mean	0.237	0.218	0.236	0.241	0.228	0.233	0.257	0.242	0.252	0.232	0.228	0.230
	SD	0.094	0.087	0.077	0.069	0.080	0.086	0.076	0.085	0.059	0.077	0.071	0.071

Appendix Table 2: Results and sample sizes for both social organizations of *F. exsecta* and *F. pressilabris*. The biochemical resources are presented as mass-corrected concentrations in TBE buffer (10 ul/ 1 mg) to allow comparisons among the differently sized species. The male triglyceride measurements are shown in grey, as they are based on a very few individuals while most males had zero values.

		F. exsecta				*F. pressilabris*			
		Monodomous		Polydomous		Monodomous		Polydomous	
		♂	♀	♂	♀	♂	♀	♂	♀
	N ind	125	112	65	7	78	29	87	71
	N nest	13	10	10	1	9	4	12	8
Weight (g)	Mean	0.0104	0.0124	0.0054	0.0099	0.0045	0.0043	0.0039	0.0040
	SD	0.0023	0.0013	0.0014	0.0006	0.0008	0.0004	0.0008	0.0004
Abdomen ratio	Mean	0.374	0.347	0.397	0.370	0.373	0.374	0.373	0.350
	SD	0.024	0.032	0.029	0.032	0.029	0.037	0.025	0.038
Thorax ratio	Mean	0.540	0.489	0.500	0.457	0.517	0.422	0.509	0.443
	SD	0.023	0.024	0.027	0.025	0.030	0.027	0.025	0.028
	N ind	54	47	19	7	35	14	35	29
	N nest	13	10	9	1	8	4	10	7
Muscle ratio	Mean	0.293	0.210	0.243	0.200	0.217	0.116	0.235	0.151
	SD	0.032	0.025	0.046	0.035	0.061	0.031	0.038	0.036
	N ind	32	22	47	0	8	11	24	32
	N nest	6	6	10	0	2	3	6	7
Protein (mg/ml)	Mean	3.182	2.476	3.086		4.025	2.876	3.184	2.370
	SD	1.700	1.558	2.169		0.874	1.492	1.359	0.564
Glycogen (mg/ml)	Mean	0.920	1.005	0.835		1.199	0.269	0.825	0.356
	SD	0.707	1.154	0.489		1.302	0.174	0.544	0.260
Free glucose (mg/ml)	Mean	1.361	0.650	1.195		1.259	0.519	1.103	0.233
	SD	0.819	0.434	0.562		0.372	0.148	0.588	0.186
Triglyceride (mg/ml)	Mean	0.016	0.091	0.015		0.083	0.371	0.058	0.227
	SD	0.089	0.238	0.067		0.089	0.397	0.093	0.253

Appendix Table 3: Differences in variances between measured classes in body proportion and biochemical data tested with F test. Social: comparison between monodomous and polydomous social structure, F values smaller than 1 indicate that the polydomous have a higher variance. Sex: comparison between males and queens, F value smaller than 1 indicates the queens have a higher variance. Non-significant results are presented in grey.

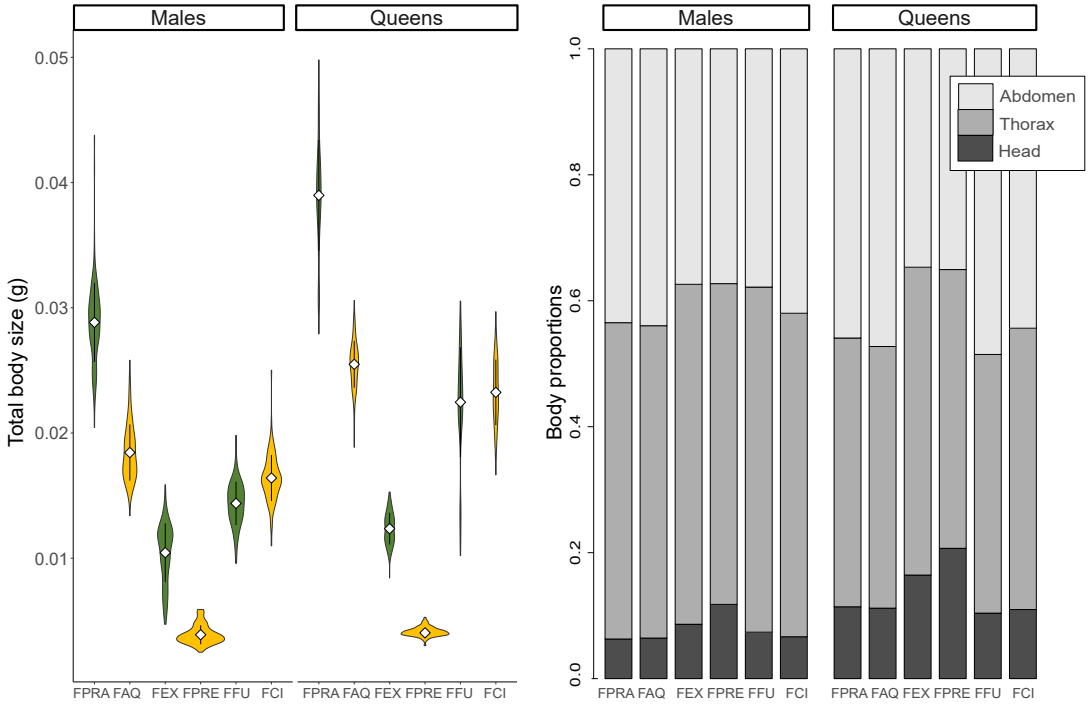
	Variance of data	
Measurement	**Social**	**Sex**
Total body weight	F = 1.8562, p = 5.773e-15	F = 0.50838, p < 2.2e-16
Abdomen ratio	F = 1.3152, p = 0.0004893	F = 0.36987, p < 2.2e-16
Thorax ratio	F = 1.365, p = 7.592e-05	F = 0.65512, p = 6.97e-08
Muscle ratio	F = 0.7809, p = 0.04132	F = 1.1613, p = 0.2193
Protein concentration	F = 1.2954, p = 0.07299	F = 2.1479, p = 1.619e-07
Glycogen concentration	F = 1.2675, p = 0.1005	F = 0.34272, p = 3.163e-13
Triglyceride concentration	F = 2.7938, p = 9.232e-07	NA

Appendix Table 4: Analyses of the microscopical muscle structure measurements. In all of the analyses, the social organization (monodomous/polydomous), sex, and their interaction were used as fixed effects, and the species, nest and individual identity as nested random effects. For the analyses of organelle profiles also the image was used as a random effect, nested within individual. The Benjamini-Hochberg procedure (BH, 1995) was used to correct the parameter p-values to decrease the false discovery rate. Tests where the p-value is smaller than the BH critical value are considered significant. Non-significant results are presented in grey.

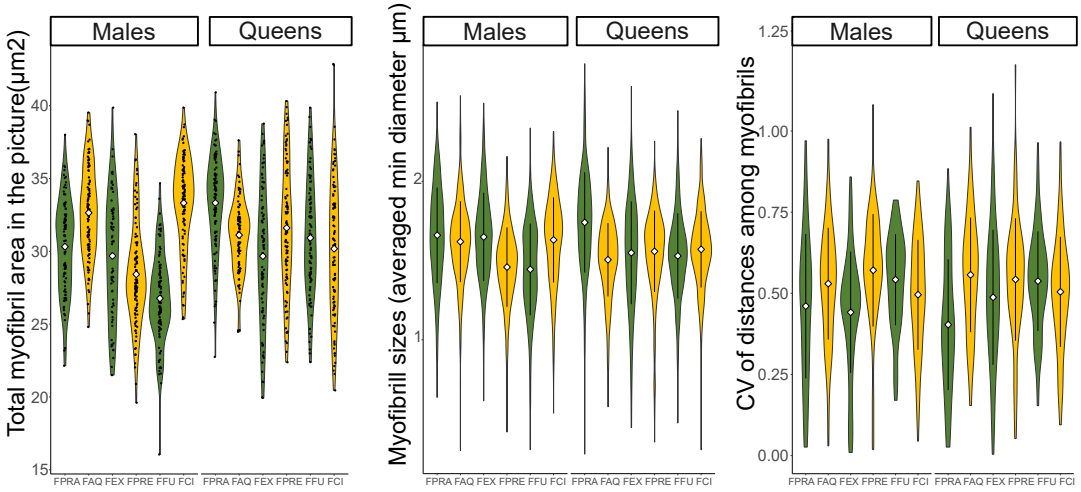
	Response					
	Parameter names	β	SE	z/t-value	p	BH
Mitochondria	**Total area**			**LMM**		
	Intercept (Monodomous males)	28.0731	0.7306	38.427	1.17E-09	
	Polydomous	-1.012	1.0178	-0.994	0.3538	0.033
	Queens	-0.2542	0.6954	-0.366	0.7155	0.044
	Interaction	1.6143	0.9679	1.668	0.0986	0.021
	Area of profiles			**GLMM (gamma)**		
	Intercept (Monodomous males)	1.00034	0.09202	10.871	<2E-16	
	Polydomous	0.28439	0.13004	2.187	0.0288	0.016
	Queens	0.18922	0.10799	1.752	0.0797	0.018
	Interaction	-0.27135	0.15301	-1.773	0.0762	0.018
	Shape of profiles			**GLMM (gamma)**		
	Intercept (Monodomous males)	3.4187	0.1089	31.389	<2E-16	
	Polydomous	0.192	0.1536	1.25	0.211	0.026
	Queens	-0.1755	0.1184	-1.483	0.138	0.023
	Interaction	0.1183	0.1678	0.705	0.481	0.038
	Presense of high degradation			**GLMM (binomial)**		
	Intercept (Monodomous males)	-3.20694	0.22549	-14.222	<2E-16	
	Polydomous	0.221	0.31471	0.702	0.483	0.039
	Queens	-0.13272	0.3158	-0.42	0.674	0.043
	Interaction	0.08644	0.44557	0.194	0.846	0.048
Myofibrils	**Total area**			**LMM**		
	Intercept (Monodomous males)	28.8324	0.9405	30.657	1.24E-10	
	Polydomous	2.5937	1.3122	1.977	0.0801	0.019
	Queens	2.514	1.0691	2.351	0.026	0.014
	Interaction	-3.003	1.4965	-2.007	0.0548	0.017
	Diameter of profiles			**LMM**		
	Intercept (Monodomous males)	1.62538	0.05602	29.013	1.66E-07	
	Polydomous	-0.02046	0.07919	-0.258	0.805	0.046
	Queens	0.03296	0.04646	0.71	0.484	0.04
	Interaction	-0.06492	0.06587	-0.986	0.333	0.031
	CV of distances among myofibrils			**LMM**		
	Intercept (Monodomous males)	0.2395166	0.0069459	34.483	1.01E-12	
	Polydomous	0.0003942	0.0096782	0.041	0.968	0.049
	Queens	-0.0118959	0.0087021	-1.367	0.183	0.024
	Interaction	0.009038	0.01219	0.741	0.465	0.038

	Response Parameter names	LMM results				
F. exsecta	**Total body mass**	**β**	**SE**	**t-value**	**p**	**BH**
	Intercept (Monodomous males)	1.04E-02	3.86E-04	26.99	<2E-16	
	Polydomous	-5.12E-03	6.36E-04	-8.041	1.14E-08	0.006
	Queens	1.72E-03	2.72E-04	6.318	9.44E-10	0.005
	Interaction	6.79E-04	6.41E-04	1.058	0.291	0.029
	Abdomen ratio					
	Intercept (Monodomous males)	0.377616	0.0046	82.091	<2E-16	
	Polydomous	0.020074	0.007456	2.692	0.0117	0.012
	Queens	-0.032768	0.005131	-6.387	1.59E-09	0.005
	Interaction	-0.001338	0.013032	-0.103	0.9183	0.049
	Thorax ratio					
	Intercept (Monodomous males)	0.536703	0.004107	130.694	<2E-16	
	Polydomous	-0.036948	0.00666	-5.548	6.33E-06	0.008
	Queens	-0.047137	0.004474	-10.536	<2E-16	0.003
	Interaction	0.009073	0.011279	0.804	0.422	0.037
	Muscle ratio					
	Intercept (Monodomous males)	0.293243	0.005652	51.879	<2E-16	
	Polydomous	-0.050535	0.010393	-4.862	2.83E-05	0.008
	Queens	-0.084247	0.007383	-11.411	<2E-16	0.003
	Interaction	0.042619	0.018148	2.348	0.0213	0.013
	Protein concentration (males)					
	Intercept (Monodomous)	2.9106	0.7667	3.796	0.00279	
	Polydomous	0.4785	0.9756	0.49	0.6329	0.042
	Glycogen concentration (males)					
	Intercept (Monodomous)	1.0233	0.1872	5.466	8.32E-05	
	Polydomous	-0.2827	0.2387	-1.185	0.255	0.028
F. pressilabris	**Total body mass**					
	Intercept (Monodomous males)	0.0048142	0.0002092	23.01	<2E-16	
	Polydomous	-0.000663	0.0002666	-2.487	0.01952	0.012
	Queens	-0.0009556	0.0003056	-3.128	0.00261	0.011
	Interaction	0.0005731	0.0003444	1.664	0.09975	0.021
	Abdomen ratio					
	Intercept (Monodomous males)	0.373827	0.007141	52.347	<2E-16	
	Polydomous	0.002422	0.00933	0.26	0.7969	0.045
	Queens	0.003228	0.012185	0.265	0.7925	0.045
	Interaction	-0.031836	0.014503	-2.195	0.0331	0.016
	Thorax ratio					
	Intercept (Monodomous males)	0.515276	0.006241	82.562	<2E-16	
	Polydomous	-0.007287	0.008157	-0.893	0.3785	0.034
	Queens	-0.094866	0.010661	-8.898	6.06E-11	0.004
	Interaction	0.028861	0.012698	2.273	0.0275	0.015
	Muscle ratio					
	Intercept (Monodomous males)	0.2194	0.0115	19.08	2.51E-15	
	Polydomous	0.01557	0.01564	0.995	0.329	0.03
	Queens	-0.10093	0.02057	-4.906	4.76E-05	0.009
	Interaction	0.01525	0.02564	0.595	0.557	0.041
	Protein concentration					
	Intercept (Monodomous males)	4.0248	0.6594	6.103	3.09E-05	
	Polydomous	-0.9023	0.7505	-1.202	0.248	0.027
	Queens	-0.9169	0.8553	-1.072	0.302	0.029
	Interaction	0.2239	0.9583	0.234	0.818	0.047
	Glycogen concentration					
	Intercept (Monodomous males)	1.2	0.5039	2.379	0.0321	
	Polydomous	-0.8048	0.5443	-1.479	0.1614	0.023
	Queens	-0.8964	0.6505	-1.378	0.1899	0.025
	Interaction	1.22	0.6506	1.88	0.0811	0.02
	Triglyceride concentration (queens)					
	Intercept (Monodomous)	0.4072	0.1911	2.13	0.0657	
	Polydomous	-0.2026	0.2283	-0.887	0.4008	0.035

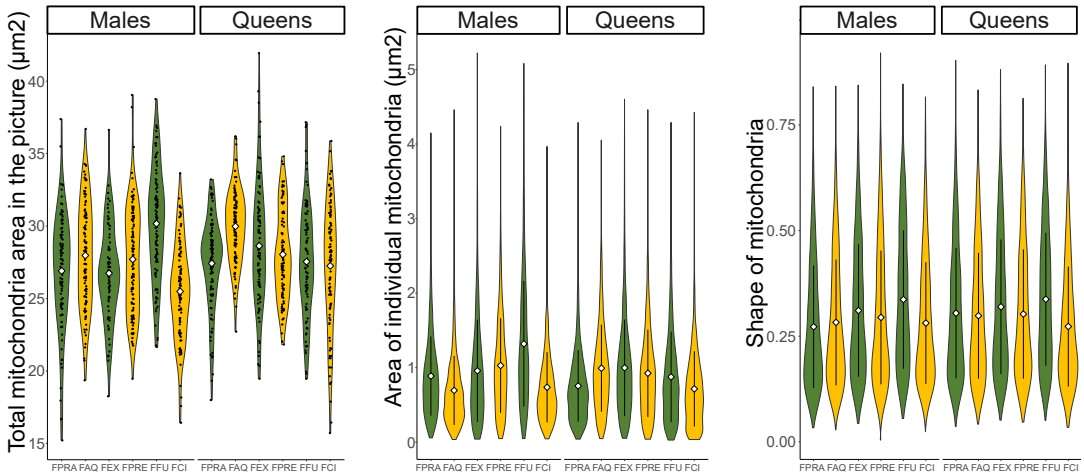
Appendix Table 5 (previous page): Analyses of the body proportions and biochemical measurements within *F. exsecta* and *F. pressilabris*. The social organization (monodomous/polydomous), sex, and their interaction were used as fixed effects. In the cases when there is data only for a single sex (all biochemical measurements for *F. exsecta*, triglyceride for *F. pressilabris*), only the social organization is used as fixed effect. The nest was used as random effect. The Benjamini-Hochberg procedure (BH, 1995) was used to correct the parameter p-values to decrease the false discovery rate. Tests where the p-value is smaller than the BH critical value are considered significant. Non-significant results are presented in grey.



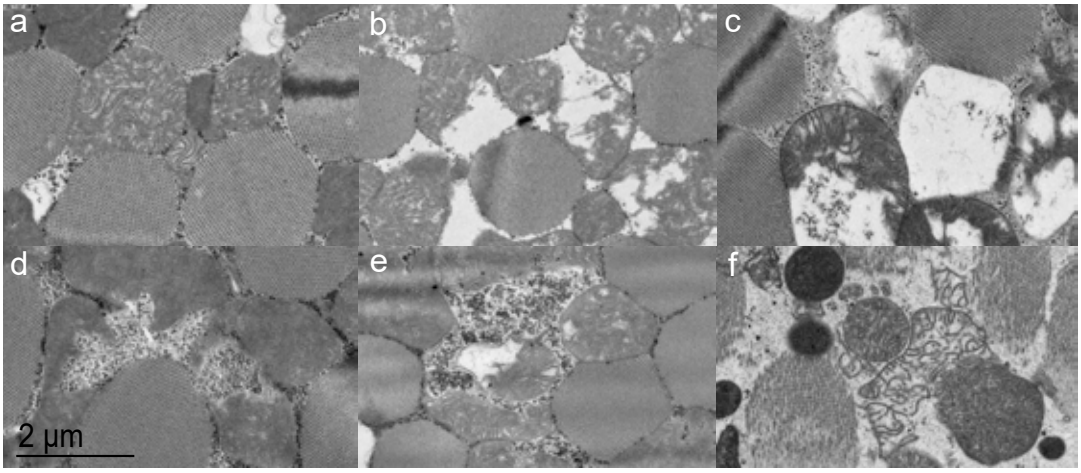
Appendix Figure 1: Left panel: Body mass by species and by sex. All data are visualized as density plots (violin plots), the mean is visualized with a diamond shape and +- standard deviation with whiskers. The social organization of each species is highlighted with color: dark green = monodomous, yellow = polydomous. The data of *F. exsecta* and *F. pressilabris* used for this figure include only the main populations that have the typical social organization. For within-species variation of these two species, see Figure 6 in the manuscript. **Right panel: The relative average body proportions by species and by sex.** The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = F. *aquilonia*, FEX = *F. exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F. cinerea*.



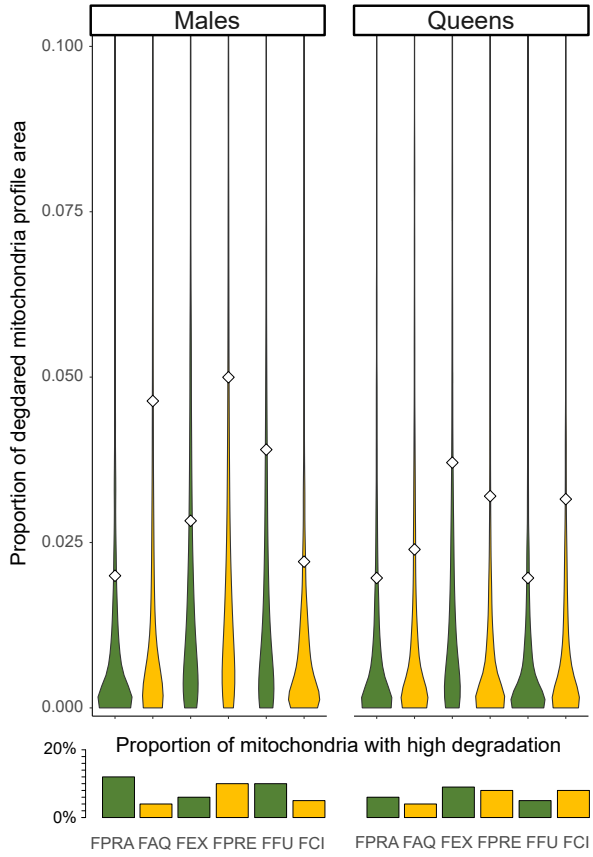
Appendix Figure 2. Results of the myofibril measurements.
a) Total myofibril area per TEM image (image area=69.2 µm2) with all data points, b) size of individual myofibrils as diameters with mean and standard deviation, c) organization of myofibrils per TEM image as coefficient of variation, with mean and standard deviation.
All data are visualized as density plots (violin plots), the mean is visualized with a diamond shape. The social organization of each species is highlighted with color: dark green = monodomous, yellow = polydomous. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*.



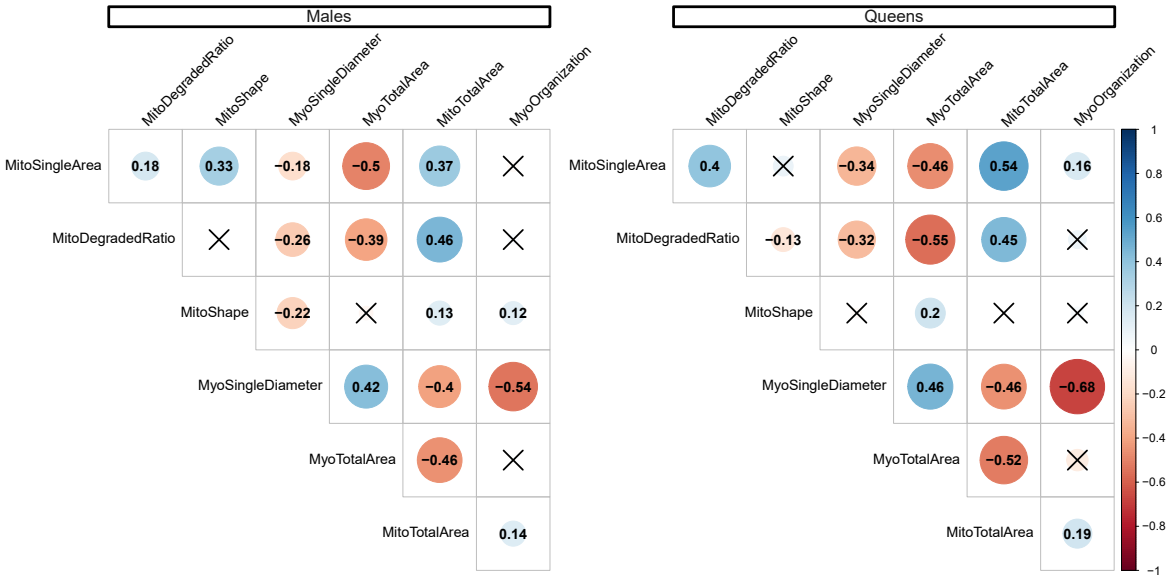
Appendix Figure 3. Results of the mitochondria measurements.
a) Total mitochondria area per TEM image (image area=69.2 µm2) with all data points, b) area of individual mitochondria with mean and standard deviation, c) mitochondrion profile shape, with mean and standard deviation. 0= round, longer shapes, especially > 0.5 indicate fused and tubular, strong mitochondria.
All data are visualized as density plots (violin plots), the mean is visualized with a diamond shape. The social organization of each species is highlighted with color: dark green = monodomous, yellow = polydomous. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*.



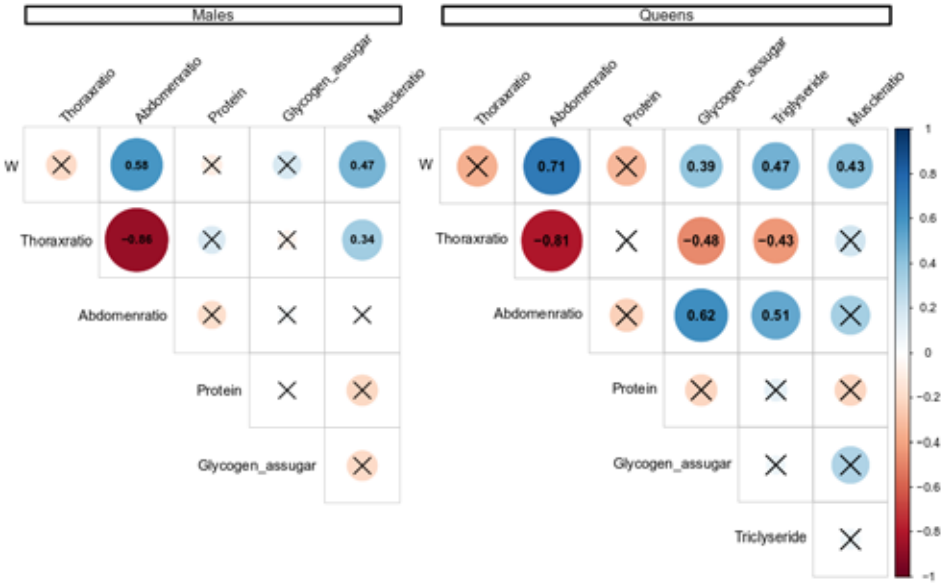
Appendix Figure 4. Examples of mitochondria structures that get values for degradation: a) slightly loose cristae structures unlike the usually denser cristae in our specimens – this is not true degradation but gives low values with our thresholding, b&c) empty area within mitochondria membranes, d&e) empty area filled with glycogen inside mitochondria membranes, f) extremely degraded mitochondria with broken membranes. Small values of degradation are common in our specimens and likely normal with no effects for flight ability. High values (>10% of the mitochondria profile area) are likely related to decrease of muscle function.



Appendix Figure 5. Mitochondria degradation for each species and both sexes. Upper panel shows the proportion of degraded area per total area of mitochondria profiles. Most mitochondria have low values for degradation (mean= 0.03). The y-axis of the upper panel is cut at 0.1, and the lower panel shows the proportion of data left out from the upper panel: the percentage of mitochondria having higher than 0.1 proportion of degraded area. Upper panel data are visualized as kernel density plots, the mean is visualized with a diamond shape.
The social organization of each species is highlighted with color: dark green = monodomous, yellow = polydomous. The species are abbreviated as follows: FPRA = *F. pratensis*, FAQ = *F. aquilonia*, FEX = *F.exsecta*, FPRE = *F. pressilabris*, FFU = *F. fusca*, FCI = *F.cinerea*.



Appendix Figure 6. Correlation coefficients among the microscopical muscle structure variables. Blue signifies positive correlations, red signifies negative correlations. X marks statistically non-significant correlations (p>0.01).



Appendix Figure 7: Correlation coefficients among the morphological and biochemical variables. Blue signifies positive correlations, red signifies negative correlations. X marks statistically non-significant correlations (p>0.01).

Appendix Figure 8: Here you can draw your own figure. The manuscript clearly does not have enough of them otherwise.

Limited dispersal and an unexpected aggression pattern in a native supercolonial ant

Superkoloniaalisen muurahaisen rajoittunut levittäytyminen ja odottamaton aggressiivisuuskuvio

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*** contributed equally / jaettu päävastuu**

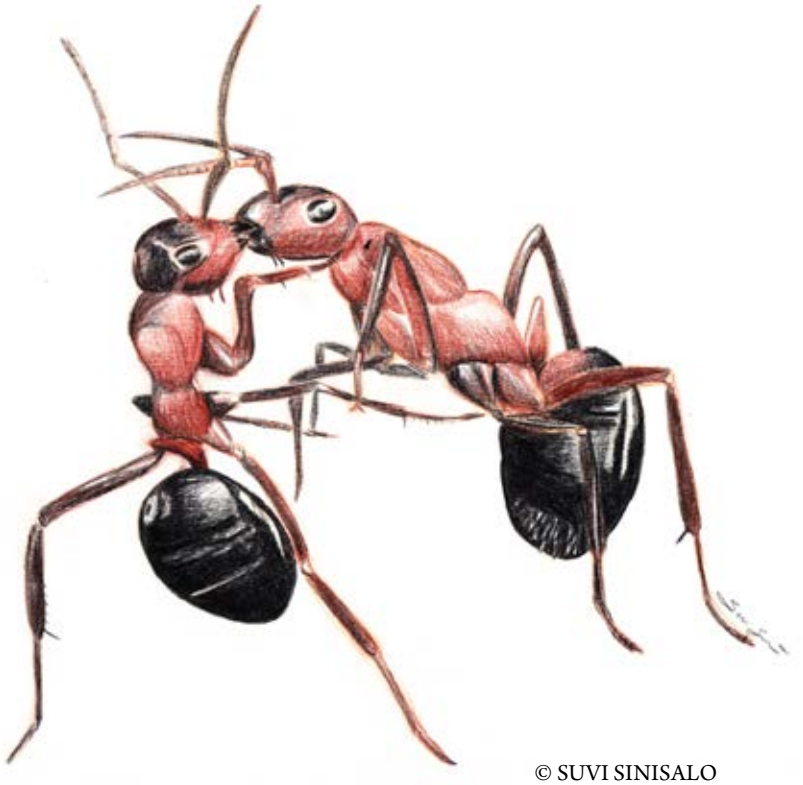
Ecology and Evolution 2020, 00:1–15

IV

TIIVISTELMÄ

Ymmärtääksemme sosiaalisten ryhmien toimintaa tarvitsemme tutkimuksia siitä, kuinka yksilöt liikkuvat ympäristössään ja vuorovaikuttavat toistensa kanssa. Muurahaisten superkoloniat ovat esimerkki äärimmäisestä yhteistyöstä: ne voivat koostua tuhansista toisiinsa yhteydessä olevista pesistä, joiden jäsenet tekevät yhteistyötä suurten välimatkojen poikki. Silti superkoloniaalisten (tai unikoloniaalisten) yhteiskuntien sisäistä rakennetta on harvoin tutkittu yhtaikaa sekä geneettisesti että käyttäytymisanalyyseilla. Tässä työssä esittelemme kaljuloviniskan (*Formica pressilabris*) tiiviin superkoloniankaltaisen pesärykelmän, joka koostuu yli 1300 pesästä.

Teimme aggressiivisuusanalyysejä ja havaitsimme, että vaikka aggressiota on kaiken kaikkiaan vähän, oletetun superkolonian sisällä on silti jonkin verran aggressiivista käytöstä. Sen esiintyminen lisääntyy, mitä kauempaa tutkittavasta pesästä kohdattu yksilö on, mikä sopii yhteen populaation geneettisen rakenteen kanssa. Populaation sisällä läheiset pesät ovat geneettisesti samankaltaisempia kuin kaukaisemmat pesät, minkä analysoimme käyttämällä kymmentä DNA-mikrosatelliittijaksoa. Havaitsemamme aggressiivisuus ei silti noudata mitään selkeää kuviota, eikä alueen sisällä ole mahdollista rajata erillisiä kolonioita sen perusteella. Geneettinen aineisto näyttää, että geenivirta on rajoittunutta superkoloniassa. Tuloksemme osoittavat, että *Formica*-superkolonia ei välttämättä ole yksi selkeä yksikkö vaan se saattaa myös olla mukautuvampi, aggressiivisten ja ystävällisten vuorovaikutusten mosaiikki. Tämä korostaa pesien välisten vuorovaikutusten havainnoinnin tärkeyttä superkolonioita tutkittaessa.



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ORIGINAL RESEARCH

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Limited dispersal and an unexpected aggression pattern in a native supercolonial ant

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Abstract

Understanding how social groups function requires studies on how individuals move across the landscape and interact with each other. Ant supercolonies are extreme cooperative units that may consist of thousands of interconnected nests, and their individuals cooperate over large spatial scales. However, the inner structure of suggested supercolonial (or unicolonial) societies has rarely been extensively studied using both genetic and behavioral analyses. We describe a dense supercolony-like aggregation of more than 1,300 nests of the ant *Formica* (*Coptoformica*) *pressilabris*. We performed aggression assays and found that, while aggression levels were generally low, there was some aggression within the assumed supercolony. The occurrence of aggression increased with distance from the focal nest, in accordance with the genetically viscous population structure we observe by using 10 DNA microsatellite markers. However, the aggressive interactions do not follow any clear pattern that would allow specifying colony borders within the area. The genetic data indicate limited gene flow within and away from the supercolony. Our results show that a *Formica* supercolony is not necessarily a single unit but can be a more fluid mosaic of aggressive and amicable interactions instead, highlighting the need to study internest interactions in detail when describing supercolonies.

KEYWORDS
aggression assay, dispersal, Hymenoptera, nestmate recognition, polydomy, polygyny

1 | INTRODUCTION

Cooperation in social groups can be favored when interacting individuals are related, or otherwise share alleles for cooperative behavior (Hamilton, 1964a; 1964b). Indeed, highly cooperative groups across all domains of life are usually family groups, where relatedness among group members is high (Bourke, 2011). However, the benefits of cooperation may be canceled out if relatives simultaneously compete with each other locally. The balance between kin-selected cooperation and harmful competition with relatives is delicate, and especially the roles of spatial patterns and dispersal have received considerable attention (e.g., Kümmerli, Gardner, West, & Griffin, 2009; Platt & Bever, 2009; Queller, 1992; Taylor, 1992; West, Pen, & Griffin, 2002). However, the spatial scales of cooperation and competition remain understudied in natural populations. They can be especially difficult to interpret in genetically viscous populations,

Hakala and Ittonen contributed equally to the article.

where limited dispersal leads to relatives aggregating together, leading to both increased kin competition and more possibilities for cooperation among relatives (Queller, 1992; Taylor, 1992). Understanding the relevant spatial scales of cooperation and competition requires knowledge on how individuals move across landscapes and interact with each other.

Ants are a good example of obligate eusociality, where a reproductive caste depends on help from a sterile worker caste (Crespi & Yanega, 1995). In several ant species, colony structures have grown beyond the ancestral simple family unit, from which eusociality originally evolved (Boomsma, 2009). In many ant taxa, dispersal is limited, and new nests are formed by budding from a parent nest, instead of long-range dispersal by flying sexual offspring (Cronin, Molet, Doums, Monnin, & Peeters, 2013). In some of these taxa, this leads to the formation of polydomous colonies where several nests are interconnected and work together as a single colony (Debout, Schatz, Elias, & McKey, 2007). Further, polydomy is often associated with polygyny (multiple reproducing queens per colony) and the colonies can grow extremely large and have extremely high numbers of queens. This leads to colony members being unrelated at the local scale (Boomsma, Huszár, & Pedersen, 2014; Helanterä, Strassmann, Carrillo, & Queller, 2009), which challenges traditional definitions of colonies as kin-selected cooperative units. Very large polydomous and polygynous colonies are often called supercolonies (Helanterä et al., 2009). In a supercolony, the cooperative colony spans larger spatial scales than a single individual can cross (Helanterä et al., 2009; Pedersen, Krieger, Vogel, Giraud, & Keller, 2006), but individuals still recognize and treat each other as members of the same colony when brought together (Moffett, 2012). As an extreme example, a genetically homogenous single colony of the Argentine ant *Linepithema humile* has spread over the whole globe, but individuals living on different continents still behaved as one colony in behavioral experiments (Brandt, Wilgenburg, & Tsutsui, 2009; Holway, Suarez, & Case, 1998; van Wilgenburg, Torres, & Tsutsui, 2010).

Although the existence of supercolonies is something of an evolutionary paradox due to low relatedness within these cooperative units (Giraud, Pedersen, & Keller, 2002), they are ecologically very dominant (Human & Gordon, 1996). This has led to many supercolonial ant species becoming harmful invasive pests (GISD, 2019), the best-studied example being the above-mentioned Argentine ant (Giraud et al., 2002; Tsutsui & Case, 2001). It forms massive supercolonies, especially in its invasive ranges, where it has been able to spread without much competition (Tsutsui & Suarez, 2003; Wetterer, Wild, Suarez, Roura-Pscual, & Espadaler, 2009), and smaller supercolonies in its native ranges (Pedersen et al., 2006; Vogel, Pedersen, D'Ettorre, Lehmann, & Keller, 2009). Similarly, polygynous and polydomous colonies of the fire ant (*Solenopsis invicta*) reach very large sizes and densities, and are ecologically dominant in their invasive range (Ascunce et al., 2011; Ross, Vargo, & Keller, 1996). Supercoloniality has evolved independently in several other taxa across the ant phylogeny, some of which are not invasive (Helanterä et al., 2009). Studying the spatial scale and inner social organization of supercolonial societies in all of these taxa would give a fuller understanding of the evolution and maintenance of such high levels of cooperation (Robinson, 2014).

The ability of ant individuals to distinguish between group members and outsiders makes it possible to define the borders of supercolonies (Moffett, 2012). Individuals recognize their colonymates using olfactory cues such as cuticular hydrocarbons that can be both genetically determined and acquired from the environment and food (Ginzel & Blomquist, 2016; Howard, 1993; Vander Meer & Morel, 1998). As ants usually behave aggressively toward intruders, aggression assays are commonly used to study nest- and colonymate recognition, and provide a simple way to infer colony boundaries, and thus also spatial scales of potential cooperation (Roulston, Buczkowski, & Silverman, 2003). Even the largest supercolonies lack internest aggression within the colony, while they do behave aggressively toward other conspecific (super)colonies (Giraud et al., 2002; Holway et al., 1998; Thomas, Payne-Makrisâ, Suarez, Tsutsui, & Holway, 2007; Tsutsui, Suarez, Holway, & Case, 2000). However, not all supercolonies and supercolonial species reported in the literature have been rigorously tested for internest aggression or resource sharing (Hoffmann, 2014), but instead some of them have been described as supercolonial based only on genetic data and the spatial organization of nests (Helanterä et al., 2009).

Formica ants offer excellent possibilities for studying spatial scales of cooperation and the evolution of social organization, as this genus has large variation in social organization, from simple family units all the way to very large supercolonies (Ellis & Robinson, 2014; Helanterä et al., 2009; Rosengren & Pamilo, 1983; Rosengren, Sundström, & Fortelius, 1993). The largest reported example is a 45,000 nest supercolony of *Formica yessensis* (Higashi & Yamauchi, 1979), while supercolonies in other *Formica* species range from tens to a few thousands of nests (Markó, Czekes, Eros, Csata, & Szász-Len, 2012). In some cases, populations of the same species vary in their social structure, and, for example, in *Formica exsecta*, it seems that polygynous, polydomous, and supercolonial populations can arise from monogynous background populations (Seppä, Gyllenstrand, Corander, & Pamilo, 2004). *Formica* populations tend to be genetically viscous; that is, spatially close nests are more closely related than spatially distant ones, which is especially true in polygynous species and populations where young queens are often philopatric (Chapuisat, Goudet, & Keller, 1997; Rosengren et al., 1993; Sundström, Seppä, & Pamilo, 2005). While patterns of genetic variation in supercolonial *Formica* ants have been studied in detail before (Chapuisat et al., 1997; Elias, Rosengren, & Sundström, 2005; Holzer, Keller, & Chapuisat, 2009; Seppä et al., 2004; Seppä, Johansson, Gyllenstrand, Pálsson, & Pamilo, 2012; Sundström et al., 2005), these studies have rarely combined genetic data with behavioral experiments to assess the scale of cooperation, or potential for it. Recognition behavior and internest aggression has been tested in some species (Chapuisat, Bernasconi, Hoehn, & Reuter, 2005; Holzer, Chapuisat, Kremer, Finet, & Keller, 2006; Kidokoro-Kobayashi et al., 2012; Martin, Helanterä, Kiss, Lee, & Drijfhout, 2009; Pohl, Ziemen, & Witte, 2018), but overall the behavioral structure of highly polydomous *Formica* colonies remains understudied.

We investigate the nature and spatial scale of supercoloniality in the highly polydomous ant *Formica pressilabris* using behavioral

assays and DNA microsatellite data. We test the hypothesis that behavioral colony borders correspond to the spatial and genetic structuring of a large nest aggregation, which is an underlying assumption of many previous studies. Based on this hypothesis, we expect nests at a densely populated *F. pressilabris* site to either belong to one supercolony without internest aggression, or possible internest aggression to occur between spatially or genetically distinct supercolonies competing with each other. We use genetic data to infer dispersal patterns within and outside of this supercolonial nest aggregation. As supercoloniality has previously been linked to limited dispersal, our hypothesis is that the dense nest aggregation is genetically somewhat separated from three other closely located study sites, and competition thus largely local. Additionally, we expect the philopatry of daughter queens to lead to genetic viscosity within our supercolonial site.

2 | MATERIAL AND METHODS

2.1 | Study species and sites

Formica (*Coptoformica*) *pressilabris* (Figure 1) is a mound-building ant that lives on meadows and banks, builds nests of grass, and tends aphids for its main energy supply (Schultz & Seifert, 2007; Seifert, 2000) It founds new nests via temporary social parasitism with other *Formica* (*Serviformica*) species as its host, or via budding from a parent nest (Czechowski, 1975; Kutter, 1969). While monogynous colonies have been reported, secondary polygyny, where daughter queens stay in their natal nests, is common. A single nest can have hundreds of queens and grow up to over one meter in diameter, which is exceptionally large for such a small *Formica* species (Collingwood, 1979; Czechowski, 1975; Pamilo & Rosengren, 1984; Rosengren et al., 1993; Seifert, 2000). Colonies are also commonly polydomous with several interconnected nests and no aggression between nests (Collingwood, 1979; Czechowski, 1975; Seifert, 2000). Nest turnover is high in polydomous colonies; that is, new satellite nests are built regularly while old ones are abandoned (Bönsel, 2007).

FIGURE 1 The study species, *Formica pressilabris*

We sampled a *F. pressilabris* population in Southern Finland in 2016. The sampled area consists of one 9-ha abandoned field (Särkkilen) with a large continuous nest aggregation (hereafter referred to as the supercolony site), and three closely located smaller sites with smaller nest aggregations (Storgård, Lillgård, Storsand; Figure 2). The three largest sites are former cropland, set aside for years or decades and now vegetated mainly by tall grass as well as some bushes and young trees. The smallest site is an edge area between cropland and forest. To assess whether the colonization potential of the area was fully used, we also extensively, although not exhaustively, searched for nests in other suitable habitats inside a 1 km radius from the supercolony site, but did not find additional colonies. Relatedness among worker nestmates estimated in a part of the supercolony site (r = .21 ± .02, determined with DNA microsatellites, Schultner, Gardner, Karhunen, & Helanterä, 2014) indicates polygyny and/or polydomy, that is, movement of individuals among nests. In our sampling, we counted nest mounds separated by more than 20 cm as separate nests. For our behavioral experiments and genetic analysis, we identified four spatially distinct parts of the supercolony site, with a clear gap between parts II and III (Figure 2) and less clear gaps between the parts I and II, and III and IV, making the latter two divisions somewhat arbitrary (Figure 2). We calculated nest densities first for the whole supercolony site and then separately for each of the four parts on it, as well as for the three smaller sites, using QGIS 3.4.1 (QGIS Development Team, 2018). For the whole supercolony site, we used the complete open area of the field for the calculation, whereas for its four parts and the three separate fields we used the areas of the polygons obtained by drawing straight lines between the outermost nests belonging to the respective areas. The age of the study population is not known, but the supercolony site has not been cultivated since the 1970s (landowner P. Forsbom, personal communication). Thus, the *F. pressilabris* population may have occupied the site for up to five decades.

2.2 | Behavioral assays

We performed behavioral assays in order to determine whether workers from the supercolony site behave differently toward their nestmates, conspecifics from other close and distant nests at the same site, conspecifics from other sites, and allospecific ants. These treatments are hereafter referred to as control, neighbor, distant, outside, and allospecific, respectively. We expected no aggression within the supercolony site, and that if any aggression were observed, it would occur across the nest-free gap in the middle of the site (Figure 2), as the gap may form a barrier between two separate supercolonies. We expected more aggression toward individuals from other sites than toward individuals from within the supercolony site. The closely related species *F. exsecta* should always be faced with aggression, as *F. pressilabris* has previously been shown to behave very aggressively against it (Czechowski, 1971).

We collected workers from 16 *F. pressilabris* nests at the supercolony site, covering all four parts (Figure 2). Additionally, we collected *F.*

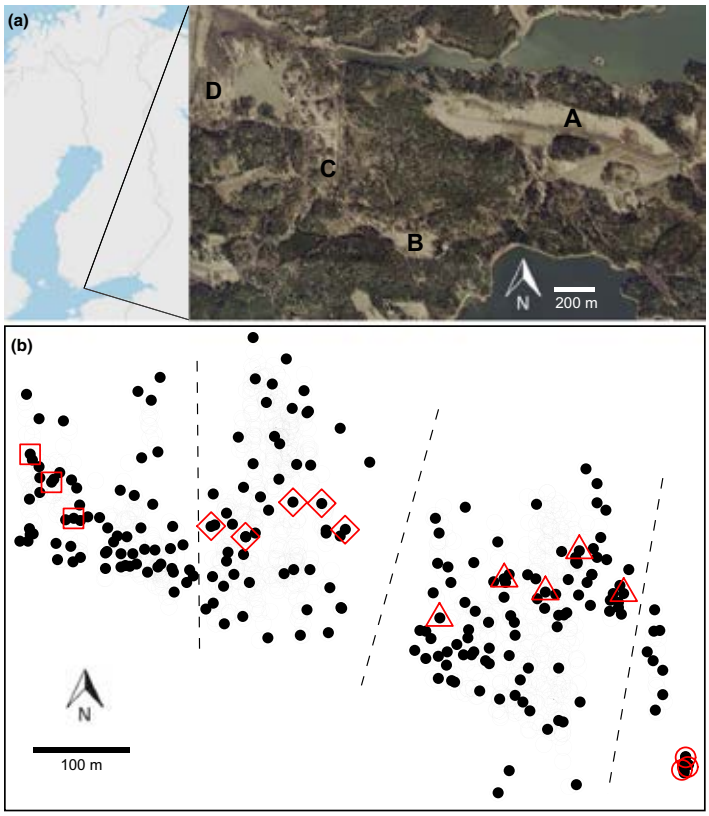

FIGURE 2 (a) The study area. The sampled subpopulations are indicated with letters. A: The supercolony field Särkkilen; B–D: The smaller fields, Storsand, Lillgård, and Storgård, respectively. (b) Locations of the nests on the supercolony field (circles). The nests used for genotyping are marked with filled circles, and the nests used for the behavioral experiments with red symbols. The red squares, diamonds, triangles, and circles represent parts I, II, III, and IV, respectively (see text). There are relatively large nest-free gaps between the parts (70 m between II and III; 31 m between III and IV), except for parts I and II, which are separated by a narrower (18 m) area dominated by several *Formica exsecta* nests. Dashed lines mark the borders between the parts. The red circles in part IV have been slightly moved apart in order to show them all. Aerial image from National Land Survey of Finland NLS Orthophotos database 04/2019 (CC BY-SA 4.0)

pressilabris workers from four nests at one of the smaller study sites, and one nest at another location approximately 30 km SW of the study population (Tvärminne). We also sampled two *F. exsecta* nests from the main study area and one from Tvärminne. We collected 2–5 L of nest material and a minimum of 300 workers from each nest on June 29 and 30. In the laboratory, we immediately divided all 16 focal nests from the supercolony site into two boxes to control for the possibility that physical separation in the laboratory causes aggression in the assays. We reared the laboratory nests in room temperature for three to eight days, kept them moist, and fed them daily with a Bhatkar–Whitcomb diet (Bhatkar & Whitcomb, 1970).

We tested the reaction of workers from each of the 16 supercolony site nests against control, neighbor, distant, outside, and allospecific individuals (Figure 3). We replicated each of the five treatments five times per nest with new arenas and new individuals, except for the allospecific tests. The latter we performed in the same arenas with the same individuals as the same-nest control treatments, as there never was any aggression in the control treatments. Our preliminary experiments showed that *F. pressilabris* workers act passively in standard one-on-one aggression assays on neutral arenas, usually showing no interest toward each other. Therefore, we used experimental arenas (6.5-cm-diameter fluon-coated, newly purchased plastic cups) with 15 workers on their own nest material, simulating natural conditions with nestmates and familiar odors present. In an assay like this, the observed behavior is expected to correlate with the natural nest defense behavior, revealing whether the workers would allow visitors to enter their nest or not, as even submissive ant species defend their nests against intruders (Savolainen & Vepsäläinen, 1988; Vepsäläinen & Pisarski, 1982). If an assay this sensitive does not show aggression, this can be interpreted as very strong potential for cooperation: When ants are willing to share their nest, they probably cooperate in other ways, too. After letting the 15 focal ants calm down (when they had stopped running around and did not show signs of alert, such as opening their mandibles), we introduced one worker from another laboratory nest box. We recorded the actions of the ants for one minute from the introduction, using a Canon EOS 550D DSLR camera with a Canon EF 100 mm f/2,8 macro lens. The distance between the ants and the lens was 48.5 cm. Ants did not react toward the camera. We performed the behavioral assays on July 3–7, 2016.

One of the authors (MI) watched and transcribed the videos in a randomized order at half speed and blindly regarding which nests and which treatments were represented in each video. The durations of antennation, trophallaxis, and biting events against

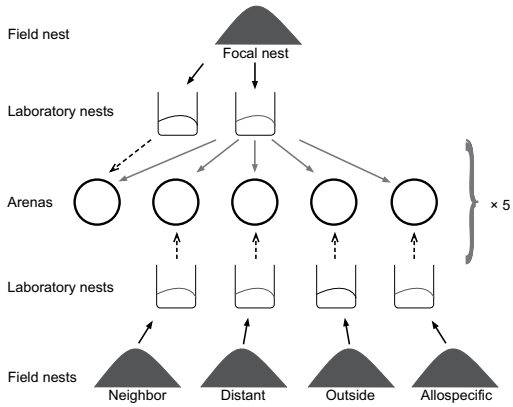

FIGURE 3 The design of the behavioral experiment. In each assay, we tested the reactions of 15 ants from a focal nest toward one ant from another laboratory nest box. We collected ants and nest material into laboratory nest boxes (black arrows) and put 15 workers from a focal nest into each of five experimental arenas (gray arrows). Then, we introduced one worker from another laboratory nest into the arenas (dashed arrows). For each focal nest, we replicated this procedure five times with new ants and arenas. Control = introduced ant from the same nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta*

the one introduced worker were recorded using the software JWatcher 1.0. We could not reliably score behavior frequencies or minor signs of aggression such as mandible opening due to the large number of individuals on the arena and the small size of the ants. The scoring was hierarchical in the sense that when even one of the fifteen focal individuals was aggressive, we did not score nonaggressive behavior at the same time, because aggression-related pheromones could affect the behavior of other individuals. In our analyses, we combined trophallaxis and antennation as nonaggressive inspecting behavior, because the two were sometimes hard to separate from each other and both have been shown to increase when individuals recognize the opponent as a non-nestmate in polydomous *Formica paralugubris* (Chapuisat et al., 2005; Holzer et al., 2006). Seven out of the 400 videos could not be analyzed due to damaged files.

2.3 | Statistical analysis of behavioral data

We analyzed the presence and absence of aggression explained by the different treatment classes with a binomial generalized linear mixed model (GLMM). In all of our models, we included both the nest of the focal workers and the nest of the introduced worker as random effects to account for the nonindependence of samples coming from the same nest. We excluded the "control" treatment as no aggression occurred in the within-nest controls. We used a beta GLMM to analyze the duration of aggression among the treatment classes where it occurred. For the videos where no aggression occurred, we also used a beta GLMM to analyze the duration of nonaggressive inspecting behavior (antennation and trophallaxis combined). For this analysis, we excluded the treatment level "allospecific" due to low sample size (n = 3), and substituted five samples with a value of 0 (= no inspecting behavior) with a value of 1 (= a millisecond of inspecting behavior) to allow the use of the beta distribution, which cannot contain zeros. For the beta GLMM's, we measured the duration of aggression or inspecting behavior as the proportion of the total time that the introduced ant was in sight.

We further tested whether the geographical distance between two separate nests explains the presence or duration of aggression, or the duration of nonaggressive inspecting behaviors. We analyzed a subset of our behavioral data within the supercolony site (treatment levels "neighbor" and "distant"), using a binomial GLMM for the presence of aggression and beta GLMM for the duration of aggression and inspecting behavior. Geographical and genetic distances are collinear in our data (see below), and the effect of these two variables cannot be fully separated in our results. Therefore, we used only geographical distance as an explanatory variable in our analysis. As we did the genetic analysis only for a single individual per nest, using genetic distance would be more problematic in connection to the nest-level behavioral data. Additionally, conspecific aggression in ants correlates mostly with chemical distance (Martin, Vitikainen, Drijfhout, & Jackson, 2012) which may have both genetic and environmental components (Ginzel & Blomquist, 2016; Vander Meer & Morel, 1998). Thus, as geographical distance contains information of both genetic and environmental factors, we deemed it more biologically relevant than genetic distance. We analyzed the behavioral data in R (R Core Team, 2013) with the package glmmTMB (Bolker et al., 2009).

2.4 | DNA microsatellite genotyping and population genetics

To estimate gene flow among and within the four sites, we genotyped a single worker from 285 different nests, including 233 nests from the supercolony site (Figure 2) and all nests from the three smaller study sites. We extracted the DNA using NucleoSpin Tissue extraction kits (Macherey-Nagel) and genotyped the samples with 14 DNA microsatellite markers originally developed for other Formica species (Chapuisat, 1996; Gyllenstrand, Gertsch, & Pamilo, 2002; Hasegawa & Imai, 2004; Trontti, Tay, & Sundström, 2003) using the protocol designed by Hakala et al. (2018). We scored the DNA microsatellite alleles with the software GeneMapper 5 (Applied Biosystems).

We analyzed linkage disequilibrium and Hardy–Weinberg equilibrium using GenePop on the Web (Raymond & Rousset, 1995; Rousset, 2008) and calculated allelic richness values using the PopGenReport 3.0.0 package (Adamack & Gruber, 2014) in R. We calculated allele frequencies, linear genetic distances between nests, and heterozygosity values using GenAlEx 6.502 (Peakall

& Smouse, 2012). We calculated F_{ST} values among all sampled sites, and among the four parts within the supercolony field using AMOVA's in GenAlEx 6.502 (Peakall & Smouse, 2012). To test for genetic viscosity, we performed Mantel tests using the package ecodist 2.0.1 (Goslee & Urban, 2007) in R.

We analyzed the genetic structure of the population with a Bayesian approach using the software STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000), which clusters individual genotypes by the probability of similarity. To determine the most likely number of clusters (K), the analysis was run with K ranging from 1 to 7, using the admixture model and correlated allele frequencies. For each K value, we ran the analysis ten times with a burn-in of 100,000 steps for a run length of 300,000 steps. We estimated the most likely number of clusters by applying the delta K method with plotting the mean and standard deviation of the mean likelihood L(K) for each run in STRUCTURE HARVESTER (Earl & vonHoldt, 2012; Evanno, Regnaut, & Goudet, 2005). As the mathematical model used by STRUCTURE is not ideal with unbalanced sampling and groups with low sample sizes or patterns of isolation by distance (Kalinowski, 2011; Puechmaille, 2016), we repeated the genetic mixture analysis with a similar software, BAPS 6.0 (Corander & Marttinen, 2006; Corander, Siren, & Arjas, 2008; Corander, Waldmann, & Sillanpää, 2003). BAPS was allowed to find the most probable number of clusters with repeated runs (10 times K1–K7). As BAPS was unable to find any stable clustering without a spatial prior, we repeated the analysis as a spatial analysis with geographical coordinates. Subsequently, we performed an admixture analysis with the results of the spatial analysis.

3 | RESULTS

3.1 | Mapping of the study area

The supercolony site had more than 1,300 nests, and the nest densities ranged from 254 to 401 nests/ha in the different parts of the site. The three smaller sites had 7, 16, and 29 nests, and the nest densities were 426, 25, and 25 nests/ha, respectively (Figure 2, Table A1). We could not directly observe worker movement between nests, because *F. pressilabris* workers walk mostly on the ground surface under the grass cover or on grass stems, forming no visible paths between the nests. All parts of the supercolony site had many dense aggregations with nests situated very closely together. Often these aggregations had one or two large main nests and a few smaller ones. The distances between nest aggregations were often short, and the field is overall almost uniformly occupied by the species.

3.2 | Behavioral assays

There was no aggression in the same-nest controls, while the allospecific treatment with *F. exsecta* had aggression in 72 out of 75 assays (Figure 4). In the neighbor, distant, and outside treatments, there was clearly more aggression than in the completely nonaggressive control treatments. Aggression occurred significantly more often in the allospecific than in any other treatment (compared to Neighbor: $Z = 6.71$, $SE = 0.73$, $p < .001$; Distant: $Z = 5.64$, $SE = 0.71$, $p < .001$; Outside: $Z = 5.08$, $SE = 0.72$, $p < .001$). The workers were also more often aggressive toward conspecific ants from distant nests than those from neighbor nests ($Z = 2.23$, $SE = 0.40$, $p = .026$). However, there was no significant difference between the aggression faced by ants from distant and outside nests (full test statistics in Table A2). The behavioral patterns were not consistent among the five replicates; instead, there were aggressive and nonaggressive interactions among and within all parts of the supercolony site. There were plenty of nonaggressive replicates also between distant nest pairs (Figure 5).

In the assays where aggression occurred, its duration did not significantly differ among the treatments, except in one of the pairwise comparisons, where the effect size remained small (Figure 6). However, the allospecific treatment always had long aggression

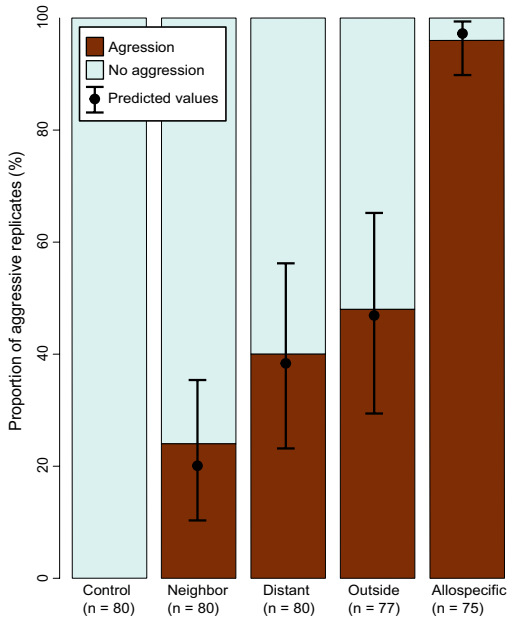

FIGURE 4 Presence and absence of aggression by treatment with model predictions for the four treatment classes that we included in the analysis (binomial GLMM). The control treatment, which had no aggression, was excluded from the model. Among those treatment classes that were included, all other pairwise differences were significant, except the difference between distant and outside. Control = introduced ant from the same nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta*

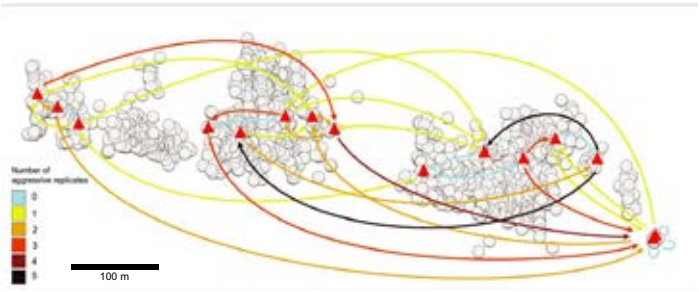

FIGURE 5 The number of replicates (out of five) with aggression for each nest pair within the supercolony site. The nests used in the aggression assays are shown as red triangles, and other nests as gray circles. The colored arrows show the number of aggressive replicates (see legend), and arrowheads show the direction of aggression, pointing toward the nest of the introduced ant

FIGURE 6 Duration of aggression (when present) by treatment as proportion of the total observation time. All data points, density plot, and median and quartile plot (box plot) shown. Only the difference between "outside" and "allospecific" is statistically significant (beta GLMM: $X = 2.43$, $SE = 0.23$, $p = .015$). Control = introduced ant from the same nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta*

durations (>25% of the assay time), whereas all within-species treatments also included shorter durations (<25% of the assay time). In the assays without aggression, significantly less inspecting behavior was targeted toward the control than any of the other treatments (compared to Neighbor: $Z = 5.45$, $SE = 0.19$, $p < .001$; Distant: $Z = 5.07$, $SE = 0.20$, $p < .001$; Outside: $Z = 3.74$, $SE = 0.34$, $p < .001$), while none of the other treatments differed from each other (Figure 7, Table A2).

Within the supercolony site, the occurrence of aggression between two nests increased with geographical distance (Figure 8a, binomial GLMM, $z = 2.85$, $SE = 1.20$, $p = .004$), but its duration did not change with distance (Figure 8b, beta GLMM $z = -0.334$, $SE = 0.94$, $p = .74$). The duration of inspecting behavior between nests within the supercolony field did not change with increasing distance (beta GLMM, $z = 0.36$, $SE = 0.66$, $p = .72$).

3.3 | Population genetics

Of the original 14 DNA microsatellite markers, we used 10 for further analysis, as four had too much missing data or significant heterozygote deficiency at all study sites (details in Tables S1–S6). The pairwise F_{ST} values show that the four parts of the supercolony site differed genetically from each other and that this differentiation was on a level similar to the differentiation among the other study sites (Table 1). Mantel tests showed minor but significant genetic viscosity when analyzing all samples in the study area ($R = 0.06$, 95% CI = 0.04, 0.08; $p = .038$), and also when analyzing only the samples from the supercolony site (R = 0.1, 95% CI = 0.09, 0.12; $p \leq .001$).

In the Bayesian clustering for the study area (Figure 9a,b), the optimal number of genetic clusters was two according to STRUCTURE HARVESTER ($\Delta K = 99.9$, details in Table S6 (Evanno et al., 2005)) and three according to BAPS (posterior probability = 0.981). However, the obtained clusters did not correspond to the different locations, as both analyses showed some sites to contain a mixture of individuals belonging to different clusters. The results from STRUCTURE revealed strong genetic admixture among individuals, whereas BAPS found admixture only in a few individuals. The four sampled sites seem to belong to a single population with gene flow among the sites.

4 | DISCUSSION

We found minor genetic viscosity on a small spatial scale, both within the supercolonial site and in its close surroundings. This indicates limited dispersal within the study area, as expected if new

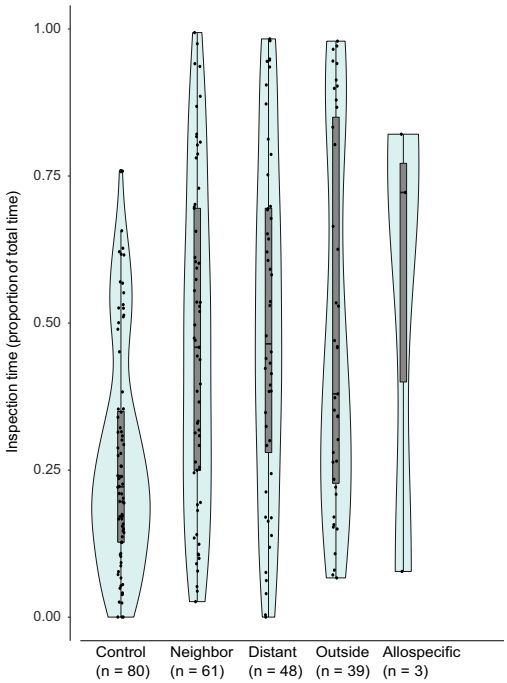

FIGURE 7 Duration of inspection behavior (antennation and trophallaxis) by treatment in the nonaggressive samples as proportion of the total observation time. All data points, density plot, and median and quartile plot (box plot) shown. Only the treatment Control is significantly different from other classes (beta GLMM, allospecific treatment not included in the model due to $n = 3$). Control = introduced ant from the same nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta*

nests are formed mostly by budding from parent nests. However, the fact that the different sites are not genetically more differentiated than different parts of the supercolony site indicates that some dispersal by flight also occurs. Behavioral experiments within the densely populated supercolony site show a similar behavioral pattern: The overall aggression level is low, workers mostly tolerate visitors from other nests in conditions simulating their own nest environment, and geographically close nests are better tolerated than nests further away. This suggests potential for cooperation among adjacent nests, and this potential slightly decreases the further nests are apart. However, because we observed some aggression within the supercolony site, it does not seem to consist of a single, distinct supercolony. There might be a mosaic of multiple supercolonies at the site, but as our data do not reveal any clear-cut behavioral borders, it is possible that the different colonies are somewhat connected over the whole spatial scale.

4.1 | Gene flow and dispersal

Our population genetic data indicate limited dispersal within the supercolony site. Even though within-nest relatedness is low, probably due to polygyny and mixing of individuals among adjacent nests (Schultner et al., 2014), individuals do not seem to mix effectively across the entire supercolony site, not even winged sexual individuals. Such genetic viscosity at a site less than 1 km across shows that these ants disperse mostly over very short distances. Local mating between nestmates or individuals from neighboring nests must be common, as the population would otherwise not remain even weakly genetically viscous. *F. exsecta*, which is closely related and ecologically similar to *F. pressilabris*, has similar levels of genetic viscosity in its supercolonies (Seppä et al., 2012). In *Formica paralugubris*, a supercolony was more viscous than the surrounding nonsupercolonial population, which, just as our results, suggests that supercoloniality is linked to locally reduced dispersal (Chapuisat et al., 1997). Importantly, data on gene flow, such as in the above-mentioned cases, do not provide any information about failed dispersal attempts. Workers in existing nests have a key role in determining which sexual individuals can establish themselves as reproducers at the supercolony site. Aggressive behavior toward individuals from distant nests could make their establishment as reproducers hard, even if they tried.

On a slightly larger spatial scale, the supercolony site is not genetically distinct from surrounding smaller polydomous colonies. Instead, pairwise F_{ST} values among the small sites and among the different parts of the supercolony site are in the same range. Our data suggest that longer-range dispersal among different sites is frequent enough to keep population structuring low (Table 1), although dispersal seems to be limited within the supercolony site. Ongoing long-range dispersal ensures that the supercolony is not a closed population, and extends the scale of competition beyond single sites, which may give some selective advantage to the individuals from the supercolony on larger spatial scales (Kennedy, Uller, & Helanterä, 2014; Pedersen et al., 2006). This contrasts with previous findings in *F. exsecta*, where polydomous colonies were genetically more different from surrounding monodomous and monogynous colonies than these were from each other (Gyllenstrand, Seppä, & Pamilo, 2005; Seppä et al., 2004), suggesting that polydomous colonies could form closed populations without much dispersal outwards.

Unfortunately, with our data we cannot assess whether dispersal is sex-biased. Male-biased dispersal is common in polygynous ants (Hakala, Seppä, & Helanterä, 2019) and in *Formica* overall (Sundström et al., 2005). In socially parasitic *Formica* species, such as our study species, queen dispersal is further complicated by the fact that queens cannot found their nests alone, but have to parasitize a nest of their host species or possibly another nest of their own species (Buschinger, 2009; Czechowski, 1975). Reduced queen dispersal, or queen dispersal predominantly among existing colonies instead of founding new ones, would impair the colonization potential even if dispersal abilities were good, as suggested by gene flow among sites. Indeed, there are plenty of empty potential habitat

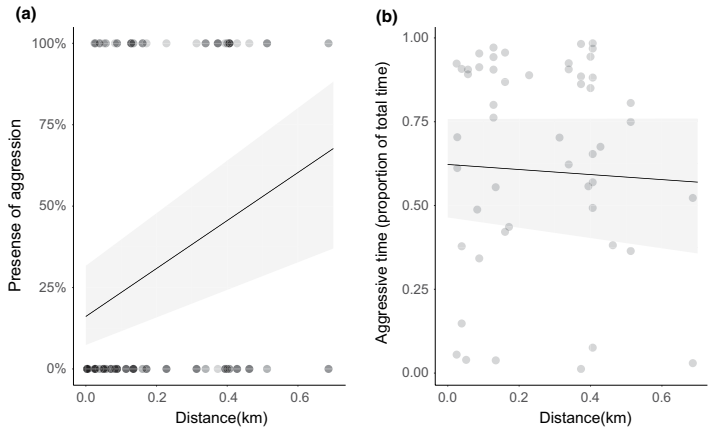

FIGURE 8 (a) Presence of aggression between different nests at the supercolony site as a function of internest distance, data (dots), and prediction (line with 95% confidence intervals) according to a binomial GLMM). *N* = 160. Darker dots indicate several data points on top of each other. (b) Duration of aggression (when present) between different nests as a function of distance on the supercolony field, data (dots), and nonsignificant prediction (line with confidence intervals according to beta GLMM). *N* = 51

TABLE 1 Pairwise F_{ST} values between subpopulations from an AMOVA with 999 permutations

	Särkkilen I					
		Särkkilen II				
Särkkilen II	0.015		Särkkilen III			
Särkkilen III	0.048	0.062		Särkkilen IV		
Särkkilen IV	0.065	0.074	0.031		Storsand	
Storsand	0.041	0.042	0.074	0.102		Lillgård
Lillgård	O.056	0.065	0.061	0.103	0.095	
Storgård	0.034	0.037	0.054	0.080	0.039	0.059

Note: The supercolony site (Särkkilen) is divided into four parts according to Figure 2b. All values are significantly different from zero ($p \leq .001$).

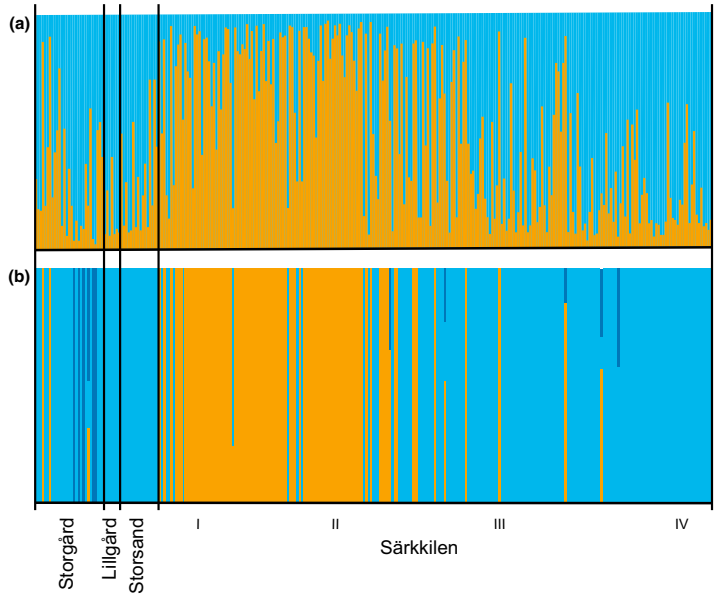

FIGURE 9 (a) Bayesian clustering of all genotyped individuals from the four study sites (I–IV = the four different parts of the supercolony site) with the software STRUCTURE. Samples organized from west to east. Optimal *K* = 2 with strong admixture. (b) Bayesian clustering of the same data with the software BAPS. Optimal *K* = 3 with less admixture

patches around the supercolony in our study area, which speaks for limited colonization. Overall, the dispersal strategy of *F. pressilabris* seems twofold: Risky long-range dispersal combined with high levels of queen philopatry and potential to spread locally through short-range dispersal by foot. Such a dual strategy seems to be the rule in polygynous and polydomous *Formica* (Sundström et al., 2005) and exists in other ant taxa too, for example, in *Crematogaster pygmaea* (Hamidi et al., 2017).

4.2 | Aggression patterns and potential for cooperation

Our main study site is, to our knowledge, the largest described nest aggregation of *Formica pressilabris* and among the largest of any species in the *Coptoformica* subgenus (Czechowski, 1971, 1975; Markó et al., 2012). The nest densities (Table A1) are well in line with previously reported values for large polydomous systems of *Formica* (Markó et al., 2012). While we are not aware of any reported nest densities for monodomous *F. pressilabris*, Pamilo and Rosengren (1984) reported clearly lower values (1.16–3.11 nests/ha) for three monodomous *F. exsecta* populations. The nest densities and observed high tolerance for introduced workers make us confident that the supercolony field is polydomous to a large degree. However, our behavioral assays still suggest that this nest aggregation is not a uniformly cooperative supercolony. Aggression generally increases with distance, but there are plenty of exceptions and no distinguishable colony borders (Figure 5). More detailed data on genetic and chemical similarity of the nests would be required for dissecting the ultimate reasons and mechanisms for this pattern. This behavioral pattern resembles a phenomenon already suggested possible by Moffett (2010, 2012): a social equivalent of a ring species, where all individuals that meet each other in natural settings interact peacefully, and all of the nests can thus be considered to belong to one colony, but individuals may act aggressively when experimentally brought together from distant parts of the range.

In contrast to our data, dense and spatially distinct polydomous nest aggregations often show a complete lack of aggression, for example, in both native and introduced argentine ants (Björkman-Chiswell, Wilgenburg, Thomas, Swearer, & Elgar, 2008; Giraud et al., 2002; Vogel et al., 2009), introduced *Myrmica rubra* (Chen, O'sullivan, & Adams, 2018), and native *Formica* (Chapuisat et al., 2005; Holzer et al., 2006; Kidokoro-Kobayashi et al., 2012; Pohl et al., 2018). However, some previous studies have also shown aggression within large polydomous *Formica exsecta* colonies (Katzerke, Neumann, Pirk, Bliss, & Moritz, 2006; Pisarski, 1982). Observations of seasonal and resource-dependent differences in aggression levels (Katzerke et al., 2006; Mabelis, 1979, 1984; Sorvari & Hakkarainen, 2004), and seasonal variation in supercolony genetic structure (Elias et al., 2005; Schultner, Saramäki, & Helanterä, 2016), suggest that further temporal analysis of aggression patterns in supercolonial *Formica* is needed.

In addition to the current study, positive correlations between aggression and spatial distance have been reported, for example, within polydomous sites of *F. exsecta* (Katzerke et al., 2006) and *Myrmica rubra* (Fürst, Durey, & Nash, 2012; Garnas, Drummond, & Groden, 2007), although later Chen et al. (2018) did not find such a correlation in *M. rubra*. Importantly, Chen et al. (2018) had, through a previous set of aggression assays, assigned colony borders prior to testing for this relationship and suggest that the correlations between geographical distance and aggression found by Garnas et al. (2007) and Fürst et al. (2012) may be attributed to mixing nest pairs belonging to the same and different colonies in one analysis. Our supercolonial site may indeed consist of multiple supercolonies instead of one, but the lack of clear patterns and the overall low levels of aggression revealed in our study (Figure 5) lend little support to the existence of clear and persistent borders at our study site. Finally, aggression has been shown to increase with internest distance also in monodomous species (Beye, Neumann, Chapuisat, Pamilo, & Moritz, 1998) and among distinct colonies or sites (Holzer et al., 2006; Pirk, Neumann, Moritz, & Pamilo, 2001; Rosengren, Cherix, & Pamilo, 1986; Zinck, Hora, Châline, & Jaisson, 2008), making these kinds of behavioral patterns hard to interpret.

The different behavioral assay methods used in many of the studies discussed above make direct comparisons of the results difficult (Roulston et al., 2003). Our method, where 15 individuals on their own nest material met one introduced ant, is very sensitive to aggression as it simulates an alien ant suddenly appearing in a nest, and there are many ants that can react. Based on our pilot experiments, we consider it likely that *F. pressilabris* would have shown even less, if any, aggression in some more commonly used assay types, such as one-on-one tests on neutral arenas. Even in the absence of aggression, our results show that workers spend more time inspecting any non-nestmates than nestmates. Thus, workers may distinguish between nestmates and more distant individuals, as is also suggested by increased antennation and trophallaxis in *F. paralugubris* (Chapuisat et al., 2005; Holzer et al., 2006), and increased antennation in argentine ants (Björkman-Chiswell et al., 2008).

While a lack of aggression between nests is commonly interpreted as a sign of shared colony identity, it does not necessarily mean that two nests share resources (Buczkowski, 2012; Giraud et al., 2002; Heller, Ingram, & Gordon, 2008). In large nest aggregations, it is relevant to ask what the true spatial scale of cooperation and competition is (Pedersen, 2012). At our supercolony site, aggression did not always occur even when testing over the nest-free gap in the middle of the field, showing that workers are willing to let individuals from relatively far away enter their nests (Figure 5). Czechowski (1975) found that workers can move at least 20–30 m between nests in supercolonies of *F. pressilabris*, but such movements were considerably rarer than movements among nearer nests. In *F. exsecta*, workers from polydomous colonies forage on trees less than ten meters from a central nest (Sorvari, 2009). Thus, given the width of the gap (~70 m) at our main study site, worker movements over it should be rare, and we consider true

cooperation, such as transfer of brood or resources, over this gap unlikely. If resources are shared over relatively limited distances also at our study site, the two field halves should be considered functionally separate colonies, even though they are not clearly distinct based on either genetic or behavioral data. We agree with Heller et al. (2008) and Lester and Gruber (2012) in their arguments that functional cooperation and resource sharing is a crucial component when considering the evolution and maintenance of supercolonies. To be able to assess true relatedness among cooperating individuals, we need to understand which parts of assumed supercolonies truly cooperate, and whether there are seasonal or resource-dependent patterns. Without this knowledge, it is not possible to assess whether competition happens more within or among assumed supercolonies.

The genetic viscosity corresponds to the behavioral pattern where workers from nearby nests were allowed to enter the nest material more than distant workers. This suggests that limited dispersal does result in cooperation among relatives in *Formica* supercolonies. As our genetic data suggest that competition over reproduction is not exclusively local, local cooperation even under low but positive relatedness may help maximizing reproductive success on a larger spatial scale. The *F. pressilabris* nest aggregation described in this study is extremely dense and seemingly supercolonial. However, it defies usual definitions of ant colonies as single cooperative units with clear borders. Based on our behavioral data, discrimination in *F. pressilabris* is fluid, which begs for further studies on the functional connectedness of the nests, and the cooperative behavior in more natural settings in the field. Truly understanding the nature of supercoloniality requires more functional studies focusing on resource sharing and competitive dynamics—in all ant taxa exhibiting this fascinating lifestyle.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors designed and conceived the study. MI collected the data. SMH and MI analyzed the data, prepared figures and tables, and drafted the manuscript. All authors reviewed drafts and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data used in this study are archived in the Dryad Digital Repository: https://doi.org/10.5061/dryad.ffbg79cr2.

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REFERENCES

Adamack, A. T., & Gruber, B. (2014). PopGenReport: Simplifying basic population genetic analyses in R. *Methods in Ecology and Evolution*, *5*(4), 384–387. https://doi.org/10.1111/2041-210X.12158

Ascunce, M. S., Yang, C.-C., Oakey, J., Calcaterra, L., Wu, W.-J., Shih, C.-J. … Shoemaker, D. (2011). Global invasion history of the fire ant Solenopsis invicta. *Science*, *331*(6020), 1066–1068.

Beye, M., Neumann, P., Chapuisat, M., Pamilo, P., & Moritz, R. F. A. (1998). Nestmate recognition and the genetic relatedness of nests in the ant Formica pratensis. *Behavioral Ecology and Sociobiology*, *43*(1), 67–72. https://doi.org/10.1007/s002650050467

Bhatkar, A., & Whitcomb, W. H. (1970). Artificial diet for rearing various species of ants. *The Florida Entomologist*, *53*(4), 229–232.

Björkman-Chiswell, B. T., van Wilgenburg, E., Thomas, M. L., Swearer, S. E., & Elgar, M. A. (2008). Absence of aggression but not nestmate recognition in an Australian population of the Argentine ant Linepithema humile. *Insectes Sociaux*, *55*(2), 207–212. https://doi.org/10.1007/s00040-008-0990-9

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S.-S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135.

Bönsel, A. (2007). Nest turnover in a colony of Formica pressilabris Nylander, 1846 as related to habitat quality (Hymenoptera: Formicidae). *Opuscula Zoologica Fluminensia*, *222*, 1–12.

Boomsma, J. J. (2009). Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1533), 3191–3207. https://doi.org/10.1098/rstb.2009.0101

Boomsma, J. J., Huszár, D. B., & Pedersen, J. S. (2014). The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. *Animal Behavior*, *92*, 241–252. https://doi.org/10.1016/j.anbehav.2014.03.005

Bourke, A. F. G. (2011). *Principles of social evolution*, *Oxford series of ecology and evolution*. Oxford: Oxford University Press.

Brandt, M., van Wilgenburg, E., & Tsutsui, N. D. (2009). Global-scale analyses of chemical ecology and population genetics in the Argentine ant. *Molecular Ecology*, *18*(5), 997–1005.

Buczkowski, G. (2012). Colony spatial structure in polydomous ants: Complimentary approaches reveal different patterns. *Insectes Sociaux*, *59*(2), 241–250. https://doi.org/10.1007/s00040-011-0211-9

Buschinger, A. (2009). Social parasitism among ants: A review (Hymenoptera: Formicidae). *Myrmecological News*, *12*(3), 219–235.

Chapuisat, M. (1996). Characterization of microsatellite loci in Formica lugubris B and their variability in other ant species. *Molecular Ecology*, *5*(4), 599–601. https://doi.org/10.1111/j.1365-294X.1996.tb00354.x

Chapuisat, M., Bernasconi, C., Hoehn, S., & Reuter, M. (2005). Nestmate recognition in the unicolonial ant Formica paralugubris. *Behavioral Ecology*, *16*(1), 15–19. https://doi.org/10.1093/beheco/arh128

Chapuisat, M., Goudet, J., & Keller, L. (1997). Microsatellites reveal high population viscosity and limited dispersal in the ant Formica paralugubris. *Evolution*, *51*(2), 475–482.

Chen, W., O'sullivan, Á., & Adams, E. S. (2018). Intraspecific aggression and the colony structure of the invasive ant *Myrmica rubra*. *Ecological Entomology*, *43*(2), 263–272. https://doi.org/10.1111/een.12500

Collingwood, C. (1979). The Formicidae (Hymenoptera) of Fennoscandia and Denmark. *Fauna Entomolgica Scandinavica*, *8*, 64.

Corander, J., & Marttinen, P. (2006). Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, *15*(10), 2833–2843. https://doi.org/10.1111/j.1365-294X.2006.02994.x

Corander, J., Siren, J., & Arjas, E. (2008). Bayesian spatial modeling of genetic population structure. *Computational Statistics*, *23*(1), 111–129. https://doi.org/10.1007/s00180-007-0072-x

Corander, J., Waldmann, P., & Sillanpää, M. J. (2003). Bayesian analysis of genetic differentiation between populations. *Genetics*, *163*(1), 367–374.

Crespi, B. J., & Yanega, D. (1995). The definition of eusociality. *Behavioral Ecology*, *6*(1), 109–115.

Cronin, A. L., Molet, M., Doums, C., Monnin, T., & Peeters, C. (2013). Recurrent evolution of dependent colony foundation across eusocial insects. *Annual Review of Entomology*, *58*(1), 37–55. https://doi.org/10.1146/annurev-ento-120811-153643

Czechowski, W. (1971). Competition between Formica exsecta Nyl. and Formica pressilabris Nyl. (Hymenoptera, Formicidae). *Annales Zoologici*, *17*, 273–286.

Czechowski, W. (1975). Bionomics of Formica (Coptoformica) pressilabris Nyl. (Hymenoptera, Formicidae). *Annales Zoologici*, *33*(8), 103–125.

Debout, G., Schatz, B., Elias, M., & McKey, D. (2007). Polydomy in ants: What we know, what we think we know, and what remains to be done. *Biological Journal of the Linnean Society*, *9*(2), 319–348.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Elias, M., Rosengren, R., & Sundström, L. (2005). Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant Formica truncorum. *Behavioral Ecology and Sociobiology*, *57*(4), 339–349. https://doi.org/10.1007/S00265-004-0864-8

Ellis, S., & Robinson, E. J. H. (2014). Polydomy in red wood ants. *Insectes Sociaux*, *61*(2), 111–122. https://doi.org/10.1007/s00040-013-0337-z

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fürst, M. A., Durey, M., & Nash, D. R. (2012). Testing the adjustable threshold model for intruder recognition on Myrmica ants in the context of a social parasite. *Proceedings of the Royal Society B. Biological Sciences*, *279*(1728), 516–522. https://doi.org/10.1098/rspb.2011.0581

Garnas, J. R., Drummond, F. A., & Groden, E. (2007). Intercolony aggression within and among local populations of the invasive ant, *Myrmica rubra* (Hymenoptera: Formicidae), in Coastal Maine. *Environmental Entomology*, *36*(1), 105–113. https://doi.org/10.1603/0046-225x(2007)36[105:iawaal]2.0.co;2

Ginzel, M. D., & Blomquist, G. J. (2016). Insect hydrocarbons: Biochemistry and chemical ecology. In E. Cohen, & B. Moussian (Eds.), *Extracellular composite matrices in arthropods* (pp. 221–252). Cham, Switzerland: Springer.

Giraud, T., Pedersen, J. S., & Keller, L. (2002). Evolution of supercolonies: The Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 6075–6079. https://doi.org/10.1073/pnas.092694199

GISD (Global Invasive Species Database) (2019). Retrieved from http://www.issg.org/database

Goslee, S. C., & Urban, D. L. (2007). The ecodist Package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, *22*(7), 1–19. https://doi.org/10.18637/jss.v022.i07

Gyllenstrand, N., Gertsch, P. J., & Pamilo, P. (2002). Polymorphic microsatellite DNA markers in the ant Formica exsecta. *Molecular Ecology Notes*, *2*(1), 67–69. https://doi.org/10.1046/j.1471-8286.2002.00152.x

Gyllenstrand, N., Seppä, P., & Pamilo, P. (2005). Restricted gene flow between two social forms in the ant Formica truncorum. *Journal of Evolutionary Biology*, *18*(4), 978–984. https://doi.org/10.1111/j.1420-9101.2005.00908.x

Hakala, S. M., Seppä, P., Heikkilä, M., Punttila, P., Sorvari, J., & Helanterä, H. (2018). Genetic analysis reveals Finnish Formica fennica populations do not form a separate genetic entity from F. exsecta. *PeerJ*, *6*, e6013. https://doi.org/10.7717/peerj.6013

Hakala, S. M., Seppä, P., & Helanterä, H. (2019). Evolution of dispersal in ants (Hymenoptera: Formicidae): A review on the dispersal strategies of sessile superorganisms. *Myrmecological News*, *29*, 35–55. https://doi.org/10.25849/myrmecol.news_029:035

Hamidi, R., de Biseau, J., Bourguignon, T., Martins Segundo, G. B., Fontenelle, M. T. M. B., & Quinet, Y. (2017). Dispersal strategies in the highly polygynous ant Crematogaster (Orthocrema) pygmaea Forel (Formicidae: Myrmicinae). *PLoS ONE*, *12*(6), 1–22.

Hamilton, W. D. (1964a). The genetical evolution of social behaviour I. *Journal of Theoretical Biology*, *7*, 1–16.

Hamilton, W. D. (1964b). The genetical evolution of social behaviour II. *Journal of Theoretical Biology*, *7*, 17–52.

Hasegawa, E., & Imai, S. (2004). Characterization of microsatellite loci in red wood ants Formica (s. str.) spp. and the related genus Polyergus. *Molecular Ecology Notes*, *4*(2), 200–203. https://doi.org/10.1111/j.1471-8286.2004.00614.x

Helanterä, H., Strassmann, J. E., Carrillo, J., & Queller, D. C. (2009). Unicolonial ants: Where do they come from, what are they and where are they going? *Trends in Ecology & Evolution*, *24*(6), 341–349. https://doi.org/10.1016/j.tree.2009.01.013

Heller, N. E., Ingram, K. K., & Gordon, D. M. (2008). Nest connectivity and colony structure in unicolonial Argentine ants. *Insectes Sociaux*, *55*(4), 397–403. https://doi.org/10.1007/s00040-008-1019-0

Higashi, S., & Yamauchi, K. (1979). Influence of a supercolonial ant Formica (Formica) yessensis Forel on the distribution of other ants in Ishikari coast. *Japanese Journal of Ecology*, *29*(3), 257–264.

Hoffmann, B. D. (2014). Quantification of supercolonial traits in the yellow crazy ant, Anoplolepis gracilipes. *Journal of Insect Science*, *14*(25), 1–21. https://doi.org/10.1673/031.014.25

Holway, D. A., Suarez, A. V., & Case, T. J. (1998). Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, *282*(5390), 949–952.

Holzer, B., Chapuisat, M., Kremer, N., Finet, C., & Keller, L. (2006). Unicoloniality, recognition and genetic differentiation in a native Formica ant. *Journal of Evolutionary Biology*, *19*(6), 2031–2039. https://doi.org/10.1111/j.1420-9101.2006.01133.x

Holzer, B., Keller, L., & Chapuisat, M. (2009). Genetic clusters and sex-biased gene flow in a unicolonial Formica ant. *BMC Evolutionary Biology*, *9*(1), 69. https://doi.org/10.1186/1471-2148-9-69

Howard, R. W. (1993). Cuticular hydrocarbons and chemical communication. In D. W. Stanley-Samuelson, & D. R. Nelson (Eds.), *Insect lipids: Chemistry, biochemistry and biology* (pp. 179–226). Lincoln, NE: Univ. Neb. Press.

Human, K., & Gordon, D. M. (1996). Exploitation and interference competition between the invasive Argentine ant, Linepithema humile, and native ant species. *Oecologia*, *105*(3), 405–412.

Kalinowski, S. T. (2011). The computer program STRUCTURE does not reliably identify the main genetic clusters within species: Simulations and implications for human population structure. *Heredity*, *106*(4), 625–632. https://doi.org/10.1038/hdy.2010.95

Katzerke, A., Neumann, P., Pirk, C. W. W., Bliss, P., & Moritz, R. F. A. (2006). Seasonal nestmate recognition in the ant Formica exsecta. *Behavioral Ecology and Sociobiology*, *61*(1), 143–150. https://doi.org/10.1007/s00265-006-0245-6

Kennedy, P., Uller, T., & Helanterä, H. (2014). Are ant supercolonies crucibles of a new major transition in evolution? *Journal of Evolutionary Biology*, *27*(9), 1784–1796. https://doi.org/10.1111/jeb.12434

Kidokoro-Kobayashi, M., Iwakura, M., Fujiwara-Tsujii, N., Fujiwara, S., Sakura, M., Sakamoto, H., Higashi, S., Hefetz, A., & Ozaki, M. (2012). Chemical discrimination and aggressiveness via cuticular hydrocarbons in a supercolony-forming ant, Formica yessensis. *PLoS ONE*, *7*(10), 1–14. https://doi.org/10.1371/journal.pone.0046840

Kümmerli, R., Gardner, A., West, S., & Griffin, A. (2009). Limited dispersal, budding dispersal, and cooperation: An experimental study. *Evolution*, *63*(4), 939–949. https://doi.org/10.1111/j.1558-5646.2008.00548.x

Kutter, H. (1969). *Die sozialparasitischen Ameisen der Schweiz* . Zürich, Switzerland: Naturforschenden Gesellschaft in Zürich.

Lester, P., & Gruber, M. A. (2012). Comment on Moffett: "Supercolonies of billions in an invasive ant: What is a society?". *Behavioral Ecology*, *23*(5), 934–935. https://doi.org/10.1093/beheco/ars047

Mabelis, A. (1979). The relationship between aggression and predation in the red wood ant (Formica polyctena Först.). *Netherlands Journal of Zoology*, *29*(4), 451–620.

Mabelis, A. (1984). Aggression in wood ants (Formica polyctena Foerst., Hymenoptera, Formicidae). *Aggressive Behavior*, *10*(1), 47–53.

Markó, B., Czekes, Z., Eros, K., Csata, E., & Szász-Len, A. M. (2012). The largest polydomous system of Formica ants (Hymenoptera: Formicidae) in Europe discovered thus far in Romania. *North-Western Journal of Zoology*, *8*(2), 287–291.

Martin, S. J., Helanterä, H., Kiss, K., Lee, Y. R., & Drijfhout, F. P. (2009). Polygyny reduces rather than increases nestmate discrimination cue diversity in Formica exsecta ants. *Insectes Sociaux*, *56*(4), 375–383. https://doi.org/10.1007/s00040-009-0035-z

Martin, S. J., Vitikainen, E., Drijfhout, F. P., & Jackson, D. (2012). Conspecific ant aggression is correlated with chemical distance, but not with genetic or spatial distance. *Behavior Genetics*, *42*(2), 323–331. https://doi.org/10.1007/s10519-011-9503-0

Moffett, M. W. (2010). *Adventures among ants*. Berkeley, CA: University of California Press.

Moffett, M. W. (2012). Supercolonies of billions in an invasive ant: What is a society? *Behavioral Ecology*, *23*(5), 925–933. https://doi.org/10.1093/beheco/ars043

Pamilo, P., & Rosengren, R. (1984). Evolution of nesting strategies of ants: Genetic evidence from different population types of Formica ants. *Biological Journal of the Linnean Society*, *21*(3), 331–348. https://doi.org/10.1111/j.1095-8312.1984.tb00370.x

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539.

Pedersen, J. S. (2012). The logic of hypersocial colonies. *Behavioral Ecology*, *23*(5), 934–935. https://doi.org/10.1093/beheco/ars047

Pedersen, J. S., Krieger, M. J. B., Vogel, V., Giraud, T., & Keller, L. (2006). Native supercolonies of unrelated individuals in the invasive argentine ant. *Evolution*, *60*(4), 782–791. https://doi.org/10.1111/j.0014-3820.2006.tb01156.x

Pirk, C. W. W., Neumann, P., Moritz, R. F. A., & Pamilo, P. (2001). Intranest relatedness and nestmate recognition in the meadow ant Formica pratensis (R.). *Behavioral Ecology and Sociobiology*, *49*(5), 366–374. https://doi.org/10.1007/s002650000315

Pisarski, B. (1982). Influence de la structure sociale sur le comportement agressif des ouvrières de Formica (Coptoformica) exsecta. *Memorabilia Zoologica*, *38*, 113–136.

Platt, T. G., & Bever, J. D. (2009). Kin competition and the evolution of cooperation. *Trends in Ecology & Evolution*, *24*(7), 370–377. https://doi.org/10.1016/j.tree.2009.02.009

Pohl, A., Ziemen, V., & Witte, V. (2018). Mass occurrence and dominant behavior of the European ant species Formica fuscocinerea (Forel). *Journal of Insect Behavior*, *31*(1), 12–28. https://doi.org/10.1007/s10905-017-9654-9

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3), 608–627. https://doi.org/10.1111/1755-0998.12512

QGIS Development Team (2018). *QGIS Geographic Information System. Open Source Geospatial Foundation Project*. Retrieved from http://qgis.osgeo.org

Queller, D. C. (1992). Does population viscosity promote kin selection? *Trends in Ecology & Evolution*, *7*(10), 322–324. https://doi.org/10.1016/0169-5347(92)90120-Z

R Core Team (2013). *A language and environment for statistical computing*. Vienna Austria: R Foundation for Statistical Computing.

Raymond, M., & Rousset, F. (1995). GENEPOP (Version 1.2): Population genetics software for exact tests and Ecumenicism. *Journal of Heredity*, *86*(3), 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Robinson, E. J. (2014). Polydomy: The organisation and adaptive function of complex nest systems in ants. *Current Opinion in Insect Science*, *5*(1), 37–43. https://doi.org/10.1016/j.cois.2014.09.002

Rosengren, R., Cherix, D., & Pamilo, P. (1986). Insular ecology of the red wood ant Formica truncorum Fabr. II. Distribution, reproductive strategy and competition. *Mitteilungen Der Schweizerischen Entomologischen Gesellschaft*, *59*, 63–94.

Rosengren, R., & Pamilo, P. (1983). The evolution of polygyny and polydomy in mound-building Formica ants. *Acta Entomologica Fennica*, *42*, 65–77.

Rosengren, R., Sundström, L., & Fortelius, W. (1993). Monogyny and polygyny in Formica ants: The result of alternative dispersal tactics. In L. Keller (Ed.), *Queen number and sociality in insects* (pp. 308–333). Oxford, UK: Oxford University Press.

Ross, K. G., Vargo, E. L., & Keller, L. (1996). Social evolution in a new environment: The case of introduced fire ants. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(7), 3021–3025.

Roulston, T. H., Buczkowski, G., & Silverman, J. (2003). Nestmate discrimination in ants: Effect of bioassay on aggressive behavior. *Insectes Sociaux*, *50*(2), 151–159. https://doi.org/10.1007/s00040-003-0624-1

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Savolainen, R., & Vepsäläinen, K. (1988). A competition hierarchy among boreal ants: Impact on resource partitioning and community structure. *Oikos*, *51*(2), 135–155.

Schultner, E., Gardner, A., Karhunen, M., & Helanterä, H. (2014). Ant larvae as players in social conflict: Relatedness and individual identity mediate cannibalism intensity. *American Naturalist*, *184*(6), E161–E174. https://doi.org/10.1086/678459

Schultner, E., Saramäki, J., & Helanterä, H. (2016). Genetic structure of native ant supercolonies varies in space and time. *Molecular Ecology*, *25*(24), 6196–6213. https://doi.org/10.1111/mec.13912

Schultz, R., & Seifert, B. (2007). The distribution of the subgenus Coptoformica Müller, 1923 (Hymenoptera: Formicidae) in the Palaearctic Region. *Myrmecological News*, *10*, 11–18.

Seifert, B. (2000). A taxonomic revision of the ant subgenus Coptoformica Mueller, 1923 (Hymenoptera, Formicidae). *Zoosystema*, *22*(3), 517–568.

Seppä, P., Gyllenstrand, N., Corander, J., & Pamilo, P. (2004). Coexistence of the social types: Genetic population structure in the ant Formica exsecta. *Evolution*, *58*(11), 2462–2471.

Seppä, P., Johansson, H., Gyllenstrand, N., Pálsson, S., & Pamilo, P. (2012). Mosaic structure of native ant supercolonies. *Molecular Ecology*, *21*(23), 5880–5891. https://doi.org/10.1111/mec.12070

Sorvari, J. (2009). Foraging distances and potentiality in forest pest insect control: An example with two candidate ants (Hymenoptera: Formicidae). *Myrmecological News*, *12*, 211–215.

Sorvari, J., & Hakkarainen, H. (2004). Habitat-related aggressive behaviour between neighbouring colonies of the polydomous wood ant Formica aquilonia. *Animal Behavior*, *67*(1), 151–153. https://doi.org/10.1016/j.anbehav.2003.03.009

Sundström, L., Seppä, P., & Pamilo, P. (2005). Genetic population structure and dispersal patterns in Formica ants — A review. *Annales Zoologici Fennici*, *42*, 163–177.

Taylor, P. D. (1992). Altruism in viscous populations – An inclusive fitness model. *Evolutionary Ecology*, *6*(4), 352–356.

Thomas, M. L., Payne-Makrisâ, C. M., Suarez, A. V., Tsutsui, N. D., & Holway, D. A. (2007). Contact between supercolonies elevates aggression in Argentine ants. *Insectes Sociaux*, *54*(3), 225–233.

Trontti, K., Tay, W. T., & Sundström, L. (2003). Polymorphic microsatellite markers for the ant *Plagiolepis pygmaea*. *Molecular Ecology Notes*, *3*(4), 575–577. https://doi.org/10.1046/j.1471-8286.2003.00516.x

Tsutsui, N. D., & Case, T. J. (2001). Population genetics and colony structure of the Argentine ant (Linepithema humile) in its native and introduced ranges. *Evolution*, *55*(5), 976–985.

Tsutsui, N. D., & Suarez, A. V. (2003). The colony structure and population biology of invasive ants. *Conservation Biology*, *17*(1), 48–58. https://doi.org/10.1046/j.1523-1739.2003.02018.x

Tsutsui, N. D., Suarez, A. V., Holway, D. A., & Case, T. J. (2000). Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(11), 5948–5953.

van Wilgenburg, E., Torres, C. W., & Tsutsui, N. D. (2010). The global expansion of a single ant supercolony. *Evolutionary Applications*, *3*(2), 136–143. https://doi.org/10.1111/j.1752-4571.2009.00114.x

Vander Meer, R. K., & Morel, L. (1998). In R. K. Vander Meer, M. Breed, M. Winston, & K. E. Espelie (Eds.), *Pheromone communication in social insects* (pp. 79–103). Boulder, CO: Westview Press.

Vepsäläinen, K., & Pisarski, B. (1982). Assembly of island ant communities. *Annales Zoologici Fennici*, *19*(4), 327–335.

Vogel, V., Pedersen, J. S., D'Ettorre, P., Lehmann, L., & Keller, L. (2009). Dynamics and genetic structure of argentine ant supercolonies in their native range. *Evolution*, *63*(6), 1627–1639. https://doi.org/10.1111/j.1558-5646.2009.00628.x

West, S., Pen, I., & Griffin, A. (2002). Cooperation and competition between relatives. *Science*, *296*(5565), 72–75. https://doi.org/10.1126/science.1065507

Wetterer, J. K., Wild, A. L. W., Suarez, A. V. S., Roura-Pscual, N. R., & Espadaler, X. E. (2009). Worldwide spread of the Argentine ant, Linepithema humile (Hymenoptera: Formicidae). *Myrmecological News*, *12*, 187–194.

Zinck, L., Hora, R. R., Châline, N., & Jaisson, P. (2008). Low intraspecific aggression level in the polydomous and facultative polygynous ant Ectatomma tuberculatum. *Entomologia Experimentalis et Applicata*, *126*(3), 211–216. https://doi.org/10.1111/j.1570-7458.2007.00654.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

TABLE A1 Number of nests, area, and nest density for the studied subpopulations as well as separately for the four parts within the supercolony site

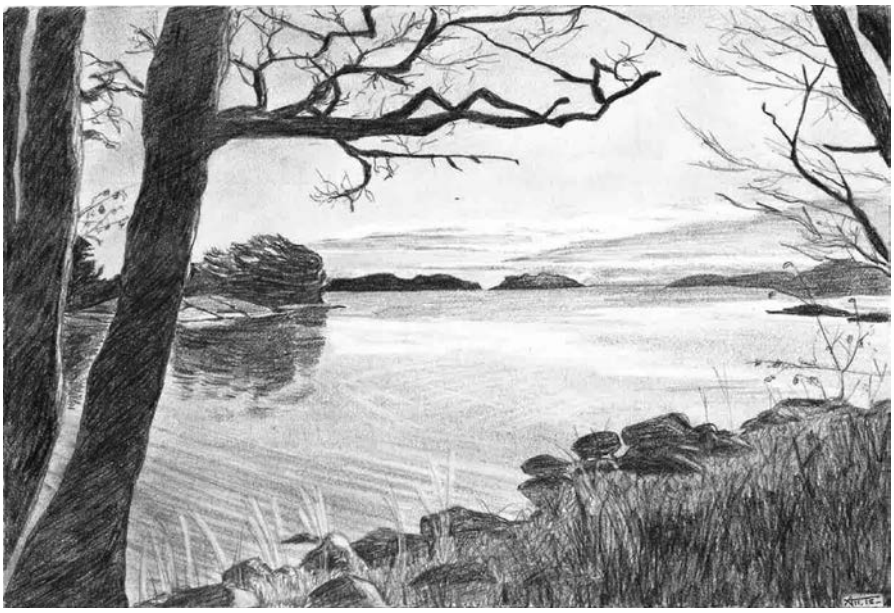
Part	Nests	Area (ha)	nests/ha
Supercolony site	1,343	5.93	226.48
Part I	281	1.11	253.78
Part II	519	1.29	400.98
Part III	519	1.87	277.66
Part IV	24	0.09	275.76
Storsand	16	0.63	25.26
Lillgård	7	0.02	425.81
Storgård	29	1.17	24.87

Note: The area of the supercolony site is the area of all the open, and thus potentially habitable, parts of the field. The areas of the individual parts of the supercolony site and the smaller sites are defined as the polygon formed by drawing straight lines between the outermost nests.

TABLE A2 Pairwise comparisons between the factor levels of the fixed effects included in the models

Response Pairwise comparisons	*SE*	*z*-Value	*p*
Presence of aggression	**GLMM (binomial)**		
Neighbor—Distant	0.401	2.231	.026
Neighbor—Outside	0.432	2.887	.004
Neighbor—Allospecific	0.729	6.709	<.001
Distant—Outside	0.406	0.866	.386
Distant—Allospecific	0.708	5.639	<.001
Outside—Allospecific	0.718	5.075	<.001
Duration of aggression	**GLMM (beta)**		
Neighbor—Distant	0.317	−0.290	.772
Neighbor—Outside	0.310	−0.713	.476
Neighbor—Allospecific	0.299	1.111	.267
Distant—Outside	0.261	−0.494	.621
Distant—Allospecific	0.238	1.780	.075
Outside—Allospecific	0.227	2.434	.015
Duration of antennation	**GLMM (beta)**		
Control—Neighbor	0.195	5.454	<.001
Control—Distant	0.200	5.071	<.001
Control—Outside	0.336	3.737	<.001
Neighbor—Distant	0.210	−0.231	.818
Neighbor—Outside	0.334	0.578	.563
Distant—Outside	0.343	0.703	.482

Note: Fixed effect levels: Control = introduced ant from the same nest, Neighbor = introduced ant from the same part of the supercolony field, Distant = introduced ant from a different part from the supercolony field, Outside = introduced ant from another field, Allospecific = introduced ant of a different species, *F. exsecta*. The aggression models do not include the level "Control," for it had no aggression. The model for antennation does not include the level "Allospecific" due to it having aggression in all but three samples and thus too small a sample size for analyzing the nonaggressive behaviors. In all of the models, the nest IDs of both the host and the visitor were included as crossed random effects.



“My dear People, began Bilbo…"

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