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Ecological genetics of a supergene controlling ant social organization

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Ecological genetics of a supergene controlling ant social organization

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Prof. Paul Franken

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Abstract

Supergenes control various complex phenotypes, yet in many cases the mechanisms contributing to maintain such genetic polymorphisms remain poorly understood. In the Alpine silver ant *Formica selysi*, a supergene determines social organization, a fundamental trait for the evolution of sociality by kin selection in insects. The supergene has two haplotypes, Sm and Sp. In single queen (monogynous) colonies, all females and males have the genotypes Sm/Sm and Sm, respectively. In contrast, all individuals produced by multiple queen (polygynous) colonies bear at least one copy of the Sp haplotype. In this thesis, I explore three mechanisms that might be involved in the maintenance of the polymorphism at this supergene controlling ant social organization. In the first chapter, I used fine-scale population genomic data to investigate the mating and dispersal strategies of each sex from each social form. The mating pattern observed in mature colonies was asymmetrical, suggesting unidirectional male-mediated gene flow from the monogynous to the polygynous social form. Queens showed signs of restricted dispersal and queens from polygynous colonies tended to be mated with relatives. In the second chapter, I investigated the causes of a transmission ratio distortion observed in polygynous colonies. Heterozygous queens are expected to produce Sm males, and, when mated with Sm males from monogynous colonies, Sm/Sm females, yet such individuals are absent from polygynous colonies. I found that the Sp haplotype is a maternal offspring killer, causing the death of progenies from heterozygous queens that did not inherit Sp. In the third chapter, I tested whether mate preferences and genetic incompatibilities explain the asymmetrical mating pattern documented in the first chapter. There was no evidence for genetic or behavioral barriers between social forms. Queens of monogynous origin were more likely to mate and more fertile than queens of polygynous origin, suggesting that queens of alternative social forms differ in their reproductive strategies. Altogether, this thesis sheds light on some key mechanisms contributing to the maintenance of polymorphism at a supergene controlling ant social organization.

Résumé

Les supergènes contrôlent de multiples phénotypes complexes, mais dans de nombreux cas les mécanismes qui contribuent à maintenir ces polymorphismes génétiques sont encore mal compris. Chez la fourmi Alpine argentée *Formica selysi*, un supergène détermine l'organisation sociale, un trait fondamental dans l'évolution de la socialité par la sélection de parentèle chez les insectes. Le supergène a deux haplotypes, Sm et Sp. Dans les colonies à une seule reine (monogyne), toutes les femelles et les mâles ont le génotype Sm/Sm et Sm, respectivement. A l'inverse, tous les individus produits par les colonies à plusieurs reines (polygynes) portent au moins une copie de l'haplotype Sp. Dans cette thèse, j'ai exploré trois mécanismes susceptibles d'être impliqué dans la maintenance du polymorphisme au supergène contrôlant l'organisation sociale chez une fourmi. Dans le premier chapitre, j'ai utilisé des données de génomique des populations à une échelle fine pour explorer les stratégies d'accouplement et de dispersion de chaque sexe et chaque forme sociale. Le pattern d'accouplement observé dans les colonies matures était asymétrique, suggérant un flux de gènes unidirectionnel induit par les mâles de la forme sociale monogyne à polygyne. Les reines ont montré des signes de dispersion limitée, et les reines des colonies polygynes avaient tendance à être accouplées avec des mâles apparentés. Dans le deuxième chapitre, j'ai exploré les causes du biais de transmission observé dans les colonies polygynes. Les reines hétérozygotes devraient produire des mâles Sm ainsi que des femelles Sm/Sm lorsqu'elles sont accouplées avec des mâles Sm, mais ces génotypes ne sont pas présents dans les individus produits par les colonies polygynes. J'ai trouvé que l'haplotype Sp est un élément génétique égoïste, causant la mort des descendants de reines hétérozygotes qui n'héritent pas Sp. Dans le troisième chapitre, j'ai testé si les préférences d'accouplement et les incompatibilités génétiques expliquent le pattern d'accouplement asymétrique documenté dans le premier chapitre. Il n'y avait pas d'indices de barrières génétiques ou comportementales entre les formes sociales. Les reines d'origine monogyne avaient plus de chances de s'accoupler et étaient plus fertile que les reines d'origine polygyne, ce qui suggère que les reines de forme sociales alternatives diffèrent dans leurs stratégies reproductrices. Dans l'ensemble, cette thèse met en lumière certains mécanismes clés contribuant à la maintenance du polymorphisme au supergène contrôlant l'organisation sociale chez une fourmi.

General introduction

Social organization

Social organization, here defined as the number of breeders within colonies, is a very labile trait in ants (Bourke & Franks, 1995). In particular, colony queen number varies tremendously within and between species (Crozier & Pamilo, 1996). This variation in social organization has long been a puzzle in evolutionary biology (Keller, 1995, Hamilton, 1972). Indeed, social organization shapes the genetic composition of the colony, and therefore the degree of relatedness among nestmates, which is central for the evolution of eusociality by kin selection (Hamilton, 1964). Single queen (monogynous) colonies, which maximize intra-colony relatedness, are ancestral in ants (Hughes et al., 2008a). Yet, multiple queen (polygynous) colonies evolved multiple times, raising questions on the evolution and maintenance of such societies with low intra-colony relatedness (Hughes et al., 2008b, Keller, 1995, Hamilton, 1972).

Unraveling the causes and consequences of intra-specific variation in social organization can help to understand fundamental evolutionary principles generating phenotypic diversity (Ross, 2001, Schradin, 2013). Social organization is a complex phenotype, i.e. an association of many co-adapted behavioral, physiological and morphological traits (Keller, 1995, Rosset & Chapuisat, 2007, Araujo & Tschinkel, 2010, Meunier et al., 2011, Purcell & Chapuisat, 2012, Purcell & Chapuisat, 2014). Individuals originating from single-queen and multiple-queen colonies typically differ in body size, behavior, mating and dispersal (DeHeer et al., 1999, Lawson et al., 2012, Meunier & Chapuisat, 2009). Such differences may lead to reproductive isolation between the two social forms, and some authors have suggested that social polymorphism is a transient

state toward speciation (Ross & Keller, 1995a, Pamilo et al., 1997). More generally, co-adapted traits involve more than one locus. This raises an interesting question pertaining to the mechanisms underlying the polymorphism, because co-adapted alleles are likely to be disrupted by recombination, creating maladaptive phenotypes (Pinho & Hey, 2010).

Maintenance of complex phenotypes

Several mechanisms can produce intra-specific variation in complex phenotypes. Phenotypic plasticity in response to variable environment may generate alternative phenotypes. For instance, phenotypic plasticity underlies variation in social organization in the sweat bee *Halictus rubincundus*. Using transplant experiments, researchers showed that the social phenotype of these bees, solitary or social, was a plastic response to the length of the season allowing for brood production (Field et al., 2010). The alternative morphological, behavioral and reproductive castes that characterize the eusocial insects represent another striking example of phenotypic plasticity. In general, female-destined eggs with identical genomes can develop into either a queen or a worker (Corona et al., 2016, but see Schwander & Keller, 2008).

Current research seeks to understand the epigenetic modifications modulating this reproductive division of labor, which is the hallmark of eusociality (Corona et al., 2016, Yan et al., 2014). Alternative DNA methylation patterns between castes have been documented in ants (Bonasio et al., 2012, Alvarado et al., 2015) and bees (Foret et al., 2012, Lyko et al., 2010, Herb et al., 2012), although more studies with greater statistical power are still needed to demonstrate the importance of DNA methylation for caste differentiation (Libbrecht et al., 2016). Another epigenetic modification, histone acetylation, modulates worker caste polymorphism in

Camponotus floridanus (Simola et al., 2016), suggesting that multiple epigenetic modifications are likely to contribute to the regulation of plastic complex phenotypes.

Intra-specific variation in complex phenotypes may also result from genetic variation at pleiotropic genes or at supergenes (Saltz et al., 2017). These two forms of genetic architecture are not mutually exclusive, and the regulation of some complex phenotypes leans on both mechanisms (e.g. Carbone et al., 2006). A pleiotropic gene affects multiple traits, for instance, genes controlling hormone production are often pleotropic. Division in labor is primarily controlled by the expression of one pleiotropic gene coding for vitellogenin in the honeybee *Apis mellifera* and another pleiotropic gene coding for juvenile hormone in the leaf-cutting ant *Acromyrmex octospinosus* (Nelson et al., 2007, Norman & Hughes, 2016).

Supergenes

There is growing evidence that many complex phenotypes are regulated by supergenes. Supergenes are clusters of co-adapted loci in a region of reduced recombination (Schwander et al., 2014, Thompson & Jiggins, 2014, Charlesworth, 2016). The co-adapted loci within the supergene are transmitted as a single Mendelian unit, avoiding the formation of intermediate, maladaptive phenotypes. Co-adapted loci must be located in close physical proximity, which may be achieved by functional mutation of a neighboring locus, duplication or translocation of loci (Schwander et al., 2014). Suppressed recombination between co-adapted loci is generally due to inversions but can also be caused by the genomic location of the loci and epigenetic mechanisms (Schwander et al., 2014).

The idea that complex phenotypes were controlled by multiple loci kept in tight linkage was first proposed by Fisher in 1930 to explain mimetic polymorphism in *Papilio* butterflies (Fisher,

1930). Soon after, Dobzhansky and Sturtevant (1937) demonstrated that chromosomal inversions were common, providing a mechanism for the evolution of tight linkage and supporting the idea that supergenes might be prevalent in nature.

The best-known supergenes are heteromorphic sex chromosomes controlling sex determination (Charlesworth, 2016, Abbott et al., 2017). Sex chromosomes have evolved independently multiple times (Matsubara et al., 2006, Marshall Graves & Peichel, 2010, Bachtrog et al., 2011), yet the steps involved in their evolution are similar. Sex-determination loci and sexually antagonistic genes first appear on autosomes. Then reduced recombination through inversions and further recruitment of sex specific genes lock co-adapted loci together and expand the supergene (Bachtrog et al., 2011, Charlesworth et al., 2005).

Since the advent of the genomic era, multiple studies have shown that supergenes are involved in the regulation of other types of complex phenotypes (Schwander et al., 2014). Supergenes underlie spectacular polymorphisms associated with Müllerian mimicry in the butterfly *Heliconius numata* (Joron et al., 2006), plumage and social behavior in the white-throated sparrow (Tuttle et al., 2016), male morphs and mating strategies in the ruff (Küpper et al., 2016, Lamichhaney et al., 2016), color polymorphism in the land snail *Cepaea nemoralis* (Murray & Clarke, 1976, Richards et al., 2013), self-incompatibility in plants (Li et al., 2016), autosomal drivers in the house mouse (Lyon, 2003) and the fruit fly (Larracuente & Presgraves, 2012), and sperm morphology in the zebra finch (Kim et al., 2017, Knief et al., 2017). In social insects, a supergene is associated with a high-altitude morph of honeybees (Wallberg et al., 2017) and with social organization (colony queen number) in two ant species (Wang et al., 2013, Purcell et al., 2014b).

There is no shared synteny between the supergenes controlling social organization in the fire ant *Solenopsis invicta* and the Alpine silver ant *Formica selysi*, which shows that the supergenes have evolved independently in these phylogenetically distant lineages (Purcell et al., 2014b). This provides a great opportunity to compare the two systems and unravel common principles in the evolution and maintenance of supergenes. In *S. invicta*, the supergene controlling social organization contains more than 600 genes in a non-recombining region of 13Mb. This supergene has two alternative haplotypes, SB and Sb (Wang et al., 2013). Because the Sb haplotype is homozygous lethal it can't recombine. The Sb haplotype shows reduced nucleotide diversity and accumulates repetitive elements, suggesting that it is degenerating (Pracana et al., 2017). The Sb haplotype is also a "green-beard gene" favoring its own transmission to the detriment of the rest of the genome: in polygynous colonies, queens that lack a copy of Sb are killed by workers that carry a copy of it (Keller & Ross, 1998). Despite the selective advantage of the Sb haplotype, the polymorphism may be stable in *S. invicta* because of the homozygous lethality of Sb, strong selection on sexuals, or unidirectional gene flow from the monogynous to the polygynous social forms (Shoemaker & Ross, 1996, Fritz et al., 2006).

Study species

The Alpine silver ant *F. selysi* is a pioneer ant specialized in floodplains across Europe, from the Alps to the Pyrenees (Seifert, 2002). Colonies of *F. selysi* can survive occasional floods by building living rafts and floating to the bank (Lude et al., 1999, Purcell et al., 2014a - Appendix 1). Workers use brood as a base, which increase the floatability of the rafts and the workers' ability to recover after rafting (Purcell et al., 2014a - Appendix 1). In addition, workers participating in the construction of the raft show memory and specialization in their positions (Avril et al., 2016 - Appendix 2).

Populations of *F. selysi* are composed of monogynous and polygynous colonies living in sympatry (Chapuisat et al., 2004). The social structure of the colonies rarely shifts, and the social polymorphism appears stable at the population level (Purcell & Chapuisat, 2013). However, the proportion of monogynous colonies within populations increases with altitude, which is likely due to differences in ecological optima between social forms (Purcell et al., 2015).

Variation of social organization in *F. selysi* is associated with many differences in life-history traits. Nest density, colony size, egg, queen and worker sizes, fecundity, survival rate in absence or presence of a pathogen, development time and chemical profiles are significantly different between the monogynous and the polygynous social forms (Meunier et al., 2010, Purcell & Chapuisat, 2012, Purcell et al., 2016a, Reber et al., 2008, Reber et al., 2010, Rosset & Chapuisat, 2007, Meunier et al., 2011, Schwander et al., 2005). Yet, there is little genetic differentiation between social forms at neutral markers, suggesting ongoing gene flow between them (Chapuisat et al., 2004). In line with this result, queens and males of different social forms are able to mate and found colonies, indicating that there is no genetic or behavioral incompatibilities between social forms (Reber et al., 2010).

Social organization in *F. selysi* is controlled by a supergene with two haplotypes, Sm and Sp (Purcell et al., 2014b). In monogynous colonies, only the Sm haplotype is present. In polygynous colonies, all the individuals bear at least one copy of the Sp haplotype, with workers and queens being Sp/Sp or Sp/Sm, and males Sp (**Figure 1**). The mechanisms maintaining this genetic polymorphism are not well understood.

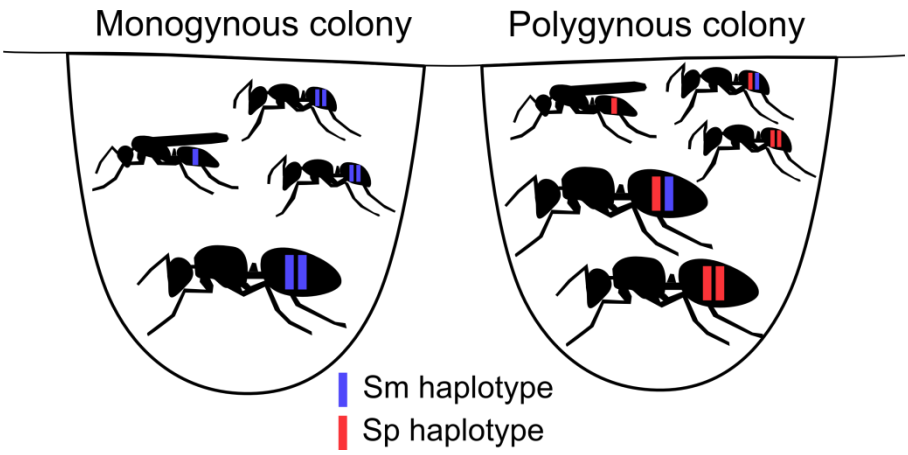

Figure 1: Genotypic distribution at the supergene in monogynous and polygynous colonies.

Aims of the PhD

The aim of this PhD is to get a better understanding of how the polymorphism at this supergene controlling social organization is maintained in *F. selysi*. Ultimately, this work provides insights into the evolutionary mechanisms and selective pressures contributing to a balanced polymorphism (Ford, 1971).

Several mechanisms may contribute to the maintenance of polymorphism at supergenes (Schwander et al., 2014, Kirkpatrick, 2010). Most of these mechanisms generate negative frequency-dependent selection, a selection regime under which the fitness of individuals bearing a specific haplotype is negatively correlated with the frequency of this haplotype in the population. For instance, obligate disassortative mating generates strong negative frequency-dependent selection that maintains the polymorphism at sex chromosomes. Likewise, disassortative mating has a critical role in the maintenance of polymorphism at supergenes controlling plumage color and social behavior in the white-throated sparrow (Tuttle et al., 2016),

self-incompatibility in *Primula vulgaris* (Li et al., 2016), and mimetic wing patterns within populations of *Heliconius numata* (Chouteau et al., 2017). In many supergenes, the positively selected variant is a recessive lethal, generating negative frequency-dependent selection that stabilizes the polymorphism (Lyon, 2003, Larracuente & Presgraves, 2012, Wang et al., 2013). Heterogeneous spatial selection, whereby the fitness of individuals bearing a specific haplotype depends on their spatial location, can also maintain diversity (Svardal et al., 2015). For example, the frequencies of multiple haplotypes at the supergene controlling Müllerian mimicry in *Heliconius* butterflies are greatly influenced by the distribution of co-mimics *Melineae* species with alternative wing patterns. The fitness of *Heliconius* individuals with a given genotype depends on the local abundance of different species of *Melineae*, which contributes to the maintenance of the supergene polymorphism over a large geographical scale (Joron et al., 1999). Finally, some polymorphic supergenes are maintained by heterozygote advantage, whereby individuals bearing a copy of each haplotype have the greatest fitness. For instance, heterozygotes males at the supergene controlling sperm morphology in the zebra finch have a higher sperm velocity and fertilization success, maintaining variation at the supergene (Knief et al., 2017, Kim et al., 2017).

In this thesis, I tested for three mechanisms that are likely to contribute to the maintenance of the polymorphism at a supergene controlling social organization in *F. selysi* (**Figure 1**). In the first chapter, I used fine-scale population genomic data to investigate the dispersal and mating strategies of queens and males from each social form. Variation in social organization is often associated with changes in dispersal and mating, at least between species (Sundström et al., 2005, Ross, 2001, Hölldobler & Wilson, 1990). Queens from monogynous species usually disperse far and found colonies independently. In contrast, queens from polygynous species often mate close

to, or within their nests, and disperse by budding. Differences between social forms in mating and dispersal strategies are likely to play an important role in the maintenance of the supergene controlling social organization in *S. invicta* (Ross & Shoemaker, 1993, Ross & Keller, 1995a, Ross & Keller, 1995b, Shoemaker & Ross, 1996, Goodisman et al., 2000). Exploring the mating and dispersal strategies in *F. selysi* will shed light on the population genetic consequences of the social polymorphism and ultimately on the maintenance of alternate haplotypes at this supergene controlling social organization.

In the second chapter, I investigated the causes of the transmission ratio distortion observed in polygynous colonies of *F. selysi*. The Sp haplotype appears to favor its own transmission over the one the alternate haplotype. Specifically, Sm males and Sm/Sm females are not produced by polygynous colonies, although they are expected to occur, given the patterns of mating documented in the first chapter. I tested whether a meiotic drive, a green-beard effect or a maternal killing effect were responsible for this transmission bias.

The genetic data from field colonies of *F. selysi* presented in the first chapter point at an asymmetric mating pattern with respect to social form. Queens of monogynous origin are always mated with males of monogynous origin. In contrast, queens of polygynous origin are mostly mated with males of polygynous origin, but 23.6% mated with males of monogynous origin. In the third chapter, I tested whether mate preferences or genetic incompatibilities between social forms caused this asymmetric mating pattern observed in field colonies. Overall, by combining genomic data from the field, crossing experiments and mate choice experiments, I documented unexpected effects of the supergene at multiple levels of biological organization, and revealed

some key mechanisms contributing to the maintenance of genetic and phenotypic social polymorphism in the Alpine silver ant.

Chapter 1:

Impact of mating system on the maintenance of polymorphism at a supergene controlling ant social organization

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Manuscript in preparation

Abstract

Genomic rearrangements that suppress recombination over large portions of the genome underlie spectacular phenotypic polymorphisms within single populations. Because alternate haplotypes at supergenes affect multiple morphological, physiological and behavioral traits, understanding how such polymorphisms are balanced is often challenging. In the Alpine silver ant *Formica selysi*, variation in social organization is controlled by a large supergene with two alternate haplotypes, Sm and Sp, maintained by multiple inversions. Sm/Sm females typically establish single-queen (monogynous) colonies, while Sm/Sp and Sp/Sp females form multiple-queen (polygynous) colonies. Here, we used fine-scale population genomic data from offspring of isolated queens to investigate the dispersal and mating strategies of each sex from each social form. Mating between social forms was asymmetrical. Queens heading mature monogynous colonies were exclusively mated with Sm males. In contrast, queens heading polygynous colonies were mated with Sm and Sp males, with a relative contribution of 23.6% of mating by Sm males that are only produced by monogynous colonies. This unusual mating pattern causes unidirectional male-mediated gene flow from the monogynous to the polygynous social form, confirmed by a higher number of private alleles in the polygynous social form. For both social forms, there were signs of restricted dispersal for queens, but not for males. Queens of polygynous origin tended to mate with relatives. Surprisingly, the supergene was associated with the rate of polyandry. Heterozygous queens were more likely to mate with more than one male, compared to homozygous queens for both haplotypes. Overall, the genetic data reveal that alternative social forms of the Alpine silver ant differ in their mating and dispersal strategies, which affects the maintenance of this genetic and phenotypic polymorphism in social organization.

Introduction

Supergenes are clusters of tightly linked loci controlling complex phenotypes (Dobzhansky, 1970, Schwander et al., 2014, Thompson & Jiggins, 2014). They underlie some of the most spectacular polymorphisms in nature, including sexes, mimetic forms in butterflies (Joron et al., 2011), mating tactics in birds (Küpper et al., 2016, Tuttle et al., 2016), and social organization in ants (Wang et al., 2013, Purcell et al., 2014b). Because alternate haplotypes at supergenes influence multiple morphological, physiological and behavioral traits, understanding how such polymorphisms are balanced is often challenging (Llaurens et al., 2017). Indeed, alternative morphs differ in many traits that potentially affect their survival, mating, dispersal and reproduction, which will in turn influence the maintenance of the genetic polymorphism (e.g. Joron & Iwasa, 2005, Chouteau et al., 2017).

A major component of the social organization of insects is the number of breeders within each colony (Ross, 2001, Bourke & Franks, 1995). The number of queens per colony and the number of matings per queen shape the degree of within colony relatedness, which is central for the evolution of altruism by kin selection (Bourke, 2011, Hamilton, 1964). In ants, the number of queens per colony varies greatly within and among species (Hölldobler & Wilson, 1977, Crozier & Pamilo, 1996). Unravelling the causes and consequences of intraspecific variation in social organization can provide insights into the fundamental evolutionary principles generating phenotypic diversity (Ross, 2001, Schradin, 2013).

Social organization and dispersal are expected to co-evolve (Mullon et al., 2018, Auld & Rubio de Casas, 2012). Variation in social organization is typically associated with shifts in dispersal and mating strategies in ants (Pamilo et al., 1997, Ross, 2001, Bourke & Franks, 1995). In

polygynous colonies, restricted dispersal of queens and re-acceptance of related queens in their natal nest may be a kin selected adaptation, because it limits the erosion of relatedness (Nonacs, 1988, Ross, 2001). Queens from polygynous species often mate near or within their natal nest, and disperse on foot and with nestmate workers to establish a new nest (Chapuisat et al., 1997a, Chapuisat & Keller, 1999, Liautard & Keller, 2001, Dekoninck et al., 2014, Holzer et al., 2009, Sanllorente et al., 2015, Rees et al., 2009). In contrast, queens from monogynous species usually disperse on the wing, mate in swarms away from their natal nest, and rely on their fat reserves to found new colonies and produce the first workers independently (Jowers et al., 2013, Timmermans et al., 2009, Suni & Gordon, 2010). In line with a shift in dispersal strategies, polygynous species tend to have higher degree of population genetic structure than monogynous species (reviewed in Sundström et al., 2005, Pamilo et al., 1997). Whether similar differences in mating and dispersal underlie intraspecific variation in social organization is less clear.

The genetic basis of intraspecific variation in social organization is rarely known. In the Alpine silver ant *Formica selysi*, social organization is controlled by a polymorphic supergene that is 14.1 Mbp long and contains 664 coding genes (Purcell et al., 2014b; Avril, Tran, & Chapuisat, unpublished results). This supergene has two non-recombining haplotypes, Sm and Sp, which differ through multiple inversion and show high levels of differentiation (Purcell et al., 2014b; Avril, Tran, & Chapuisat, unpublished results). All individuals from monogynous colonies have only the Sm haplotype, while queens, workers and male alates from polygynous colonies have at least one Sp haplotype (Purcell et al., 2014b, Purcell et al., 2016b; Avril et al., chapter 3). Alternative haplotypes are likely to influence multiple morphological, behavioral and life history traits associated with mating and dispersal strategies (Rosset & Chapuisat, 2007). For instance, *F. selysi* queens of monogynous origin are larger (Meunier & Chapuisat, 2009) and more fecund

(Reber et al., 2010; Avril et al., chapter 3) than queens of polygynous origin, which probably improves their ability to fly and found nests independently. Such differences in mating, dispersal and reproduction are likely to influence the dynamics of the genetic polymorphism.

Here, we use genomic data to infer the mating system and dispersal of queens and males from each social form in the Alpine silver ant *Formica selysi*. We examine whether differences in mating and dispersal affect the transmission of the supergene and contribute to the maintenance of the polymorphism in social organization.

Materials and Methods

Sampling and genotyping strategy

The Alpine silver ant *Formica selysi* is a socially polymorphic species that inhabits large valleys in the Alps and the Pyrenees (Chapuisat et al., 2004, Purcell et al., 2015). The ants were primarily sampled from a population located in central Valais, Switzerland (Finges: 7°36'30" E, 4°18'30" N, altitude: 565 m). The social organization of each colony (monogyny or polygyny) had been previously determined by genotyping workers at microsatellite markers (Purcell & Chapuisat, 2013) or with a PCR-RFLP assay that discriminates alternative haplotypes of the supergene (Purcell et al., 2014b). It was further confirmed by counting queens during sampling and analyzing single nucleotide polymorphisms (SNPs) located outside and within the supergene (see below).

To obtain the genotypes of queens and their mates, we genotyped offspring of single queens (= progenies) and reconstructed the parental genotypes. This strategy was chosen to circumvent the difficulty and destructive nature of sampling mature queens from monogynous colonies. A similar genotyping of progeny was applied to queens from polygynous colonies in order to obtain comparable data on mating frequency and mate genotypes for both social forms.

Progenies from monogynous queens consisted of four workers sampled from each of 63 monogynous colonies of the Finges population (Table S1, Figure S1). Progenies from polygynous queens were obtained by isolating 142 wingless reproductive queens sampled from 51 polygynous colonies of the Finges population (Table S1, Figure S1). Direct observations confirmed the presence of multiple queens in the polygynous colonies. To minimize the impact of sampling, we left at least two observed queens in each polygynous colony. Each sampled queen was placed individually in a small plastic box (15 × 13 × 6 cm), with 20 adult workers from the same parent colony. The ants were provided with a nest site, water and *ad libitum* ant food (Meunier & Chapuisat, 2009). Brood production was monitored daily. Four callow (young) workers per queen were collected for 120 queens originating from 37 polygynous colonies of the Finges population. In total, we obtained RAD-seq data for four workers per queen coming from XXX monogynous and YYY polygynous colonies, respectively (see below). These RAD-seq data were used to reconstruct the genotypes of the live queens and their mates at SNPs outside of the supergene and in the supergene (Table S1). The SNPs outside of the supergene were used to determine queen mating frequency and for all population genetic analyses. The supergene genotype was used to determine the social origin of queens and males and infer mating patterns according to social forms (Table S1).

In addition, we collected at least eight eggs per queen for all queens from polygynous colonies of the Finges population. This sample of queens with eggs was supplemented by eight queens from six polygynous colonies of a population in Derborence (7°12'56" E, 46°16'50" N, altitude: 1450 m; Table S1). At the end of the experiment, we dissected the queens and extracted the sperm contained in their spermathecae (Chapuisat, 1998). The supergene genotypes of queens, sperm and eggs from polygynous colonies were determined with a PCR-RFLP assay that discriminates three SNPs diagnostic for alternative haplotypes of the supergene (Purcell et al., 2014b). These RFLP data were used to confirm the supergene genotypes of queens and mates inferred from RAD-seq data and to supplement the mating pattern data (Table S1).

DNA was extracted from the head of queens and from the head and thorax of workers with Qiagen Blood and Tissue extraction kit (Qiagen, Hombrechtikon, Switzerland). DNA from eggs and sperm was extracted with a salting out procedure adapted from Miller (Miller et al., 1988).

Genotyping-by-sequencing

We used a genotyping-by-sequencing (RAD-seq) approach to identify SNPs in workers (Purcell et al., 2016b, Brelsford et al., 2016). The DNA was digested with the restriction enzymes MseI and SbfI. These enzymes produced a low density of SNP markers and allowed us to multiplex the 732 workers on a single lane of Illumina HiSeq 2500. The sequencing was performed at the Lausanne Genomic Technology Facility in Lausanne, Switzerland.

The genetic data were processed with the software pipeline Stacks v1.46 (Catchen et al., 2013). The raw data was demultiplexed using the process_radtags module, and 22 individuals that had low numbers of reads (< 10'000) were removed from the dataset. Reads were aligned to our reference genome (Avril et al., unpublished data) with BWA v0.7.13 (Li & Durbin, 2009). SNPs

and genotypes were called with the ref_map module of Stacks. To avoid linkage disequilibrium between adjacent markers, one SNP per RAD tag was randomly selected, using VCFtools v0.1.14 (Danecek et al., 2011). The SNPs in the supergene, which are linked, were conserved but were analyzed separately from the ones outside of the supergene. Genotypes with a quality score below 20 were treated as missing data. SNPs with a minor allele frequency below 0.01 or missing for more than 20% of the individuals were removed from the dataset. The final dataset included 271 SNPs, of which 25 were in the supergene and 246 in the rest of the genome.

Parental genotype reconstruction

For each sibship (progenies from singly mated queens), the genotypes of the queen and her male mate were reconstructed from RAD-seq data, using the computer program COLONY. For population genomic analyses we conserved the 246 generated SNPs located outside of the supergene and excluded the 25 SNPs in the supergene. Parental genotypes at given SNPs for which the posterior probability was below 0.8 were considered missing values. For multiply mated queens, the maternal and paternal genotypes could not be unambiguously reconstructed. These queens and their male mates were excluded from population genetic analyses on sex-specific dispersal (section 2 below). In total, we reconstructed the genotypes at SNPs outside of the supergene for 157 singly mated queens and their mates.

Genetic data analyses

1. Social structure, supergene genotypes, mating pattern and queen mating frequency

The social structure of each colony was inferred by direct observation of queens in the field (polygynous colonies) and by measuring the relatedness among nestmates (monogynous colonies; see below). The supergene genotype of each queen and respective male mate(s) was

inferred from the supergene genotype of the worker progeny (25 SNPs markers in the supergene obtained by RAD-seq; Table S1). For queens and mates from polygynous colonies, the supergene genotypes were further assessed with a PCR-RFLP assay of queens, sperm and eggs (Purcell et al., 2014b; Table S1).

We calculated the maximum likelihood relatedness among workers from single queens (progenies) with the algorithm of Huang et al. (2015), implemented in the software PolyRelatedness v1.6. To obtain unbiased estimate of relatedness, we used RAD-seq generated SNPs located outside of the supergene. When calculating background allele frequencies, colonies were weighted equally. We used these background allele frequencies to simulate 1'000 dataset of full-sibs, which provided us with a 95% confidence interval around the relatedness of 0.75, the expected value among offspring from singly mated queens. We inferred the pedigree relationships between workers using the maximum likelihood approach implemented in the program COLONY v2.0.6.1 (Jones & Wang, 2010). This method identifies full-sib and half-sib groups. Consensus pedigree relationships were obtained from five iterations, with a genotyping error rate set up at 0.01 per locus.

Queens were inferred to be singly mated when their worker progeny (i) had a relatedness not significantly different from 0.75; and (ii) belonged to a full-sib group in the pedigree reconstruction. Conversely, queens were assessed to be multiply mated when their worker progeny had a relatedness significantly lower than 0.75 and belonged to a half-sib group. Due to the small number of offspring genotyped, the number of mates per queen and the proportion of multiply-mated queens are minimum estimates (Pamilo, 1982, Boomsma & Ratnieks, 1996). With four offspring, there is a 0.125 probability to not sample a patriline when a queen had mated

with two equally contributing males (Boomsma & Ratnieks, 1996). However, because we genotyped the same number of offspring per queen, we can still compare the relative mating frequencies of queens belonging to alternative social forms or with alternative social genotypes.

2. Dispersal of queens and males

To get insight into dispersal patterns, we estimated the relatedness of the male mate to the queen, which equals the average inbreeding coefficient among their worker offspring (Liautard & Sundström, 2005), with the computer program PolyRelatedness v1.6. To test whether the male mate to queen relatedness differs between social forms, we used a linear mixed model with the mate to queen relatedness as response variable, queen and male social origin as fixed factors and the colonies from which queens were sampled as random factor. The model was built with the 'lme4' package (Bates et al., 2015).

We estimated the relatedness among nestmate queens, among male mates of nestmate queens and among progenies of nestmate queens with the computer program PolyRelatedness v1.6. To estimate background allele frequencies, colonies were weighted equally. We simulated 1'000 datasets of unrelated individuals and computed the 95% confidence intervals around this null hypothesis.

Dispersal of queens and males was inferred by computing isolation by distance. Kinship coefficient (Loiselle et al., 1995) between pairs of individuals was regressed against the natural logarithm of distance. Kinship coefficient was chosen because it is not affected by the ploidy of individuals, thereby allowing us to compare the magnitude of isolation by distance between sexes (Hardy et al., 2008). Regression was restricted to a maximal distance of 900 meters to ensure that

the computation was performed on a similar scale for all comparisons. Correlation between the genetic and geographic matrices was tested with a Mantel test with 10’000 permutations.

3. Gene flow between social forms

The amount of genetic differentiation between social forms was estimated using hierarchical *F*-statistics, with workers nested in sibships, sibships nested in colonies, and colonies nested in social forms. Calculation was performed with the hierfstat R package v0.04-22 (Goudet, 2005). Confidence intervals were obtained by 10’000 bootstrapping over loci.

We estimated the number of private alleles in workers from monogynous and polygynous colonies, respectively (Slatkin, 1985). To control for the effects of unequal samples sizes and hierarchical sampling, we resampled the same number of workers in the monogynous and polygynous social form, using only one individual per colony (Kalinowski, 2004). We computed the number of private alleles in each social form with the R package ‘poppr’, using 10’000 resamples (Kamvar et al., 2014). We used a permutation test to evaluate whether the number of private alleles differed significantly between social forms.

To estimate the number of immigrants per generation between social forms, we used the Bayesian approach implemented in the computer program MIGRATE v3.6.11 (Beerli & Palczewski, 2010). MIGRATE uses coalescent theory to estimate population genetic parameters under the assumption of mutation-migration-drift equilibrium. The number of immigrants per generation is calculated as the product between the mutation-scaled effective population size within a focal social form and the mutation-scaled migration rate from the focal social form to the other social form. We ran MIGRATE with 20’000 burnin and 1’000’000 iterations.

Results

1. Social structure, supergene genotypes, mating pattern and queen mating frequency

The presence of a single reproducing queen in monogynous colonies was confirmed by the relatedness among nestmate workers being close to 0.75 and by pedigrees consistent with a single queen (**Table 1**; Table S1; Figure S2). The presence of multiple queens in polygynous colonies was demonstrated by direct sampling and observation of multiple wingless reproductive queens. Social organization was perfectly associated with the genotypes of queens at the supergene: all queens from monogynous colonies were Sm/Sm, and all queens from polygynous colonies had at least one Sp haplotype, 51.3% being Sp/Sm and the rest Sp/Sp (**Table 1**; Table S1; Figure S2).

Table 1: Mating frequency of queens and social genotypes of queens and their male mates heading field colonies. See Table S1 for details.

	Polygynous colonies		**Monogynous colonies**
Queen genotype	Sp/Sp	Sp/Sm	Sm/Sm
Queens with worker progeny (RAD-seq)	56	64	63
Percentage of queens singly mated : multiply mated	92.9 : 7.1	75 : 25	90.5 : 9.5
Queens with eggs (RFLP)	71	76	0
Total number of queens	73	77	63
Percentage of mating with Sp or Sm males (Sp males : Sm males)	75.3 : 24.7	77.3 : 22.7	0 : 100

Queens heading mature monogynous colonies were invariably mated with Sm males (**Table 1**; Table S1; Figure S2). In contrast, queens in polygynous colonies were mated with Sm and Sp males (**Table 1**; Table S1; Figure S2), with a relative contribution of Sm males totaling 23.6%. Sm males are only produced by monogynous colonies (Purcell et al., 2014b; Avril et al., chapter 2 & chapter 3). This asymmetrical pattern of mating between social forms generates unidirectional male-mediated gene flow from the monogynous to the polygynous social form. The proportion of mating with Sm versus Sp males did not differ significantly between Sp/Sp and Sp/Sm queens (**Table 1**; Fisher exact test, $p = 1$).

Most queens were singly mated (**Table 1**; Table S1; Figure S2). Yet, at least 16.7% of the queens in polygynous colonies and 9.5% of the queens in monogynous colonies were mated with more than one male (**Table 1**; Fisher exact test, $p = 0.26$). The rate of multiple mating in polygynous colonies was associated with the social genotype of the queens. Heterozygous Sp/Sm queens were significantly more likely to be multiply mated than homozygous Sp/Sp and Sm/Sm queens (**Table 1**; Figure S2, Fisher exact test; $p = 0.013$).

2. Dispersal of queens and males

The relatedness between queens and their male mates depended on the social origin of queens (**Figure 1**; Linear mixed model; $F_{(2,107)} = 5.80$, $p = 0.0041$). Overall, queens from polygynous colonies tended to mate with relatives, whereas queens from monogynous colonies tended to mate with non-relatives. In particular, the relatedness of mates of polygynous origin to queens in polygynous colonies was significantly higher than the relatedness of male mates of monogynous origin to queens in monogynous colonies (**Figure 1**; TukeyHSD: $Z = 3.2$, $p = 0.004$).

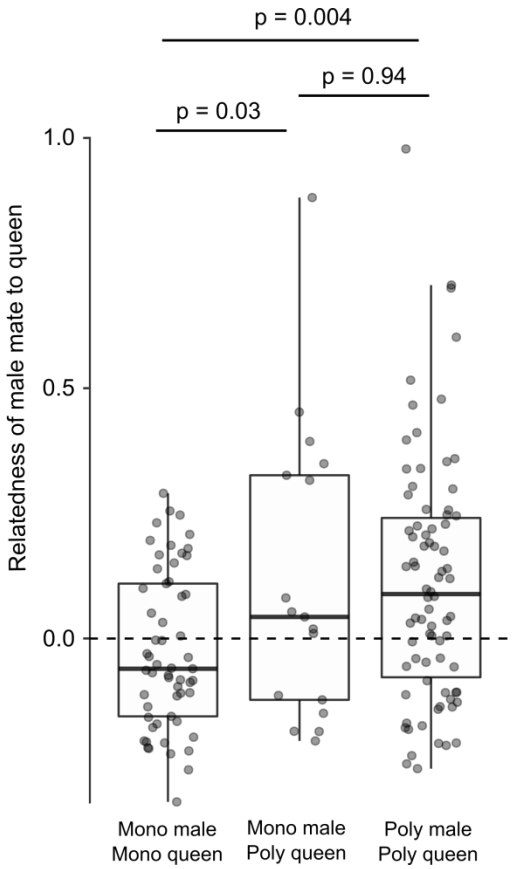

Figure 1: Relatedness of the male mates to the queens: mates of monogynous origin to queens in monogynous colonies (left bar), mates of monogynous origin to queens in polygynous colonies (central bar) and mates of polygynous origin to queens in polygynous colonies (right bar). Boxplots represent the lower and upper quartiles and whiskers the minimum and maximum values (in the limits of 1.5 × interquartile range).

Two lines of evidence indicate that queens in polygynous colonies were related, as expected if some of these queens had stayed within or close to their natal colony. First, the relatedness between workers of different sibships belonging to the same polygynous colony was significantly greater than zero ($r = 0.117 \pm 0.089$; mean ± SE; $p < 0.05$). Second, the relatedness among nestmate queens was significantly greater than zero ($r = 0.179 \pm 0.018$; mean ± SE; $p < 0.05$), while the relatedness among their mates was not ($r = 0.056 \pm 0.007$; mean ± SE; $p > 0.05$).

For both social forms, there were signs of restricted dispersal resulting in isolation by distance for queens, but not for males (**Table 2**). Indeed, the kinship coefficient decreased significantly with geographic distance for queens heading monogynous colonies and for queens heading polygynous colonies. In contrast, no significant isolation by distance was detected for males of monogynous origin, nor for males of polygynous origin (**Table 2**).

Table 2. Isolation by distance for queens and males belonging to each social form. R^2 is the correlation between kinship coefficient and geographic distance, b the slope of the regression and p the significance of the Mantel test.

	R^2	b	p
Monogynous social form			
Queens	-0.06	-0.0056	0.011
Males	-0.022	-0.000044	0.11
Polygynous social form			
Queens	-0.048	-0.0082	0.003
Males	0.022	0.0007	0.12

3. Gene flow between social forms

There was little genetic differentiation between social forms at SNPs located outside of the supergene (Fst = 0.0021, 95% confidence interval [0.0003, 0.0039]). Both the private allele analysis and estimates of migration rate between social forms were consistent with directional gene flow from the monogynous to the polygynous social form. First, he number of private alleles among workers of the polygynous social form was significantly higher than among workers of the monogynous social form (**Figure 2**). Second, the number of immigrants per generation was twofold higher from the monogynous to the polygynous social form than in the reverse direction (Nm from the monogynous to the polygynous social form: median = 8.22, 95%

confidence interval [4.39, 11.7]; Nm from the polygynous to the monogynous social form: median = 3.7, 95% confidence interval [1.82, 5.89]).

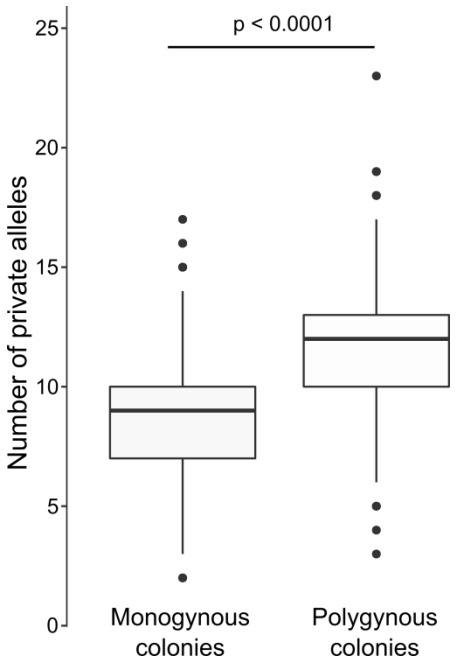

Figure 2: Number of private alleles in workers from monogynous and polygynous colonies respectively. Boxplots represent the lower and upper quartiles and whiskers the minimum and maximum values (in the limits of 1.5 × interquartile range).

Discussion

Supergenes control complex co-adapted traits, including traits involved in survival, mating system and reproduction. Because alternate supergene haplotypes can influence their own transmission in multiple ways, understanding the processes and selective pressures contributing to the maintenance of the polymorphism is a challenging task for evolutionary biologists. We investigated the mating system and dispersal strategies of queens and males belonging to

alternative social forms of the Alpine silver ant. We first confirmed that the colony social structure was perfectly associated with alternative genotypes at a large supergene. The Sp haplotype was present in all queens heading multiple-queen colonies, with Sp/Sp and Sm/Sp queens in similar proportions, while all queens heading single-queen colonies were Sm/Sm.

Mating was asymmetrical with respect to social forms, generating unidirectional male-mediated gene flow from the monogynous to the polygynous social form. Queens heading monogynous colonies were always mated with Sm males. In contrast, queens heading polygynous colonies were mated with Sp males and Sm males, with the latter contributing to 23.6% of the mating, weighting queens equally. This is an intriguing mating pattern, because polygynous colonies do not produce Sm males (Purcell et al., 2014b and Avril et al., chapter 3: in total 94 males from 21 polygynous colonies were all Sp; see Avril et al., chapter 2 for the mechanism causing the absence of Sm males in the offspring of polygynous queens). We conclude that polygynous queens mate with Sm males originating from monogynous colonies. Biased gene flow from the monogynous to the polygynous social from was confirmed by a higher number of private alleles in workers from monogynous colonies, and by a twofold higher number of immigrants per generation in that direction. Genetic differentiation between social forms was close to zero at markers located outside of the supergene, indicating that gene flow is strong enough to homogenize allelic frequencies between social forms, as already reported in previous studies of the same population (Chapuisat et al., 2004, Purcell & Chapuisat, 2013, Purcell et al., 2014b).

Unidirectional gene flow from the monogynous to the polygynous social form is likely to contribute to the maintenance of the social polymorphism, rather than lead to reproductive isolation and speciation between social forms (Crozier & Pamilo, 1996). The Sp haplotype is a

selfish genetic element, favoring its own transmission (Avril et al., chapter 2). Hence, unidirectional gene flow from the monogynous to the polygynous social form may preclude the Sp haplotype from spreading to fixation. A similar mechanism seems to be involved in the maintenance of the supergene controlling social organization in the fire ant *Solenopsis invicta* (Shoemaker & Ross, 1996).

In mature monogynous colonies, we never found queens of monogynous origin mated with males of polygynous origin. This is surprising, because in mate choice experiments we detected no mate preferences with respect to social form or mating incompatibilities between social forms (Avril et al., chapter 3). In addition, the absence of isolation by distance for males of polygynous origin indicates that these males have good dispersal abilities and might have opportunities to mate with queens originating from monogynous colonies. Sp males may be rare in the field or may show spatial or temporal segregation with Sm/Sm queens. Alternatively, it is possible that crosses between Sm/Sm queens and Sp males are selected against during independent colony founding or are quickly converted into polygynous colonies headed by multiple Sp/Sm daughter queens. More research is needed to understand why no Sp x Sm/Sm cross was detected in mature field colonies and how this absence affects the maintenance of the polymorphism at the supergene.

The proportion of polyandrous queens in monogynous colonies (9.5%) was fully consistent with the rate of polyandry measured in monogynous colonies of the same population by Chapuisat et. al. (2004). The current study provides the first estimate of polyandry for queens from polygynous colonies. It revealed an unexpected association between the supergene genotype of queens and their rate of polyandry: multiple mating was significantly more common among Sp/Sm queens than among Sp/Sp queens or Sm/Sm queens. Mating with a single Sm male is detrimental for

heterozygous queens, as half of the offspring will die due to the selfish genetic element associated with the Sp haplotype (Avril et al., chapter 2). Therefore, mating with multiple males might be a form of bet-hedging that is adaptive for heterozygous queens under certain conditions. Polyandry might also be a way to compensate for males with lower sperm quality. In line with this hypothesis, higher rates of polyandry are associated with the driving haplotypes of supergenes controlling social organization in fire ants (Lawson et al., 2012) and autosomal drive in mice (Sutter & Lindholm, 2015).

Queens of both social forms showed signs of isolation by distance, consistent with restricted dispersal. The relatedness between queens and their mates was higher for queens in polygynous colonies than for queens in monogynous colonies. In addition, nestmate queens in polygynous colonies were significantly related. This suggests that occasional intranidal mating and acceptance of daughter queens occur in polygynous colonies. For both social forms, males showed no isolation by distance, suggesting that they are better dispersers than queens.

Overall, the genetic data revealed that alternative social forms of the Alpine silver ant differed in their mating strategies and possibly in dispersal. Asymmetrical mating between social forms generates unidirectional male-mediated gene flow from the monogynous to the polygynous social form. In addition, the supergene genotype of queens was associated with their rate of polyandry. Finally, queens of both social forms showed signs of restricted dispersal, but signs of local mating and philopatry were more pronounced for polygynous queens than for monogynous ones. The differential mating and dispersal of queens and males with alternative supergene genotypes is likely to impact the maintenance of this genetic polymorphism controlling social organization.

Table S1. Sampling of queens heading monogynous and polygynous colonies, with details on the genetic analyses and inferred social genotypes for queens, their worker progeny, eggs and male mates.

Colony Id	Social structure	Population	Queen ID	Mating frequency	Worker genotype (RAD-seq)			Egg genotype (RFLP)			Queen RFLP genotype	Sperm RFLP genotype	Queen genotype consensus	Mate 1 genotype consensus	Mate 2 genotype consensus
					Sm/Sm	Sp/Sm	Sp/Sp	Sm/Sm	Sp/Sm	Sp/Sp					
102	Polygynous	Finges	102B	single		3	1		3	4	Sp/Sm	Sp	Sp/Sm	Sp	
103	Polygynous	Finges	103B	single			4			16	Sp/Sp	Sp	Sp/Sp	Sp	
			103C	multiple		2	2		3	5	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			103D	multiple		3		10	14		Sp/Sm	Sm	Sp/Sm	Sm	Sm
			103E	single			4			8	NA	NA	Sp/Sp	Sp	
			103F	single		2	2		5	3	Sp/Sm	Sp	Sp/Sm	Sp	
112	Polygynous	Finges	112B	single		1	3		1	7	Sp/Sm	Sp	Sp/Sm	Sp	
			112C	single		3	1		7	9	Sp/Sm	Sp	Sp/Sm	Sp	
			112D	single		3	1		11	5	Sp/Sm	Sp	Sp/Sm	Sp	
			112E	single		4		13	12		Sp/Sm	Sm	Sp/Sm	Sm	
115	Polygynous	Finges	115A	single		2	2		8	4	Sp/Sm	Sp	Sp/Sm	Sp	
116	Polygynous	Finges	116A	single			4			8	Sp/Sp	NA	Sp/Sp	Sp	
			116B	single		1	3		3	5	Sp/Sm	Sp	Sp/Sm	Sp	
			116C	single			4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			116D	single			4		3	5	Sp/Sm	Sp	Sp/Sm	Sp	
117	Polygynous	Finges	117A	single			4			16	Sp/Sp	NA	Sp/Sp	Sp	
			117D	single			4			16	Sp/Sp	Sp	Sp/Sp	Sp	
118	Polygynous	Finges	118A	multiple		3	1	7	9	2	NA	NA	Sp/Sm	Sp	Sm
			118B	multiple		2	2	4	9	3	Sp/Sm	Sm	Sp/Sm	Sp	Sm
			118C	single		4		7	9		Sp/Sm	Sm	Sp/Sm	Sm	
			118D	single		2	2		3	5	Sp/Sm	Sp	Sp/Sm	Sp	
			118E	single		4			3	3	Sp/Sm	Sp	Sp/Sm	Sp	
			118F	single		3	1		2	5	Sp/Sm	NA	Sp/Sm	Sp	
148	Polygynous	Finges	148A	single			4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			148B	multiple		2	2		3	5	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			148C	single		4			8		Sp/Sp	Sm	Sp/Sp	Sm	
150	Polygynous	Finges	150A	single		3	1		3	4	Sp/Sm	Sp	Sp/Sm	Sp	
			150B	single		3		20	18		Sp/Sm	Sm	Sp/Sm	Sm	
			150C	multiple		4		18	17		Sp/Sm	Sm	Sp/Sm	Sm	Sm
152	Polygynous	Finges	152B	single			4			8	Sp/Sp	Sp	Sp/Sp	Sp	
153	Polygynous	Finges	153A	single		4			14		Sp/Sp	Sm	Sp/Sp	Sm	
			153B	single			4			10	Sp/Sp	Sp	Sp/Sp	Sp	
			153C	single		1	3		3	5	Sp/Sm	Sp	Sp/Sm	Sp	
155	Polygynous	Finges	155A	single		4			5	3	NA	NA	Sp/Sm	Sp	
			155B	single			4		4	4	Sp/Sm	Sp	Sp/Sm	Sp	
			155D	single			4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			155F	single		4			8		Sp/Sp	Sm	Sp/Sp	Sm	
			155G	multiple		1	3		5	3	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			155H	single		3	1		5	3	Sp/Sm	Sp	Sp/Sm	Sp	
			155I	multiple		2	2		6	2	Sp/Sm	NA	Sp/Sm	Sp	Sp

156	Polygynous	Finges	156B	single	4			16		Sp/Sp	Sm	Sp/Sp	Sm	
			156C	single		3			16	Sp/Sp	Sp	Sp/Sp	Sp	
			156D	single	4		9	15		Sp/Sm	Sm	Sp/Sm	Sm	
			156E	single	1	3		6	2	Sp/Sm	Sp	Sp/Sm	Sp	
			156F	single	3	1		4	4	Sp/Sm	Sp	Sp/Sm	Sp	
			156G	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
157	Polygynous	Finges	157A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			157B	single	2	2		6	9	Sp/Sm	Sp	Sp/Sm	Sp	
			157C	single	4		16	21		Sp/Sm	Sm	Sp/Sm	Sm	
			157D	single		3			8	Sp/Sp	Sp	Sp/Sp	Sp	
			157E	single		4			8	Sp/Sp	NA	Sp/Sp	Sp	
			157F	single		4			8	NA	NA	Sp/Sp	Sp	
			157G	single		3			8	NA	NA	Sp/Sp	Sp	
			157H	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			157I	single	4			5	3	NA	NA	Sp/Sm	Sp	
			157J	single		3		6	2	Sp/Sm	Sp	Sp/Sm	Sp	
			157K	single	4			6	2	Sp/Sm	NA	Sp/Sm	Sp	
158	Polygynous	Finges	158A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			158B	single	1	3		5	11	Sp/Sm	Sp	Sp/Sm	Sp	
15	Polygynous	Finges	15A	single		4			16	Sp/Sp	Sp	Sp/Sp	Sp	
			15B	single	4		15	11		Sp/Sm	Sm	Sp/Sm	Sm	
			15C	single	2	2		7	1	Sp/Sm	Sp	Sp/Sm	Sp	
			15D	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			15E	single	4			6	2	Sp/Sm	NA	Sp/Sm	Sp	
191	Polygynous	Finges	191A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			191B	single		4			8	NA	NA	Sp/Sp	Sp	
			191C	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			191D	multiple		4		2	6	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			191E	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			191F	single	1	3		5	3	Sp/Sm	Sp	Sp/Sm	Sp	
			191G	single	2	2		6	2	Sp/Sm	Sp	Sp/Sm	Sp	
192	Polygynous	Finges	192A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			192B	single	4			8		Sp/Sp	Sm	Sp/Sp	Sm	
			192C	single	4			34		NA	NA	Sp/Sp	Sm	
276	Polygynous	Finges	276A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
278	Polygynous	Finges	278B	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			278C	single		4			16	Sp/Sp	Sp	Sp/Sp	Sp	
420	Polygynous	Finges	420A	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			420B	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
421	Polygynous	Finges	421A	single	4			8		Sp/Sp	Sm	Sp/Sp	Sm	
			421B	single		4			8	NA	Sp	Sp/Sp	Sp	
42	Polygynous	Finges	42A	multiple	2	2		12	16	Sp/Sm	Sp	Sp/Sm	Sp	Sp
431	Polygynous	Finges	431A	single		4			8	Sp/Sp	NA	Sp/Sp	Sp	
435	Polygynous	Finges	435A	multiple	3	1	1	4	3	Sp/Sm	Sp	Sp/Sm	Sp	Sm

43	Polygynous	Finges	43A	single	4		21	14		Sp/Sm	Sm	Sp/Sm	Sm	
			43B	single	3	1		4	4	Sp/Sm	NA	Sp/Sm	Sp	
			43C	single	3	1		4	4	Sp/Sm	Sp	Sp/Sm	Sp	
			43D	single	4		17	19		Sp/Sm	Sm	Sp/Sm	Sm	
58	Polygynous	Finges	58A	single	1	3		3	3	Sp/Sm	Sp	Sp/Sm	Sp	
			58B	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			58C	single	4			5	3	Sp/Sm	Sp	Sp/Sm	Sp	
9	Polygynous	Finges	9A	single	2	2		10	14	Sp/Sm	Sp	Sp/Sm	Sp	
			9B	single	1	3		6	2	Sp/Sm	Sp	Sp/Sm	Sp	
			9C	single	4		20	21		Sp/Sm	Sm	Sp/Sm	Sm	
			9D	multiple	2	2	5	3		Sp/Sm	NA	Sp/Sm	Sp	Sm
			9E	single	3	1		6	2	Sp/Sm	Sp	Sp/Sm	Sp	
N115	Polygynous	Finges	N115B	single	1	3				Sp/Sm	Sp	Sp/Sm	Sp	
P	Polygynous	Finges	P1	single	4			8		Sp/Sp	Sm	Sp/Sp	Sm	
			P2	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
			P3	single		4			8	Sp/Sp	Sp	Sp/Sp	Sp	
UNM1	Polygynous	Finges	UNM1A	multiple	3	1	5	3		Sp/Sm	Sp	Sp/Sm	Sp	Sm
UNM2	Polygynous	Finges	UNM2A	single	4		9	5		Sp/Sm	Sm	Sp/Sm	Sm	
			UNM2B	single		4			8	NA	NA	Sp/Sp	Sp	
			UNM2C	single	4			8		Sp/Sp	Sm	Sp/Sp	Sm	
			UNM2D	single		4			8	NA	NA	Sp/Sp	Sp	
UNM3	Polygynous	Finges	UNM3A	single	4			7		Sp/Sp	Sm	Sp/Sp	Sm	
			UNM3B	multiple	2	2		7	1	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			UNM3C	multiple	3	1			8	Sp/Sp	Sp	Sp/Sp	Sp	Sm
			UNM3D	multiple	2	1		6	2	Sp/Sp	Sp	Sp/Sp	Sp	Sm
UNM4	Polygynous	Finges	UNM4A	single		3		3	4	Sp/Sm	Sp	Sp/Sm	Sp	
UNM5	Polygynous	Finges	UNM5A	multiple	3	1		9	4	Sp/Sm	Sp	Sp/Sm	Sp	Sp
			UNM5B	multiple	4			6	2	Sp/Sm	Sp	Sp/Sm	Sp	Sp
UNM6	Polygynous	Finges	UNM6A	multiple		3			8	Sp/Sp	NA	Sp/Sp	Sp	Sp
			UNM6B	single		4				Sp/Sp	Sp	Sp/Sp	Sp	
UNM7	Polygynous	Finges	UNM7A	single	4			5	2	Sp/Sm	Sp	Sp/Sm	Sp	
			UNM7B	multiple		4			8	Sp/Sp	Sp	Sp/Sp	Sp	Sp
			UNM7C	single		3			8	Sp/Sp	Sp	Sp/Sp	Sp	
			UNM7D	single	3			8		Sp/Sp	Sm	Sp/Sp	Sm	
			UNM7E	single	4					Sp/Sp	Sm	Sp/Sp	Sm	
			UNM7F	single	4			6	2	Sp/Sm	Sp	Sp/Sm	Sp	
100	Monogynous	Finges	100	single	4							Sm/Sm	Sm	
108	Monogynous	Finges	108	single	4							Sm/Sm	Sm	
109	Monogynous	Finges	109	single	3							Sm/Sm	Sm	
119	Monogynous	Finges	119	single	4							Sm/Sm	Sm	
121	Monogynous	Finges	121	single	4							Sm/Sm	Sm	
123	Monogynous	Finges	123	single	4							Sm/Sm	Sm	
125	Monogynous	Finges	125	single	4							Sm/Sm	Sm	
13	Monogynous	Finges	13	single	4							Sm/Sm	Sm	

142	Monogynous	Finges	142	single	4				Sm/Sm	Sm	
151	Monogynous	Finges	151	single	4				Sm/Sm	Sm	
163	Monogynous	Finges	163	single	4				Sm/Sm	Sm	
165	Monogynous	Finges	165	single	4				Sm/Sm	Sm	
182	Monogynous	Finges	182	single	4				Sm/Sm	Sm	
185	Monogynous	Finges	185	single	4				Sm/Sm	Sm	
187	Monogynous	Finges	187	single	4				Sm/Sm	Sm	
188	Monogynous	Finges	188	single	4				Sm/Sm	Sm	
19	Monogynous	Finges	19	single	4				Sm/Sm	Sm	
195	Monogynous	Finges	195	single	4				Sm/Sm	Sm	
212	Monogynous	Finges	212	single	4				Sm/Sm	Sm	
217	Monogynous	Finges	217	single	4				Sm/Sm	Sm	
248	Monogynous	Finges	248	single	4				Sm/Sm	Sm	
249	Monogynous	Finges	249	single	4				Sm/Sm	Sm	
26	Monogynous	Finges	26	single	4				Sm/Sm	Sm	
403	Monogynous	Finges	403	single	4				Sm/Sm	Sm	
405	Monogynous	Finges	405	single	4				Sm/Sm	Sm	
445	Monogynous	Finges	445	single	4				Sm/Sm	Sm	
449	Monogynous	Finges	449	single	3				Sm/Sm	Sm	
450	Monogynous	Finges	450	single	4				Sm/Sm	Sm	
451	Monogynous	Finges	451	single	4				Sm/Sm	Sm	
452	Monogynous	Finges	452	multiple	4				Sm/Sm	Sm	Sm
453	Monogynous	Finges	453	single	3				Sm/Sm	Sm	
457	Monogynous	Finges	457	single	4				Sm/Sm	Sm	
459	Monogynous	Finges	459	single	4				Sm/Sm	Sm	
460	Monogynous	Finges	460	single	4				Sm/Sm	Sm	
463	Monogynous	Finges	463	single	4				Sm/Sm	Sm	
464	Monogynous	Finges	464	single	4				Sm/Sm	Sm	
465	Monogynous	Finges	465	single	4				Sm/Sm	Sm	
474	Monogynous	Finges	474	single	4				Sm/Sm	Sm	
475	Monogynous	Finges	475	single	4				Sm/Sm	Sm	
476	Monogynous	Finges	476	single	4				Sm/Sm	Sm	
477	Monogynous	Finges	477	single	4				Sm/Sm	Sm	
478	Monogynous	Finges	478	multiple	4				Sm/Sm	Sm	Sm
479	Monogynous	Finges	479	single	4				Sm/Sm	Sm	
482	Monogynous	Finges	482	multiple	4				Sm/Sm	Sm	Sm
484	Monogynous	Finges	484	single	4				Sm/Sm	Sm	
485	Monogynous	Finges	485	multiple	4				Sm/Sm	Sm	Sm
51	Monogynous	Finges	51	single	4				Sm/Sm	Sm	
62	Monogynous	Finges	62	single	4				Sm/Sm	Sm	
64	Monogynous	Finges	64	single	4				Sm/Sm	Sm	
67	Monogynous	Finges	67	single	4				Sm/Sm	Sm	
71	Monogynous	Finges	71	single	4				Sm/Sm	Sm	
82	Monogynous	Finges	82	single	4				Sm/Sm	Sm	

83	Monogynous	Finges	83	single	4					Sm/Sm	Sm	
84	Monogynous	Finges	84	single	4					Sm/Sm	Sm	
90	Monogynous	Finges	90	single	4					Sm/Sm	Sm	
91	Monogynous	Finges	91	multiple	4					Sm/Sm	Sm	Sm
92	Monogynous	Finges	92	single	4					Sm/Sm	Sm	
93	Monogynous	Finges	93	single	4					Sm/Sm	Sm	
97	Monogynous	Finges	97	single	4					Sm/Sm	Sm	
99	Monogynous	Finges	99	single	4					Sm/Sm	Sm	
c80	Monogynous	Finges	c80	single	4					Sm/Sm	Sm	
CP2	Monogynous	Finges	CP2	single	4					Sm/Sm	Sm	
CP3	Monogynous	Finges	CP3	multiple	4					Sm/Sm	Sm	Sm
102	Polygynous	Finges	102A	undetermined			8			Sp/Sp	Sm	
			102C	undetermined				8		Sp/Sp	Sp	
103	Polygynous	Finges	103A	undetermined			4	4		Sp/Sm	Sp	
112	Polygynous	Finges	112A	undetermined			7	9		Sp/Sm	Sp	
117	Polygynous	Finges	117B	undetermined				16		Sp/Sp	Sp	
			117C	undetermined				8		Sp/Sp	Sp	
118	Polygynous	Finges	118G	undetermined			3	5		Sp/Sm	Sp	
152	Polygynous	Finges	152A	undetermined			16			Sp/Sp	Sm	
155	Polygynous	Finges	155J	undetermined			6	2		Sp/Sm	Sp	
			155C	undetermined			4	4		Sp/Sm	Sp	
			155E	undetermined			5	3		Sp/Sm	Sp	
156	Polygynous	Finges	156A	undetermined				8		Sp/Sp	Sp	
207	Polygynous	Finges	207A	undetermined				8		Sp/Sp	Sp	
278	Polygynous	Finges	278A	undetermined				8		Sp/Sp	Sp	
			278D	undetermined				8		Sp/Sp	Sp	
43	Polygynous	Finges	43E	undetermined			8			Sp/Sp	Sm	
431	Polygynous	Finges	431B	undetermined				8		Sp/Sp	Sp	
79	Polygynous	Finges	79A	undetermined			4	12		Sp/Sm	Sp	
			79B	undetermined				16		Sp/Sp	Sp	
DE1	Polygynous	Derborence	DE1A	undetermined				8		Sp/Sp	Sp	
DE114	Polygynous	Derborence	DE114A	undetermined			5	3		Sp/Sm	Sp	
DE94	Polygynous	Derborence	DE94A	undetermined			3	5		Sp/Sm	Sp	
DE97	Polygynous	Derborence	DE97C	undetermined				8		Sp/Sp	Sp	
			DE97A	undetermined		9	10			Sp/Sm	Sm	
			DE97B	undetermined		7	5			Sp/Sm	Sm	
			DE97D	undetermined			5	5		Sp/Sm	Sp	
DE98	Polygynous	Derborence	DE98	undetermined			6			Sp/Sp	Sm	
UNM6	Polygynous	Finges	UNM6C	undetermined			8			Sp/Sp	Sm	
UNM1	Polygynous	Finges	UNM1B	undetermined		4	4			Sp/Sm	Sm	
UNM5	Polygynous	Finges	UNM5C	undetermined				8		Sp/Sp	Sp	

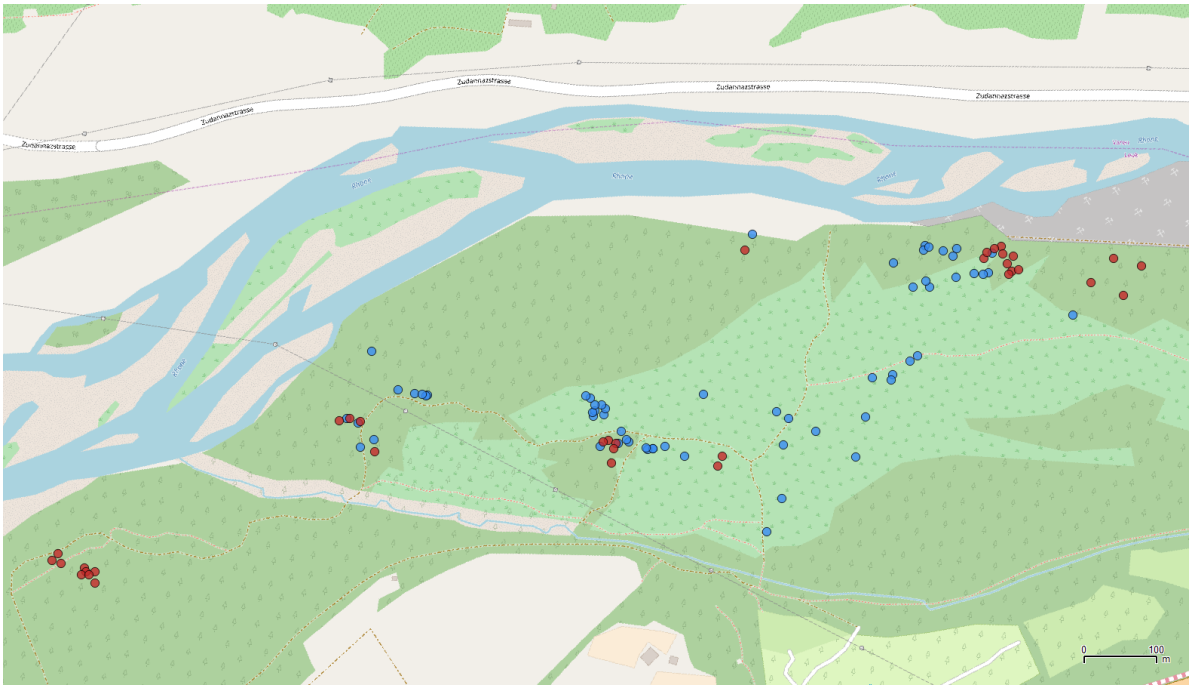

Figure S1: Map of sampled monogynous (blue) and polygynous (red) colonies from which four workers were used for RAD-seq.

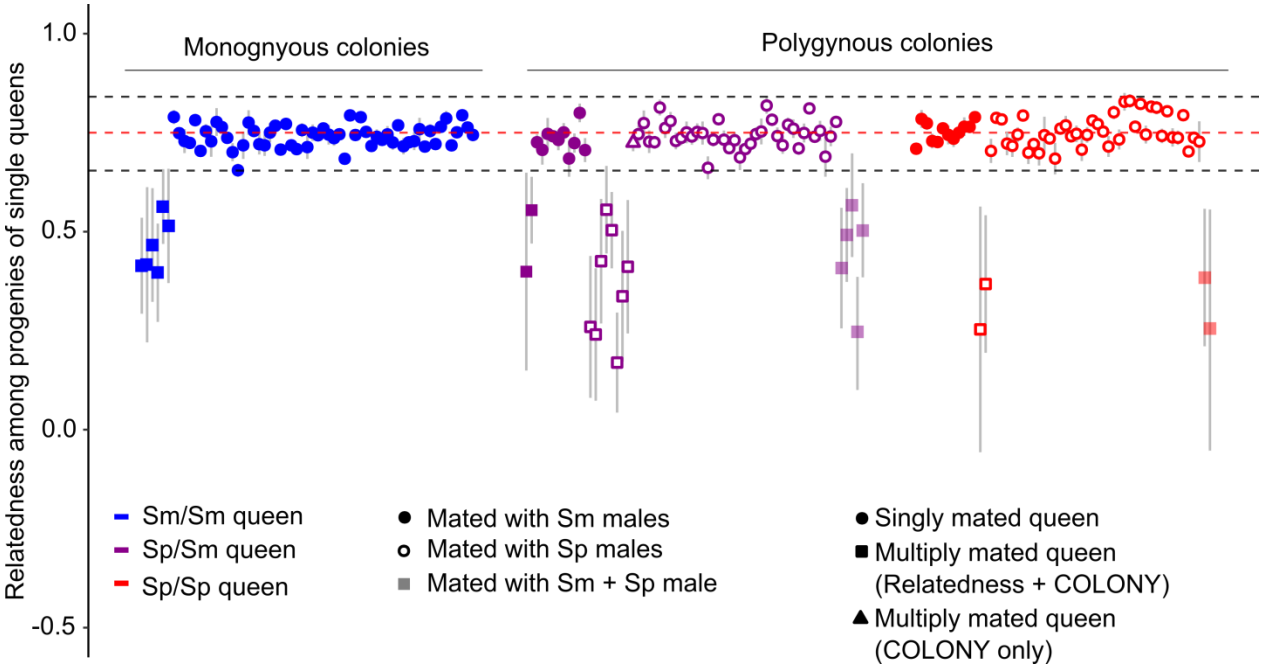

Figure S2: Social structure, supergene genotypes and queen mating frequency. The y axis shows the relatedness (mean ± SE) among progenies of single queens originating from monogynous colonies (left side of graph) and polygynous colonies (right side of graph), respectively. Red and black dotted lines indicate the mean and 95% confidence interval of the expected relatedness among progenies of a singly mated queen. Blue, purple and red symbols represent progenies of Sm/Sm queens, Sp/Sm queens, and Sp/Sp queens, respectively. Filled and open symbols represent progenies of queens mated with Sm and Sp males, respectively. Shaded symbols represent progenies of queens mated with one Sp and one Sm male. Circle stands for progenies of singly mated queen. Squares and triangle depict progenies of multiply mated queens, confirmed by both relatedness estimates and pedigree analysis, or by pedigree analysis alone, respectively.

Chapter 2

Maternal effect killing by a supergene controlling ant social organization

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Abstract

Supergenes determine major alternative social phenotypes, yet their persistence remains poorly understood. In the Alpine silver ant, colony social organization is controlled by a large supergene with two haplotypes. Here we show that the haplotype associated with polygynous organization is a maternal effect killer. Specifically, all eggs from heterozygous queens that did not inherit this haplotype failed to hatch. Hence, intragenomic conflict influences the maintenance of alternative social forms and selfish drive by one haplotype contributes to the preservation of cooperative, polygynous ant societies. More generally, selfish genetic elements frequently occur in non-recombining regions, suggesting that many supergenes are likely to be transmission ratio distorters.

Introduction

Supergenes control a variety of complex polymorphic phenotypes, including sexes, distyly, ecotypes, cryptic morphs, mating morphs and alternative forms of social organization (Thompson & Jiggins, 2014, Charlesworth, 2016, Schwander et al., 2014). The suppression of recombination in supergenes can preserve groups of co-adapted alleles and avoid maladaptive combinations of antagonistic alleles (Wright et al., 2017, Dobzhansky, 1970). But the suppression of recombination has a darker side: it exacerbates intra-genomic conflict, as co-transmitted alleles gain power to favor their own transmission over that of alternative alleles, at a cost to their bearers (Burt & Trivers, 2006, Hamilton, 1967). Segregation distorters arise through tight linkage of a distorter and target locus, followed by further accumulation of enhancer loci (Burt & Trivers, 2006, Lyttle, 1991). As tight linkage generates both supergenes and selfish genetic elements, large non-recombining genomic regions are likely not only to control complex phenotypes, but also to cause transmission ratio distortion.

The recent discovery that a supergene determines colony queen number in the Alpine silver ant *Formica selysi* provides a novel opportunity to investigate the impact of polymorphic supergenes across multiple levels of biological organization (Purcell et al., 2014b). In this species, colonies headed by a single-queen (= monogynous) and colonies headed by multiple queens (= polygynous) occur in the same populations (Chapuisat et al., 2004, Purcell & Chapuisat, 2013, Purcell et al., 2015). The two social forms differ in multiple life-history traits (Rosset & Chapuisat, 2007, Purcell et al., 2014b), yet queens and males of both social forms interbreed in the laboratory (Reber et al., 2010; Avril et al., chapter 2). There is no sign of genetic differentiation between social forms over most of the genome, except for a large, non-

recombining supergene that spans most of a chromosome (Chapuisat et al., 2004, Purcell et al., 2014b, Purcell et al., 2015; Avril et al., chapter 1). All ants from monogynous colonies invariably harbor only the Sm haplotype, with females having the Sm/Sm genotype and males the Sm haplotype. In contrast, ants produced by polygynous colonies harbor at least one copy of the Sp haplotype, with females having Sp/Sm or Sp/Sp genotypes and males carrying the Sp haplotype (Purcell et al., 2014b).

The Alpine silver ant supergene in unusual in that homozygotes for both haplotypes, Sm and Sp, are viable (Purcell et al., 2014b). This contrasts with other supergenes for social traits, in which one variant is a recessive lethal (Wang et al., 2013, Küpper et al., 2016). Another surprising feature of the Alpine silver ant supergene is the absence of Sm/Sm females and Sm alate males from polygynous colonies (Purcell et al., 2014b). As many as 51.3% of the mature queens heading polygynous colonies are Sp/Sm (Avril et al., chapter 1). These queens are expected to produce Sp and Sm males. Moreover, 22.7% of the Sp/Sm queens were mated with Sm males originating from monogynous colonies (Avril et al., chapter 1). These queens should produce Sp/Sm and Sm/Sm females. The underrepresentation of the Sm haplotype in offspring from polygynous queens points at a transmission ratio distortion and suggests that the Sp haplotype is a selfish genetic element favoring its own transmission over that of the alternative haplotype.

Transmission ratio distortion results from intragenomic conflict (Burt & Trivers, 2006). Selfish genetic elements can favor their own transmission at various stages in development, from gametogenesis to adult behavior, by causing either meiotic drive, maternal effect killing, or green beard effect (Burt & Trivers, 2006, Huang et al., 2013). Meiotic drive is characterized by the overrepresentation of one allele in the gametes of heterozygous individuals. Well-known

examples include the *t-complex* in mice (Lyon, 2003) and the *Segregation Distorter* gene complex in the fruit fly, *Drosophila melanogaster* (Larracuente & Presgraves, 2012). Maternal effect killers are genetic elements present in the parent that cause the death of the progeny that did not inherit this element (Werren, 2011). Maternal effect killers operate through a modification-rescue system, such as a toxin and antidote (Burt & Trivers, 2006, Werren, 2011). Typical maternal effect killers are the *Medea* element in red flour beetles (Lorenzen et al., 2008, Beeman et al., 1992) and both the *peel/zeel* and *sup-35/pha-1* systems in *Caenorhabdtitis elegans* (Seidel et al., 2011, Ben-David et al., 2017). Finally, a green beard effect occurs when the carrier of an allele recognizes and favors individuals that also carry this allele (Hamilton, 1964, Dawkins, 1976). Such an effect has been documented in the fire ant, where workers carrying the polygynous haplotype at the social supergene kill queens that lack this haplotype (Keller & Ross, 1998, Trible & Ross, 2016).

Here, we explore whether and how the supergene controlling social organization in the Alpine silver ant distorts the law of Mendelian segregation. We monitor the development of brood from Sp/Sm queens mated with Sm males and investigate if the Sp haplotype favors its own transmission by causing meiotic drive, maternal effect killing, or a green beard effect.

Materials and Methods

Ant sampling, rearing and genotyping

To study the causes of the transmission ratio distortion observed at the social supergene of *F. selysi*, we reared queens individually and genotyped their progeny. We sampled 134 mature

queens from 45 polygynous colonies in two field populations in Valais, Switzerland (Finges: 7°36'30" E, 4°18'30" N, altitude 565 m; Derborence: 7°12'56" E, 46°16'50" N, altitude 1450 m). Each queen was reared in a plastic box (15 × 13 × 6 cm) lined with Fluon® to prevent escape. Each rearing unit had a nest site (a glass test tube wrapped in aluminum foil, with water retained by a cotton plug at the bottom) and contained 20 workers originating from the same colony as the queen. The ants were fed *ad libitum* with standard ant food (Meunier & Chapuisat, 2009). We sampled eggs and larvae in multiple experimental conditions, as described below. We determined the social supergene genotype of eggs and larvae by genotyping three haplotype-diagnostic SNPs with a PCR-RFLP assay (Purcell et al., 2014b). DNA was extracted from eggs and larvae following a salting out procedure adapted from Miller *et al.* (1988).

The social genotype of the queens and their male mates was determined as described in detail in chapter 1 (see Table 1 and Table S1 of Avril et al., chapter 1). In short, a PCR-RFLP assay for diagnostic SNPs was applied to queens, sperm in their spermathecae and eggs. The social genotypes of most queens and mates were further confirmed by reconstructing parental genotypes from RAD-seq SNPs genotypes of four worker progeny per queen (Avril et al., chapter 1). To study transmission ratio distortion, we focused on 34 queens from 16 polygynous colonies that were heterozygous at the supergene. Of these 34 Sp/Sm queens, 16 had mated with Sm males and 18 with Sp males, respectively.

Meiotic drive

To test if the Sp haplotype was transmitted to more than 50% of the gametes, we sampled and genotyped embryonated eggs laid by Sp/Sm queens. We collected worker-destined female eggs,

which are commonly produced year-round under laboratory conditions. All eggs were less than three days old. We succeeded in genotyping 16 eggs per queen from 34 Sp/Sm queens.

Maternal effect killing

To detect post-segregational lethal maternal effects, we monitored the mortality of brood from known crosses developing in the absence of workers. If the Sp haplotype is a maternal effect killer, we expect that Sm/Sm brood laid by Sp/Sm queens mated with Sm males will not develop, while Sp/Sm brood from the same clutch will develop normally. Moreover, brood from Sm/Sm or Sp/Sp queens mated with Sm males should also develop normally. We used brood from 15 Sp/Sm queens mated with Sm males. Ten of them were mature queens sampled in polygynous colonies, while the five others resulted from experimental crosses in which young queens and males were allowed to mate in flight cages, as described in Reber *et al.* (2010). We also analyzed brood from control experimental crosses, namely eleven Sm/Sm queens mated with Sm males, and seven Sp/Sp queens mated with Sm males.

Queens were isolated for two days, and their eggs were placed individually onto sterile plastic plates, using a soft paintbrush cleaned with ethanol. The eggs were placed in an incubator (a climate chamber at 24°C and 95% relative humidity). We sampled brood at two developmental points. First, after four days in the incubator, we collected half of the surviving eggs from each queen. Hence, these eggs were four to six days old. We then monitored the remaining brood on a daily basis for twelve days, and collected all hatched larvae. All these eggs and larvae were later genotyped at the social supergene.

Green beard effect

We performed a cross fostering experiment to examine whether workers from polygynous colonies, which carry at least one Sp haplotype, selectively eliminated brood that lacked this haplotype. We introduced eggs from known crosses in rearing groups of non-nestmate workers originating from monogynous and polygynous colonies, respectively. If the Sp haplotype is causing a green beard effect, polygynous workers should kill Sm/Sm brood, while monogynous workers should spare it. Control brood of other genotypes should develop normally.

The laying queens were isolated for three days. Their eggs were transferred to small rearing units (11 × 8 × 6 cm), each containing 20 workers, and reared as described above. We formed 60 rearing units, half with workers originating from polygynous colonies, and half with workers originating from monogynous colonies, all from the Finges population (6 rearing groups per field colony). Each rearing group received 17.6 ± 4.2 (mean ± SD) eggs, as a ratio of eggs to workers close to 1:1 tends to maximize brood survival (Purcell et al., 2012).

We tested whether brood mortality depended on the genotypes of eggs, mothers and rearing workers. We introduced eggs laid by four mature Sp/Sm queens mated with Sm males into twelve rearing groups of polygynous workers and twelve rearing groups of monogynous workers, respectively. We collected the surviving eggs after three days, when these eggs were three to six days old. We further introduced eggs laid by mature Sp/Sm queens mated with Sm males into eight rearing groups of each social form, monitored brood on a daily basis for twelve days, and collected all hatched larvae. As controls, we introduced eggs laid by five Sp/Sp queens and five Sm/Sm queens, all mated with Sm males, into ten rearing groups of each social form. Again, we collected all hatched larvae. All the eggs and larvae were later genotyped.

Statistical analysis

To detect transmission ratio distortion, we examined whether the supergene genotypes of brood from Sp/Sm queens departed from the expected 1:1 Mendelian ratio. We used generalized linear mixed models (GLMM) implemented in the R package 'lme4' (Bates et al., 2015) with a binomial error distribution, as described in Ducret *et al.* (2016). In these models, the response variable was whether the offspring had inherited the Sp or Sm haplotype from its Sp/Sm mother. We constructed three separate models to determine biases during gamete production, offspring development, and behavior of caring workers, respectively. When testing for meiotic drive in eggs, we included the genotype (Sm or Sp) of the male (= the queen's mate) as fixed factor, and the queen identity as random factor. In tests of maternal effect killing, we used Sp/Sm queens that had mated with Sm males, and included brood stage (eggs or larvae) as a fixed factor, and the number of eggs laid in each replicate and the queen identity as random factors. When testing for a green-beard effect, we included the interaction between brood stage and social origin of workers as fixed factors, and the rearing group of workers and the queen identity as random factors. For each model, we used a Wald test to examine whether the intercept differed significantly from zero, revealing whether the brood genotypes deviate from the 1:1 Mendelian ratio.

In complement to identifying deviations from the 1:1 Mendelian ratio, we sought differences in brood mortality based on the cross and the rearing group. Using a GLMM with a binomial error distribution, we analyzed the mortality of brood from queens with alternative genotypes mated to Sm males, and reared either without workers, by monogynous workers or by polygynous workers, respectively. Brood mortality was the response variable. We included the interaction between queen and brood genotype as fixed factors and the queen identity, the number of eggs

incubated and the rearing group of workers as random factors. We did not detect any departure from the 1:1 Mendelian ratio in eggs laid by heterozygous queens (see results). Hence, for heterozygous queens, we estimated the mortality of each genotype by assuming that they were present in half of the eggs laid. The brood survival data showed complete separation, precluding reliable prediction of the regression estimates. Therefore, we computed GLMMs using Bayesian inference on the regression estimates with the function 'bglmer' implemented in the 'blme' package in R (Gelman et al., 2008, Rainey, 2016). We tested for pairwise differences in brood mortality with a post-hoc Tukey HSD test, as implemented in the 'multcomp' package in R. All the statistics were performed in R v3.2.0 (R Development Core Team, 2015).

Results

Meiotic drive

There was no sign of meiotic drive in the eggs laid by queens heterozygous at the supergene. The proportion of eggs carrying the Sp and Sm maternal haplotype did not depart significantly from the 1:1 Mendelian inheritance ratio, irrespective of whether the Sp/Sm queens had mated with Sm males or Sp males (**Figure 1**).

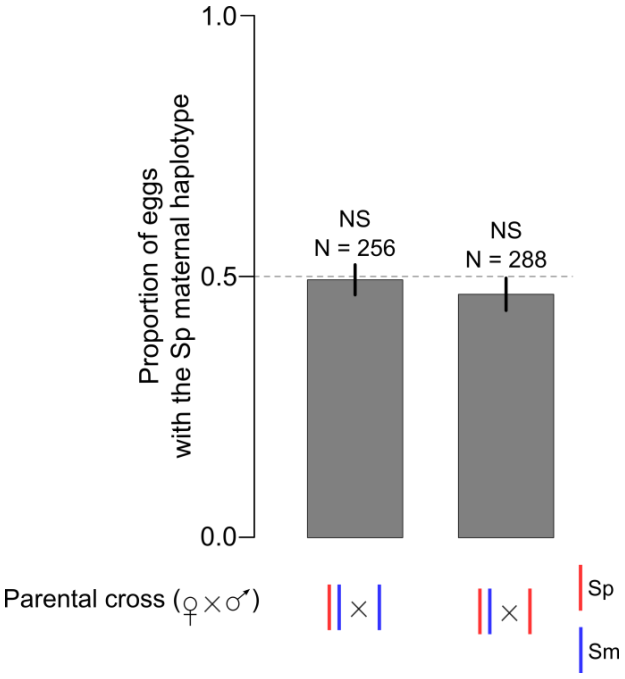

Figure 1: No evidence for meiotic drive. The proportion of eggs that inherited the Sp maternal haplotype was not significantly different from 0.5 for heterozygous Sp/Sm queens mated with Sm males (Left bar; GLMM, z = -0.238, p = 0.81) or Sp males (GLMM, z = -1.06, p = 0.29). The number of eggs genotyped is indicated above each bar.

Maternal effect killing

We monitored the development of brood from Sp/Sm queens mated with Sm males. This brood was reared in one of three treatments: in the absence of workers, by workers from monogynous colonies or by workers from polygynous colonies. There was clear evidence that the Sp haplotype was a maternal effect killer. The Sm/Sm eggs had normal survival and were found in the Mendelian ratio of 1:1 during the early stages of development, but they all failed to hatch into larvae (**Figure 2**). In contrast, Sp/Sm eggs from the same clutches hatched normally. As a result, all surviving larvae were heterozygous at the supergene (**Figure 2**).

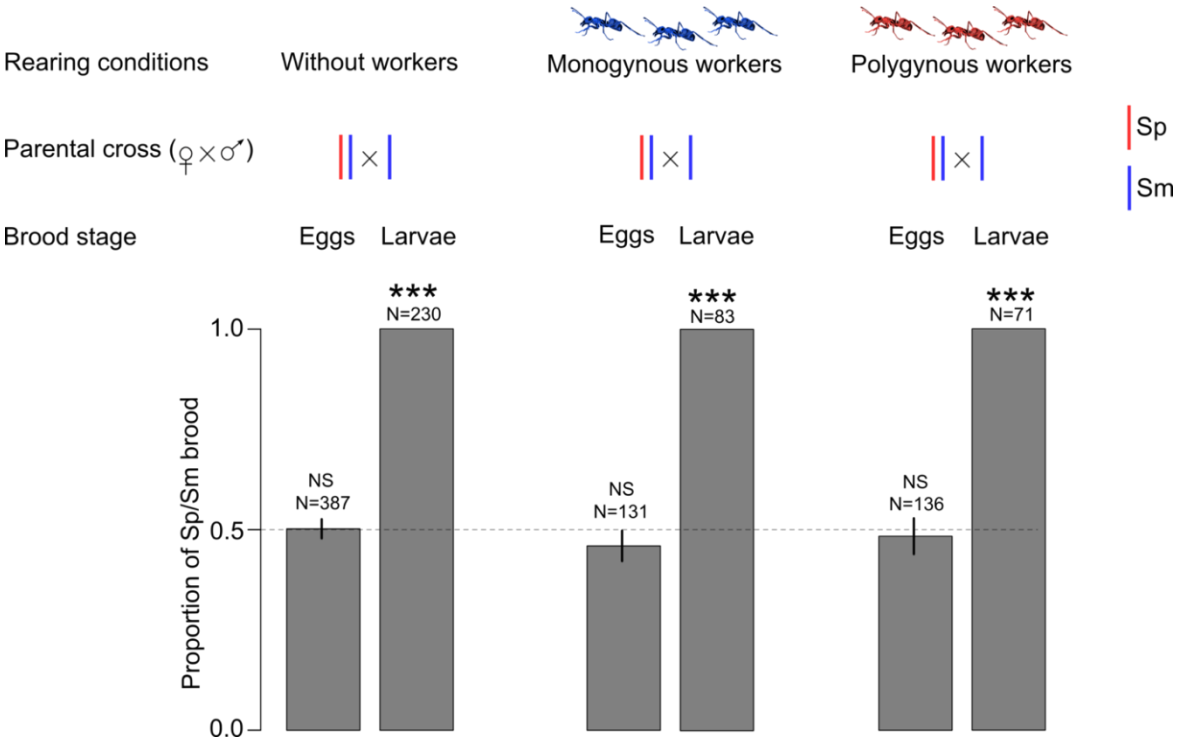

Figure 2: Evidence that the Sp haplotype is a maternal effect killer. Sm/Sm eggs laid by heterozygous queens failed to hatch into larvae, while Sp/Sm eggs from the same clutches developed normally. The number of live brood items collected and genotyped are indicated above the bars. Asterisks indicate significant departures from 1:1 Mendelian proportions (GLMM, Wald test, $p < 0.0001$ after Bonferroni correction for multiple comparisons).

The mortality of Sm/Sm brood depended on the presence of Sp in their mother. Sm/Sm eggs did not hatch into larvae when coming from Sp-carrying queens, but they developed normally when coming from queens that lacked Sp (**Figure 3**). This strong maternal effect was evidenced by the sharp contrast between the 100% mortality rate of Sm/Sm brood laid by Sp/Sm queens and the moderate mortality rate of Sm/Sm brood laid by Sm/Sm queens, which was similar to the one of brood with alternative genotypes (**Figure 3**).

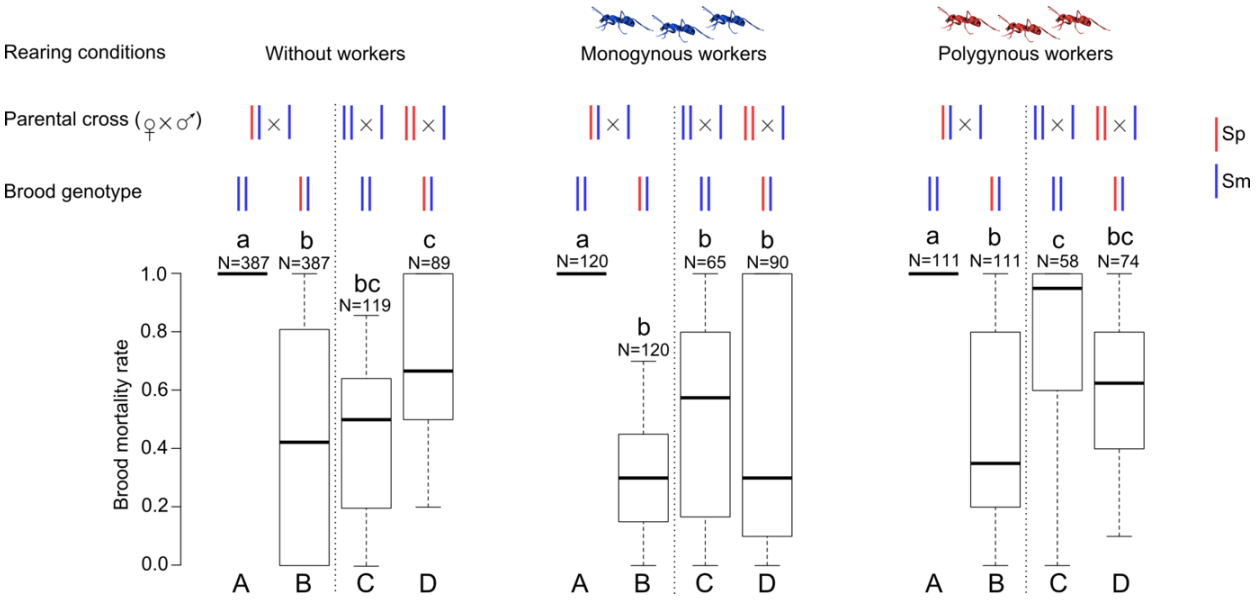

Figure 3: Evidence for lethal maternal effect. Median mortality rate until the larval stage for (**A**) Sm/Sm brood from Sp/Sm queens mated with Sm males, (**B**) Sp/Sm brood from Sp/Sm queens mated with Sm males, (**C**) Sm/Sm brood from Sm/Sm queens mated with Sm males and (**D**) Sp/Sm brood from Sp/Sp queens mated with Sm males. The number of eggs at the start of the experiments is indicated above the bars. For heterozygous queens, the number of eggs of each genotype was estimated based on Mendelian ratio, as there was no sign of meiotic drive, and the mortality rate was constrained between zero and one in each replicate. Boxplots depict the lower and upper quartiles and the whiskers encompass 1.5 times the interquartile range. The mortality rate varied significantly according to brood genotype and parental cross (GLMM; without workers: $\chi^2_{(3)} = 381$, $p < 0.0001$; reared by monogynous workers: $\chi^2_{(3)} = 164$, $p < 0.0001$; reared by polygynous workers: $\chi^2_{(3)} = 122$, $p < 0.0001$). Different small letters above bars indicate significant differences in mortality (post-hoc TukeyHSD test for multiple comparisons, $p < 0.05$). Overall, Sm/Sm brood laid by Sp/Sm queens mated with Sm males had significantly higher mortality than all other brood genotypes and parental crosses.

Green beard effect

There was no sign that the Sp haplotype was causing a green beard effect. The presence and social origin of rearing workers did not alter the 1:1 Mendelian ratio in eggs and did not explain the differential mortality of embryos with alternative genotypes (**Figure 2 and 3**). Hence, workers from polygynous colonies, that carry at least one Sp haplotype, did not selectively eliminate brood that lacked Sp. Overall, irrespective of whether the brood was unattended, attended by monogynous workers or attended by polygynous workers, all Sm/Sm eggs laid by Sp/Sm queens did not hatch into larvae, while the Sp/Sm brood from the same clutch had normal survival (**Figure 2 and 3**).

Discussion

We provide strong evidence that the haplotype associated with polygynous colonies (Sp) in the Alpine silver ant is a maternal effect killer. Queens heterozygous at the supergene (Sp/Sm) produced viable eggs that inherited each haplotype in the expected 1:1 Mendelian ratio. When heterozygous mothers had mated with Sm-carrying males, their Sp/Sm offspring developed normally, while all their Sm/Sm eggs failed to hatch into larvae. In contrast, Sm/Sm brood from homozygous mothers hatched normally. In short, presence of the Sp haplotype in a mother induced developmental arrest of her brood that did not inherit this haplotype.

The mechanism causing the death of progeny that did not inherit the Sp haplotype is not yet known, but likely involves a modification-rescue system (Burt & Trivers, 2006). We hypothesize that this very long, non-recombining genetic element contains a maternally-expressed toxin and

an embryo-expressed antidote. Such systems have been well characterized in *C. elegans*, where the toxins and antidotes are tightly linked through their adjacent position in an insertion-deletion polymorphism (Seidel et al., 2011) or in an inversion (Ben-David et al., 2017). Clusters of tightly-linked loci commonly cause other types of segregation distortion. For example, the *t*-locus in mice and the *Segregation Distorter* gene complex in *D. melanogaster* cause meiotic drive (Lyon, 2003, Larracuente & Presgraves, 2012), while the *b* haplotype in fire ants induces a green-beard effect (Keller & Ross, 1998, Trible & Ross, 2016).

Selfish genetic elements are difficult to observe, because they either spread to fixation or are repressed by other genes (Hurst & Werren, 2001). The ones that persist are often recessive lethal or near-lethal, so that negative frequency-dependent selection prevents the fixation of the driving allele (Burt & Trivers, 2006, Lyon, 2003, Keller & Ross, 1998). In the Alpine silver ant, homozygotes for both haplotypes at the social supergene are viable (Purcell et al., 2014b; this study). Yet, the social polymorphism appears stable over time and is present in most populations (Purcell et al., 2015, Purcell & Chapuisat, 2013).

Our results provide new light on the forces contributing to the maintenance of the balanced polymorphism in the Alpine silver ant. On the one hand, maternal effect killing causes a drive that favors the Sp haplotype within polygynous colonies. On the other hand, all queens in mature monogynous colonies had mated with males carrying the Sm haplotype, while 23.6% of queens in polygynous colonies had mated with males carrying the Sm haplotype (Avril et al., chapter 1). This asymmetrical mating pattern and unidirectional male-mediated gene flow may restrict the spread of the driving Sp haplotype and protect the Sm haplotype from extinction (Avril et al., chapter 1). Further investigations of the behavioral strategies and fitness of males, queens and

workers with alternative genotypes will provide a more complete picture of the dynamics of this system.

In conclusion, the suppression of recombination in large clusters of genes creates co-adapted gene complexes that selfishly distort Mendelian transmission in their favor, while promoting a cooperative, polygynous form of social organization. Supergenes controlling colony queen number have evolved independently in two highly divergent ant lineages (Purcell et al., 2014b). In the fire ant, the haplotype causing polygyny generates a green-beard effect, as workers carrying this haplotype kill queens that lack it (Keller & Ross, 1998, Trible & Ross, 2016, Huang & Wang, 2014). In the Alpine silver ant, the haplotype associated with polygynous colonies is a maternal effect killer, causing developmental arrest of brood that did not inherit this haplotype. Strikingly, in both systems intra-genomic conflict contributes to the maintenance of alternative social organizations, with the selfish drive by a supergene haplotype favoring the spread of a cooperative colony-level phenotype. More generally, many supergenes are likely to be transmission ratio distorters maintained in balanced polymorphism through antagonistic selective pressures at the gene, individual or group levels.

Acknowledgements

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Chapter 3

No role for mate preference and genetic incompatibilities in maintaining a supergene controlling ant social organization

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Manuscript in preparation

Abstract

Disassortative mating generates negative frequency-dependent selection, maintaining sex chromosomes and several supergenes that underpin complex phenotypes. Here, we explored whether mate preferences or genetic incompatibilities contribute to the maintenance of a supergene controlling variation in colony queen number in the Alpine silver ant *Formica selysi*. With mate choice experiments, we found that queens and males mated randomly with respect to social form. Moreover, for queens of both social forms, offspring production and incipient colony survival did not depend on whether the founding queen had mated with a male of the same or of the alternative social form. Overall, we found no evidence that mate preferences or genetic incompatibilities between social forms explain the asymmetric mating pattern observed in field populations. Furthermore, our experiment revealed that queens of monogynous origin were more likely to mate and more fertile, which suggests that queens of alternative social forms differ in their mating and colony founding strategies. This calls for further studies to evaluate how dispersal affects the dynamics of this polymorphism.

Introduction

Supergenes determine complex divergent phenotypes in single populations, yet in many cases explaining how the polymorphism is maintained remains a challenge (Schwander et al., 2014, Thompson & Jiggins, 2014). In absence of opposing selection pressure, natural selection will often lead to the fixation of the locally adapted supergene haplotype (Fisher, 1930, Chouteau et al., 2017). Disassortative mating between alternative morphs is a powerful mechanism to maintain balanced polymorphisms, because the rarer morph gains a reproductive advantage over the more frequent morph (Ayala & Campbell, 1974). This process explains the 1:1 sex ratio observed in many sexually reproducing species (Fisher, 1930) and the balanced polymorphism at sex chromosomes (Beukeboom & Perrin, 2014).

Disassortative mating plays a major role in maintaining polymorphic supergenes in plants and animals. *Primula vulgaris* has heteromorphic flowers that have either long style and low anthers, or short style and high anthers (heterostyly). Self-incompatibility is associated with obligate out-crossing with the alternative flower morph, which balances the frequencies of alternate allelic variants at the supergene controlling heterostyly (Li et al., 2016). In the white-throated sparrow *Zonotrichia albicolis*, near-perfect disassortative mating between alternative morphs leads to a balanced polymorphism at a supergene controlling plumage color and social behavior (Tuttle et al., 2016). Mate preferences for morphs with alternative wing-pattern also maintain the polymorphism at a supergene regulating Müllerian mimicry in the butterfly *Heliconius numata* (Chouteau et al., 2017).

Non-random mating may contribute to the maintenance of a supergene controlling alternative colony social organization in the Alpine silver ant *Formica selysi* (Purcell et al., 2014b) . In this

species, colonies can be headed by a single queen (monogynous) or by multiple queens (polygynous) and the two social forms were found in sympatry in all well-sampled populations (Chapuisat et al., 2004, Purcell & Chapuisat, 2013, Purcell et al., 2015). Two highly divergent non-recombining haplotypes at a large supergene determine colony queen number (Purcell et al., 2014b). All individuals from monogynous colonies have only the Sm haplotype, while queens, workers and male alates from polygynous colonies have at least one Sp haplotype. Both haplotypes produce normally viable homozygotes (Purcell et al., 2014b). This stands in contrast to many other supergenes where one variant is lethal when homozygote, which prevents it from reaching fixation (Wang et al., 2013, Küpper et al., 2016, Schwander et al., 2014). This unusual system raises questions on the selective pressures and evolutionary mechanisms contributing to the maintenance of the polymorphism.

Asymmetries in the pattern of mating appear to play a role in the maintenance of the social polymorphism in *F. selysi*. In monogynous field colonies, the supergene genotypes of reproductive queens and of their respective male mates reveal complete assortative mating with respect to social form: all queens are Sm/Sm and their mates are Sm (Avril et al., chapter 1; Purcell et al., 2014b). In contrast, in polygynous field colonies, 76.4% of the queens' mates have the Sp haplotype, while 23.6% have the Sm haplotype (Avril et al., chapter 1). Sm males are exclusively produced by monogynous colonies and Sp males by polygynous colonies (Purcell et al., 2014b). Hence, a fraction of queens in polygynous colonies had mated disassortatively, with males of monogynous origin. The asymmetric mating pattern observed in field colonies may be due to mate preferences or genetic incompatibilities. In particular, queens of monogynous colonies may reject Sp males, or queens of monogynous origin mated to males of polygynous

origin may fail to produce offspring. Alternatively, this asymmetric mating pattern could be due to differences between social forms in numbers and dispersal of queens and males.

The recent discovery that one variant of the supergene is a selfish genetic element further suggests that asymmetric patterns of mating contribute to stabilize the polymorphism. Sp is a maternal offspring killer causing developmental arrest of brood from heterozygous queens that did not inherit Sp (Avril et al., chapter 2). By so doing, Sp favors its own transmission over the alternative haplotype. Despite this strong drive, Sp does not go to fixation in polygynous colonies, and populations remain polymorphic (Purcell et al., 2015). We hypothesize that queen preference for Sm males or genetic incompatibilities between queens of monogynous origin and males of polygynous origin prevent the spread of the driving Sp haplotype. Here, we examine whether mate preferences or genetic incompatibilities between social forms explain the asymmetric mating pattern observed in the field and contribute to balance a polymorphic supergene controlling ant social organization.

Materials and Methods

Mate preferences

Virgin alate queens and males of *Formica selysi* were collected in summer 2015 from 42 colonies in Swiss populations (Finges: 7°36’30” E, 4°18’30” N, altitude: 565m; Derborence: 7°12’56”E, 46°16’50” N, altitude: 1450m). The social organization of each colony was previously determined based on direct observations of queens in early spring, microsatellite genotyping and RFLP genotyping of SNPs diagnostic for social form (Avril et al.,chapter 1; Purcell et al., 2014b,

Purcell & Chapuisat, 2013). Most colonies of *F. selysi* specialize in the production of one sex (Rosset & Chapuisat, 2006). When the two sexes were present, they were separated upon collection. Virgin queens or males and workers from the same parent colony were placed in plastic boxes (15 × 13 × 6 cm), and transferred to the laboratory. The ants were kept at 24°C and 50% relative humidity, and were provided with water and standard ant food *ad libitum* (Meunier & Chapuisat, 2009).

With mate choice experiments, we examined whether queens and males prefer to mate with partners of the same or the alternative social form. A virgin queen and two males from each social form were placed in a plastic box (35 × 22 × 15 cm) covered by a net. For each mating trial, the queen and males originated from different colonies of the same population. Males of alternative social forms were color-marked and the colors used were randomized between mating trials. The mating trials took placed outside in the morning, which stimulates the mating flight (Reber et al., 2010). We monitored the behavior of queens and males until mating, if any, or up to 30 minutes otherwise. Immediately after mating, the queen was isolated for incipient colony founding. Each queens was placed in a glass test tube partially filled with water retained by a cotton plug and kept in dark conditions (Brütsch et al., 2017). Males and females that did not mate were returned to their lab colonies and used in at most one other mating trial.

Genetic incompatibilities

Brood production and incipient colony survival were monitored for each mated queen. The number of eggs, larvae, cocoons and workers was counted every other day, and the status of queens (dead or alive) was recorded. To detect if some crosses suffer from genetic incompatibilities, we compared brood production (for fertile queens that survived until the end of

the experiment) and colony failure (the proportion of queens that died or did not produce any worker) between queens of monogynous or polygynous origin, mated with males of monogynous or polygynous origin, respectively.

Statistical analyses

Mate preferences and queen mating propensity were analyzed with generalized mixed effect models (GLMM), using a binomial error distribution. For mate preferences, we constructed a model in which the social origin of the queen's mate was the response variable and the social origin of the queen was included as fixed effect. Random effects comprised colony of origin of queen and males, color marks, date of mating trial, and whether the queen or males were in their first or second trial, if any. A Wald test on the intercept was used to detect significant departure from random mating. For the mating propensity of queens, we built a model in which the response variable was the mating status of the queen (mated or not) at the end of the trial. The queen social origin was included as fixed effect, while random effects comprised the date and whether the queen or males were in their first or second trial.

We explored whether genetic incompatibilities between social forms affected brood production and incipient colony survival. For brood production, we used a generalized additive mixed model (GAMM), which can model non-linear time series data (Zuur et al., 2009). The number of brood items (eggs, larvae, cocoons and workers) was the response variable. Queen social origin, male social origin and the interaction between the two factors were included as fixed effects. The post-mating date was used as the smoothing covariate. Random effects comprised queen identity and whether the queen or males were in their first or second trial. For incipient colony survival, we used a GLMM with a binomial error distribution. Colony failure rate was the binomial response

variable. Queen social origin, male social origin and the interaction between the two factors were included as fixed effects. Random effects comprised the date and the number of times queens and males were used in mating trials. All the statistics were done in R v 3.3.2 (R Development Core Team, 2015). GAMM and GLMM models were built using the 'mgcv' package v1.8 (Wood, 2011) and the 'lme4' package v1.1 (Bates et al., 2015), respectively.

Results

Mate preferences

In mate choice experiments involving a virgin queen and two males from each social form, mating occurred randomly with respect to social form. No significant preference was detected for queens of monogynous origin (**Figure 1**; GLMM binomial, $z = -0.25$, $p = 0.81$), nor for queens of polygynous origin (**Figure 1**; GLMM binomial, $z = 0.78$, $p = 0.43$). However, queens of monogynous origin were more likely to mate than queens of polygynous origin (**Figure 1**; mating occurred for 74.2 % of 62 queens of monogynous origin and 40.7 % of 59 queens of polygynous origin, respectively; GLMM binomial, $\chi^2 = 14.1$, $p < 0.001$).

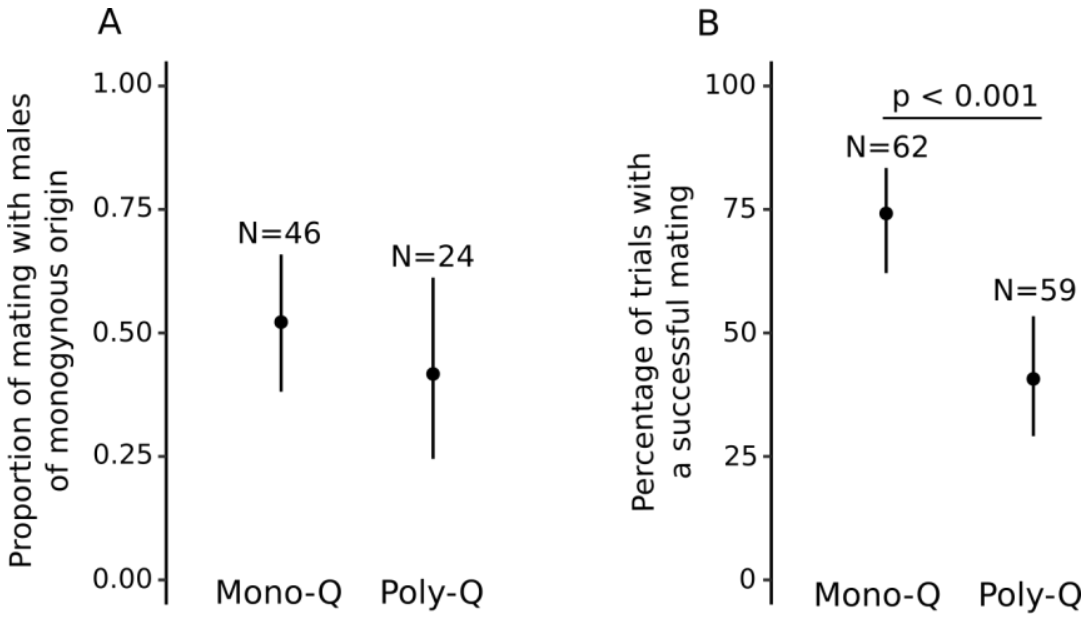

Figure 1: **Mate choice experiments** with *F. selysi* queens of monogynous origin (Mono-Q) and polygynous origin (Poly-Q), respectively. (**A**) **Mate preference**. Frequency of mating occurring with males of monogynous origin. Each queen was presented with two males of each social form, and was removed after the first mating. Bars indicate the binomial 95% confidence interval around the mean. The total number of matings is indicated above each bar. (**B**) **Mating propensity**. Percentage of queens that successfully mated across trials. Bars indicate the binomial 95% confidence interval around the mean. The total number of mating trials is indicated above each bar.

Genetic incompatibilities

Brood production in incipient colonies was not influenced by whether a queen had mated with a male of the same or the alternative social origin (**Figure 2**; Interaction between social origins of queens and their mates, for eggs: t = 0.38, p = 0.70; Larvae: t = 0.60, p = 0.55; Cocoons: t = 0.95, p = 0.34; Workers: t = 0.96, p = 0.34). Male social origin did not influence brood production by queens (Figure 2; Eggs: t = 0.47, p = 0.64; Larvae: t = 1.11, p = 0.27; Cocoons: t = 0.01, p = 0.99;

Workers: t = 0.84, p = 0.4). However, queens of monogynous origin produced more brood than queens of polygynous origin (**Figure 2**; Eggs: t = 4.6, p < 0.0001; Larvae: t = 6.5, p < 0.0001; Cocoons: t = 6.9, p < 0.0001; Workers: t = 7.4, p < 0.0001).

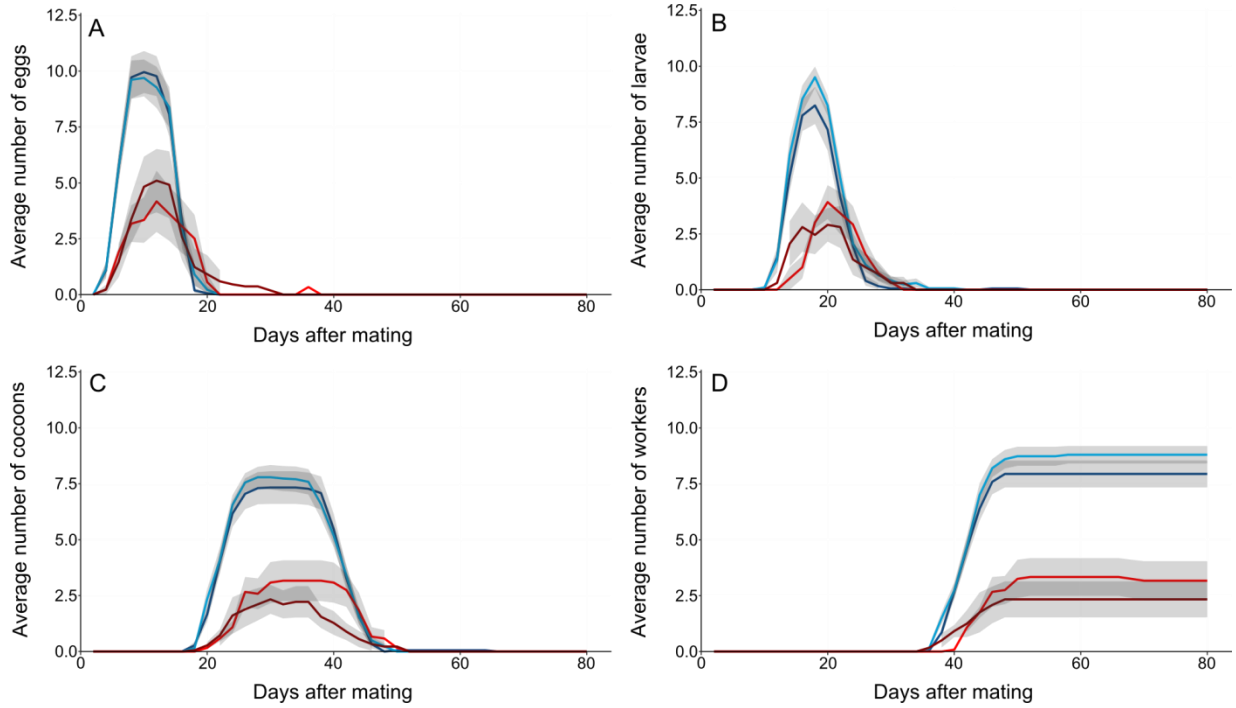

Figure 2: Brood production. Number of (**A**) eggs, (**B**) larvae, (**C**) cocoons, and (**D**) workers produced by queens of monogynous origin mated with males of monogynous origin (dark blue, N=17) or males of polygynous origin (light blue, N=15), and by queens of polygynous origin mated with males of monogynous origin (light red, N=6) or males of polygynous origin (dark red, N=6), respectively. Lines and shaded areas depict the average number of brood items and the standard error of the mean, respectively.

Colony failure did not depend on whether the founding queen had mated with a male from the same or the alternative social form (**Table 1**; GLMM, $\chi^2 = 0.05$, p = 0.81) and was not influenced by male social origin (**Table 1**; GLMM, $\chi^2 = 0.43$, p = 0.51). Colony failure rate was 1.64 times

higher for queens of polygynous origin than for queens of monogynous origin, but the difference was not statistically significant (**Table 1**; GLMM; $\chi^2 = 3.0$, $p = 0.08$).

Table 1: Proportion of incipient colonies in which the queen died or did not produce any worker. The number of colonies monitored is indicated between parentheses.

	Queens of monogynous origin		Queens of polygynous origin	
Mate social origin	monogynous	polygynous	monogynous	polygynous
Colony failure rate	0.29 (24)	0.32 (22)	0.4 (10)	0.57 (14)

Discussion

Genomic rearrangements and suppressed recombination over large portions of the genome underlie spectacular alternative phenotypes within populations (Schwander et al., 2014, Küpper et al., 2016). Alternate haplotypes at supergenes have complex phenotypic effects and the polymorphism is typically maintained by opposing selective pressures that combine recessive lethality, selfish genetic drive, natural selection or sexual selection (Schwander et al., 2014, Huang & Wang, 2014, Charlesworth, 2016, Chouteau et al., 2017). In the Alpine silver ant, genetic data indicate that all monogynous queens and a fraction of polygynous queens heading mature field colonies had mated with males originating from monogynous colonies (Purcell et al., 2014b). This asymmetric mating pattern suggests that mate preferences or genetic incompatibilities between social forms may contribute to the maintenance of the polymorphic supergene underlying social organization in this ant species.

In mate choice experiments, we found no evidence for mate preference or genetic incompatibilities associated with the social forms of *F. selysi*. Mating between queens and males occurred irrespectively of their social origin and genotype at the supergene controlling social organization. Brood production and incipient colony success were independent of whether the queen had mated with a male of the same or the alternative social form, in line with earlier results (Reber et al., 2010). Behaviorally and genetically, mating within and between social forms appeared unrestricted.

Overall, disassortative mate preference does not appear to stabilize the polymorphism at the social supergene of *F. selysi*. Because homozygotes for both haplotypes are viable, there might be no selection favoring mating between social forms. In our experiment, brood productivity and colony founding success were similar for queens mated with males originating from the same or the alternative social form. Hence, there appears to be no selective advantage for either assortative or disassortative mating with respect to social form, at least in the early stages of colony development.

Mate preference or genetic incompatibilities did not explain the asymmetric mating pattern observed in field colonies. In mate choice experiments, queens showed no preference for Sm males. In particular, Sm/Sm queens did mate with Sp males when given the opportunity, and this cross produced viable offspring. Yet, despite extensive genotyping from mature field colonies, we never detected the presence of Sm/Sm queens that had mated with Sp males. It is possible that Sm/Sm queens do not encounter Sp males in the field, due to differences in numbers, phenology or dispersal behavior (Rosset & Chapuisat, 2007). Alternatively, the Sm/Sm founder queen, when mated with a male of polygynous origin, might be quickly replaced by multiple Sp/Sm daughter queens.

Queens of alternative social forms differed in their mating and reproductive strategies. Queens of monogynous origin were more likely to mate, produced three times as many brood and were marginally more successful at independent colony founding than queens of polygynous origin, irrespective of the social origin of their mates. This difference in queen behavior and fertility suggests that queens of monogynous origin are more prone to mate outside of their nests and more successful at establishing incipient colonies without the help of workers. In contrast, queens of polygynous origin may preferentially mate close to or within their natal nest and establish new nests with the help of workers. In ants, restricted dispersal and dependent colony founding are generally associated with smaller queen body size (Keller & Passera, 1989). *F. selysi* queens of polygynous origin are smaller than queens of monogynous origin and a cross-fostering experiment showed that this difference was genetic (Rosset & Chapuisat, 2007, Meunier & Chapuisat, 2009).

An intriguing result of our experiment was that queens of polygynous origin managed to establish incipient colonies independently and succeeded in rearing a first cohort of workers. Yet, mature monogynous colonies in the field are never headed by Sp/Sm or Sp/Sp queens. Incipient colonies established by queens of polygynous origin may fail to develop into mature colonies in the harsher and more competitive conditions that prevail in the field. In line with this hypothesis, in the protected conditions of the laboratory queens of polygynous origin produced significantly fewer workers and tended to have a higher colony failure rate than queens of monogynous origin. Alternatively, incipient colonies founded by single queens of polygynous origin may rapidly recruit new daughter queens, and thus become polygynous.

Differences between social forms in the number of queens and males produced, and in their dispersal behavior, may contribute to maintain the polymorphism. On the one hand, the

supergene haplotype associated with polygyny is a maternal effect killer favoring its own transmission (Avril et al., chapter 2). On the other hand, a lower fraction of polygynous colonies produce queens and males, compared to monogynous colonies (Rosset & Chapuisat, 2007). Moreover, queens and males of polygynous origin may show more restricted dispersal and favor dependent colony founding, further limiting the spread of the Sp haplotype. In the fire ant *Solenopsis invicta*, the supergene haplotype causing polygyny is a selfish genetic element causing a green beard effect (Keller & Ross, 1998, Wang et al., 2013). Selection or unidirectional gene flow may prevent its fixation in the population (Ross, 1996, Shoemaker & Ross, 1996, Fritz et al., 2006, Goodisman et al., 2000).

Heterogeneous spatial selection for alternative forms of social organization may also contribute to stabilizing the genetic polymorphism. In *Heliconius* butterflies and *Cepaea* snails, supergenes control alternative color patterns, which are differentially selected for in different environments (Cook, 1998, Joron et al., 1999, Richards et al., 2013). Although the two social forms of *F. selysi* live in sympatry, previous work suggests that they have different ecological optima (Purcell et al., 2015). Whether heterogeneous spatial selection affects the maintenance of the polymorphism in *F. selysi* remains to be tested.

In summary, we found no evidence that mate preferences or genetic incompatibilities between social forms cause the asymmetric mating pattern observed in the field, or contribute to stabilize the polymorphism. Queens of monogynous origin were more likely to mate, more fertile and marginally more successful at independent colony founding than queens of polygynous origin, regardless of the social origin of their mates. This result likely reflects differences in the dispersal and colony founding strategies of queens from alternative social forms. Further research is

needed to evaluate the impact of dispersal on gene flow between social forms and on the dynamics of the polymorphism.

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General discussion

Main findings and perspective

Understanding how balanced polymorphisms are maintained is a major current question in evolutionary biology. In this thesis, I investigated key mechanisms that may contribute to the maintenance of a supergene controlling social organization in the Alpine silver ant *Formica selysi*.

In **chapter 1**, I used a population genomic approach to investigate the mating and dispersal patterns for queens and males of each social form. I found that mating was asymmetrical with respect to social form. Queens of monogynous origin invariably mate with Sm males of monogynous origin. In contrast, queens of polygynous origin mate with Sp males of polygynous origin, but 23.6% mate with Sm males of monogynous origin. In addition, I found evidence of restricted dispersal of queens, and queens of polygynous origin tended to mate with relatives.

In the Alpine silver ant, alternative social forms were associated with differences in dispersal and mating strategies within a single population. Whether and how similar differences affect gene flow between and within social forms at a larger scale remains to be investigated. In addition, the causes of the asymmetrical mating pattern deserve further investigations. Understanding the determinants of the mating and dispersal strategies may reveal important factors contributing to the maintenance of the polymorphism at the supergene.

In **chapter 2**, I showed that the supergene haplotype associated with polygyny was a selfish genetic element causing transmission ratio distortion. Heterozygous queens mated to Sm males never produce Sm/Sm females, because the Sp haplotype is a maternal offspring killer. Sm/Sm

offspring from Sp/Sm queens die before reaching the larval stage, while Sm/Sm offspring from Sm/Sm queens develop normally. Hence, the Sp haplotype is a selfish genetic element favoring its own transmission over the transmission of the Sm haplotype. The selfish behavior of the Sp haplotype is likely to play a central role in the maintenance of the two haplotypes at this supergene.

The molecular basis underlying the selfish behavior of the Sp haplotype remains to be investigated. Post-segregation distorters are generally based on a modification-rescue system, for example a toxin linked to its corresponding antidote (Burt & Trivers, 2006). We have obtained high quality PacBio sequences for each haplotype of the *Formica selysi* supergene. The genome assembly of the Sm form has been completed, with excellent continuity (N50 > 7.9 Mbp). We are currently assembling the genome of the Sp form. The comparison of the gene content between the Sp and Sm haplotypes, coupled with gene expression analyses of early developmental stages, may help identifying the functional and molecular basis of the maternal effect killing induced by the Sp haplotype. More generally, high quality genomes and gene expression analyses from each social form will help to understand the link between the genotype at the supergenes and the social phenotype.

Several supergenes contain selfish genetic elements (Wang et al., 2013, Larracuente & Presgraves, 2012, Lyon, 2003), which raises interesting questions about the evolutionary history of this association (Huang & Wang, 2014). Segregation distorters involve tight linkage between the modification and the rescue elements. Hence, genomic regions with suppressed recombination, like supergenes and sex chromosomes, are prime areas for the accumulation of distorters. Whether the distortion played a causal role in the evolution of the supergene remains an open question. Future studies should determine whether supergenes evolve first by integrating

co-adapted loci controlling for adaptive polymorphism, or by integrating selfish genetic elements (Huang & Wang, 2014). Comparative genomics among closely related species should help to trace the evolutionary history of selfish genetic elements within supergenes.

In **chapter 3**, we tested whether mate preference or genetic incompatibilities may contribute to the maintenance of the polymorphism. In mature field colonies, queens and males of each social form do not exhibit random mating. All queens in monogynous colonies had mated with a male of monogynous origin, while 76.4% of queens in polygynous colonies had mated with a male of polygynous origin. Yet, in mate choice experiments, queens and males mated at random with respect to social form, and there were no genetic barriers between social forms. Hence, disassortative mate preference does not contribute to stabilize the polymorphism, and factors independent of mate choice must contribute to the pattern of non-random mating observed in the field. Future research should investigate whether the two social forms differ in spatial location or timing of mating. For instance, reproductive isolation between *Pogonomyrmex rugosus* and two hybrid lineages is partly due to temporal differences in mating flight (Schwander et al., 2008).

The data in chapter 3 suggest that queens of monogynous and polygynous origin differ in their mating behavior. Indeed, a higher proportion of queens of monogynous origin did mate under our experimental settings mimicking a mating flight, in comparison to queens of polygynous origin. This difference is consistent with the results in the chapter 1, showing that queens of polygynous origin tend to be mated with relatives, which may indicate that queens of polygynous origin mate within or close to their natal nest, without engaging in a mating flight.

We didn’t find any genetic or behavioral barriers between Sm/Sm queens of monogynous origin and Sp males of polygynous origin. In addition, results from chapter 1 showed that males of

polygynous origin have good dispersal abilities. Yet, we never observe Sm/Sm queens mated with Sp males in field colonies, which calls for more studies on the mechanisms regulating the dispersal and mating behavior of queens and males of both social origin.

Maintenance of the polymorphism

Overall, our results shed light on unexpected mechanisms contributing to balance the frequencies of the two haplotypes of the supergene controlling social organization in *F. selysi*. The Sp haplotype is a selfish genetic element, and therefore has a selective advantage over the alternate Sm haplotype. However, biased gene flow from the monogynous to the polygynous social form, and restricted dispersal of queens from the polygynous social form, may counterbalance the selective advantage of the Sp haplotype. Hence, despite the absence of genetic barriers and mating preferences between social forms, neither haplotype may be able to reach fixation, resulting in what appears to be a stable social polymorphism in most populations (Purcell & Chapuisat, 2013, Purcell et al., 2015).

Other factors are likely to contribute to the maintenance of the polymorphism. For instance, a previous study indicated that the two social forms have different ecological optima (Purcell et al., 2015), so that heterogeneous spatial selection, where each social form is favored under specific environmental condition, may contribute to maintain the supergene. This selection regime has been shown to contribute to the maintenance of other supergenes (Joron et al., 1999, Lowry & Willis, 2010).

Future directions

The supergene controlling social organization in *Formica selysi* has homologues in other species of the genus *Formica* (Brelsford, Purcell and Chapuisat, unpublished results). A comparative genomic analysis of *Formica* species can shed light on how the supergene evolved, and may pinpoint key genes involved in producing alternative social forms. This type of approach has revealed that the evolution of heteromorphic sex chromosome results from successive inversions, leading to several evolutionary strata within sex chromosomes (Lahn & Page, 1999, Zhou et al., 2014). In contrast, in *Heliconius* butterflies, few narrow genomic regions are responsible for alternative color polymorphism (Van Belleghem et al., 2017).

Social organization is controlled by independently evolved supergenes in two distantly related ant lineages, *Solenopsis* and *Formica* (Wang et al., 2013, Purcell et al., 2014b). A third genus, *Leptothorax* is also likely to have a supergene controlling for the number of queens actively reproducing in the colony (Braim, 2015). This suggests that supergenes controlling social organization may be widespread in ants. Further research is needed to investigate the genetic basis of social organization in other ant species, and the role of supergenes in the evolution of other forms of complex phenotypes.

Appendix 1

Ant brood function as life preservers during floods

Purcell J., **Avril A**., Jaffuel G., Bates S., and Chapuisat M.

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Abstract

Social organisms can surmount many ecological challenges by working collectively. An impressive example of such collective behavior occurs when ants physically link together into floating ‘rafts’ to escape from flooded habitat. However, raft formation may represent a social dilemma, with some positions posing greater individual risks than others. Here, we investigate the position and function of different colony members, and the costs and benefits of this functional geometry in rafts of the floodplain-dwelling ant *Formica selysi*. By causing groups of ants to raft in the laboratory, we observe that workers are distributed throughout the raft, queens are always in the center, and 100% of brood items are placed on the base. Through a series of experiments, we show that workers and brood are extremely resistant to submersion. Both workers and brood exhibit high survival rates after they have rafted, suggesting that occupying the base of the raft is not as costly as expected. The placement of all brood on the base of one cohesive raft confers several benefits: it preserves colony integrity, takes advantage of brood buoyancy, and increases the proportion of workers that immediately recover after rafting.

Introduction

Social organisms have an advantage when responding to ecological adversity: they can react in a collective and organized way, working together to perform tasks that a solitary individual could not achieve (Gordon, 2007, Dussutour et al., 2009b, Detrain & Deneubourg, 2006). For instance, some societies respond to predators by mounting a coordinated defense, as in leaf- cutter ants, which form a defensive line featuring large major workers and teams of smaller workers to block invading army ants (Powell & Clark, 2004). Other species link their bodies together to achieve a mutual goal, as in Japanese honeybees, which will surround large predatory hornets and form an 'oven,' raising the interior temperature to kill the intruder (Ono et al., 1995). The latter case is an example of a 'collective structure.' These self-assembled collective structures can provide defense, shelter, thermoregulation, bridges over obstacles, or a means of transportation (Anderson et al., 2002). Although collective structures are widespread, particularly in the social Hymenoptera, their functional geometry, defined as the position and function of individuals within the structure, generally remains poorly understood.

In many collective structures, different castes occupy specific positions. In the 'bivouac' nests of army ants and in bee or wasp swarms, for example, workers form protective layers around more vulnerable queens and brood (Anderson et al., 2002, Schneirla, 1971, Cully & Seeley, 2004). If some positions are safe and others risky, the configuration of these structures suggests that altruism or coercion may be inherent in such self-assemblages. However, the costs

and benefits of specific positions are difficult to measure, and the position of each individual may also depend on how its particular physical properties function in the structure.

Ant rafts provide a useful model of a collective structure in which occupancy of some positions – namely positions on the raft base – may be detrimental and thus reflect altruistic self-sacrifice. Alternatively, positions may be filled based on the functional properties of individuals. Many floodplain-dwelling ant species form rafts. Colony members assemble into a floating platform by linking tarsus-tarsus or mandible-tarsus (Mlot et al., 2011, Nielsen, 2011). In the fire ant *Solenopsis invicta*, recent studies have investigated the physical properties of rafts (Mlot et al., 2011), as well as raft formation, longevity, and success rates under controlled conditions (Adams et al., 2011). Adams et al. (2011) further noted qualitatively that fire ants tended to place larger brood on the raft base, which allowed rafts to remain afloat longer than those consisting of only workers. They speculated that brood may be more buoyant than workers. The finding that rafting ants place some brood on the raft base raises the question of whether this action imposes costs on the brood and/or benefits the group.

Here, we investigate the functional geometry of rafts in the ant *Formica selysi*. These ants are abundant in floodplains throughout the Alps and the Pyrenees (**Figure 1a**), where floods can cause severe erosion and may submerge nests for days (Chapuisat et al., 2004). During floods, colonies have been observed to evacuate their nests and raft to safety (**Figure 1b**) (Lude et al., 1999). We elicited rafting behavior in the laboratory to investigate where workers, brood, and queens are positioned in the raft, and to what degree their respective positions require altruistic self-sacrifice, and/or reflect functional differences in their physical properties. In a series of experiments, we quantify for the first time the costs and benefits associated with the position of workers and brood in the rafts, and we measure their respective buoyancy.

We expect workers to protect the most vulnerable and valuable nest-mates by placing them in the center of the raft, but also to take advantage of the physical properties of each caste to build a robust and buoyant raft.

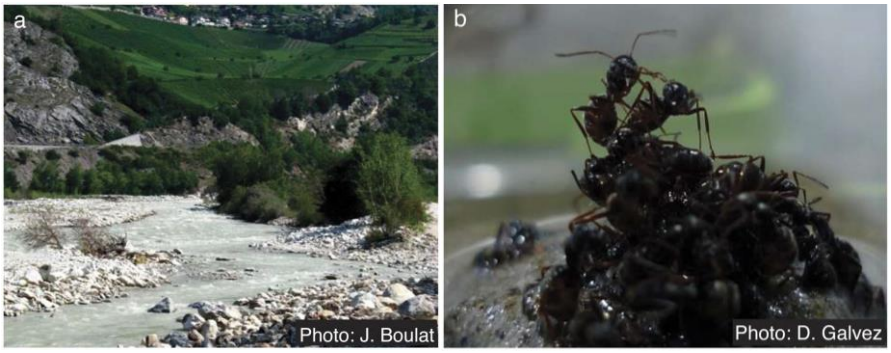

Figure 1: Photos of floodplain habitat in Valais, Switzerland (a) and incipient raft during self-assembly (b).

Materials and Methods

In 2011 and 2012, we collected workers and brood from field colonies in a large *F. selysi* population along the Rhône River in Valais, Switzerland (7°36'30"E, 46°18'30"N, altitude 565 m). We additionally collected one to two mature queens from each of five polygynous colonies. No specific permit was required to collect this ant species, which is not endangered or protected. Each individual was used in only a single experiment or trial in this study. This population has been monitored for over ten years (Purcell & Chapuisat, 2013), and large floods causing erosion and nest destruction have been observed during that time (Figure S1 in File S1) (Chapuisat et al., 2004). Flooding of the alluvial plain habitat generally occurs in the late spring through late summer, when brood is present in *F. selysi* colonies (Chapuisat et al., 2004).

We constructed an apparatus to film raft formation from above and from below simultaneously (Figure S2 in File S1). To induce rafting, we placed ants on a platform and raised the water level slowly. Initially, we investigated the placement of queens, sexual brood, and worker brood in rafts (**Table 1**). We then performed a series of experiments to better understand the geometry, function, costs and benefits of ant raft assemblages. Additional details of the rafting apparatus, study species, and methodology are provided in the supporting information.

Colony member positions

We formed groups of 60 workers collected from each of 15 field colonies, to which we added additional individuals from the same field colonies to constitute three different experimental conditions. We added: (i) one or two queens (N = 2 and N = 3, respectively), (ii) ten worker pupae (N = 5), or (iii) five sexual pupae (N = 5). Each group was then subjected to a flood, causing them to raft for 30 minutes.

Submersion tolerance of workers

We submerged three workers from each of 14 field colonies, and investigated their resistance to staying underwater. The experimental apparatus consisted of a glass tube that we placed in a large water container, ensuring that no bubbles remained in the tube. We then placed workers individually in the glass tube, so that ants were not able to float to the surface. Following an eight hour submersion, we removed workers to a filter-paper lined box and measured their survival and recovery time.

Table 1: Summary of results from rafting experiments. Each trial involved 60 workers, and each group of workers (and brood) rafted only once. The same groups of workers and brood were used for the raft recovery and rafting tolerance of brood experiments: after the initial raft trials, we observed the raft recovery and later monitored brood eclosion.

	group composition	trials	raft duration	positions	comparison
Queen position	1 queen 2 queens	2 3	30 min.	Queens in center of raft, workers above, on base, and on sides of raft. 25-50% of workers in contact with the water.	
Sexual brood position	5 brood	5	30 min.	Sexual brood on raft base, workers throughout but few on base.	
Worker brood position	10 brood	5	30 min.	Worker brood on raft base, workers throughout but few on base.	
Rafting tolerance of brood	-- 10 brood	10 10	3 hrs.	Brood on base if present, workers throughout the raft. More workers in contact with water in the absence of brood.	Survival of brood that rafted *versus* brood provided to worker groups after rafting: 83% *versus* 79%, paired t-test $t_9 = 0.74$, $p = 0.48$
Buoyant materials choice	10 brood + 10 wood cylinders	10	30 min.	Brood on base. In some cases, wood cylinders passively included on the peripheral base of the raft, workers throughout but few on base	Mean numbers of brood items *versus* wood cylinders collected: 9.8±0.2 (standard error) *versus* 1.1±0.5 and incorporated in the raft: 9.8±0.2 *versus* 3.8±0.6, paired Wilcoxon tests V = 55, df = 9, p = 0.0055
Raft recovery	-- 10 brood	10 10	3 hrs.	Brood on base if present, workers throughout the raft. More workers in contact with water in the absence of brood.	Mean time to disassemble rafts with brood *versus* without brood: 326±37 seconds ± standard error *versus* 230±29 seconds, paired t-test $t_9 = 1.60$, p = 0.14; Mean number of unresponsive workers after rafting with brood *versus* without brood: 0.6±0.2 *versus* 3±0.8, paired t-test $t_9 = 3.09$, p = 0.013

Rafting tolerance of brood

For each of ten field colonies, we formed two experimental groups of 60 workers and allowed them to raft for three hours; for each colony, one of the groups had ten nest-mate brood items during rafting, while the other group received ten nest-mate brood items after rafting (**Table 1**). We used a combination of pupae and larvae during this experiment, but found no difference in survival between the two (Binomial test $p = 0.51$), so we combined them in our subsequent analyses. After rafting, the experimental groups (each with 60 workers and 10 brood items) were placed in boxes (15×13×6 cm) containing one plaster nest and *ad libitum* access to standard ant food and water. The groups were monitored at least five times per week until all brood had either eclosed to adulthood or died. We used a paired t-test to investigate whether brood that experienced rafting exhibited a different survival rate than brood that did not experience rafting.

Buoyancy of workers and brood

We placed individual workers, larvae and pupae from each of eight field colonies in solutions with increasing concentrations of detergent for two minutes and recorded whether they remained afloat (Table S1 in File S1). Detergent decreases the surface tension of water, which reduces buoyancy caused by air trapped on the hydrophobic body surface (Mlot et al., 2011).

Buoyant materials choice experiment

To test whether workers prefer brood over other buoyant material to form a raft base, we provided them with both brood items and pieces of wood of similar dimensions and weight as the brood (**Table 1**). We collected workers and brood from 10 field colonies to form 10

replicates. We let groups of 60 workers settle on the watch glass, and placed 10 pupae and 10 wood cylinders at equal distances from the largest group of workers. We then measured the number of pupae and wood cylinders that were actively collected and the number incorporated into the raft (either actively or passively) during 30 minutes of rafting, and compared these measures using paired Wilcoxon tests.

Raft recovery

We compared the recovery time of workers from rafts with brood to those from rafts without brood. For each of ten field colonies, we formed two experimental groups: one with 60 workers and the other with 60 workers plus 10 brood items. We filmed the behavior of groups for one hour after three hours of rafting and used paired t-tests to compare the time to disassemble the raft and the number of unresponsive workers.

Results

Raft formation

When only workers are present, rafts are initiated by a single group of workers (about 60–80% of the 60 individuals in our trials) that remain close to one another and begin to form a pile consisting of 2–3 layers of workers as the water level rises. The remaining workers walk between the water's edge and the group, or engage in trophallaxis, self-grooming or allo-grooming away from the group. These individuals either climb onto the pile or join the outer edge of the aggregation when the water level rises to the raft level. The picture is similar when queens or brood are present. Workers quickly and actively collect brood

items from the platform, place them in a single pile and aggregate on top of them. The brood is often repositioned during this phase. As the water level rises, and early in the raft assembly process, queens gradually move to occupy the center of the pile of workers. Brood are held in the mandibles of the workers and maintained on the base of the pile. As above, some workers remain mobile until the water level reaches the raft when queens or brood are present. When they begin to float, rafts have 3–4 layers of workers. *Formica selysi* was reluctant to raft, both in the field (Lude et al., 1999) and in the laboratory (see supporting information).

Raft geometry

Adult queens always occupied the center of the raft (**Table 1**). The placement of queens ensured that they were neither touching the water nor exposed from above. In contrast, workers systematically placed all sexual and worker-destined brood on the base of the raft (**Table 1**; see Movie S1, S2). When brood items were present, very few workers occupied a position on the raft base, but without brood, 25–50% of workers had at least partial contact with water.

Costs of submersion and rafting

The cost of rafting was lower than expected, because both workers and brood were highly resistant to submersion in water. After spending eight hours completely under water, 79% of *F. selysi* workers recovered. On average, workers began to move 66 ± 3 minutes (mean ± SE) and began to walk 77 ± 4 minutes after removal from water. Given that workers in rafts usually were not completely submerged, rafting may cause little or no direct mortality to workers, even when they occupy the raft base. However, workers on the raft base need a significant period of time to recover after rafting (see ‘Benefits of raft geometry’ section).

Similarly, brood that spent 3 hours on a raft base did not appear to pay a significant cost; brood that rafted survived until eclosion at the same rate as those that did not (**Table 1**).

Function of raft geometry

Larvae and pupae (with and without cocoons) were significantly more buoyant than workers (Table S1 in File S1), which most likely explains why workers place brood on the raft base. Workers preferred to use brood over wood cylinders, which are also highly buoyant (**Table 1**, Table S1 in File S1). Wood cylinders were sometimes incorporated into the raft when they were encountered after raft formation.

Benefits of raft geometry

After rafting, the workers released each other and began to move away from the aggregation. The rafts disassembled with workers from the top and sides of the raft departing first. Brood and unresponsive workers were generally moved to a dry location within 20 minutes and groomed extensively. Rafts with brood tended to take more time to disassemble than rafts without brood, but the difference was not significant (**Table 1**; **Figure 2**). On the other hand, rafts composed of workers and brood had significantly fewer unresponsive workers than those with workers alone (**Table 1**; **Figure 2**).

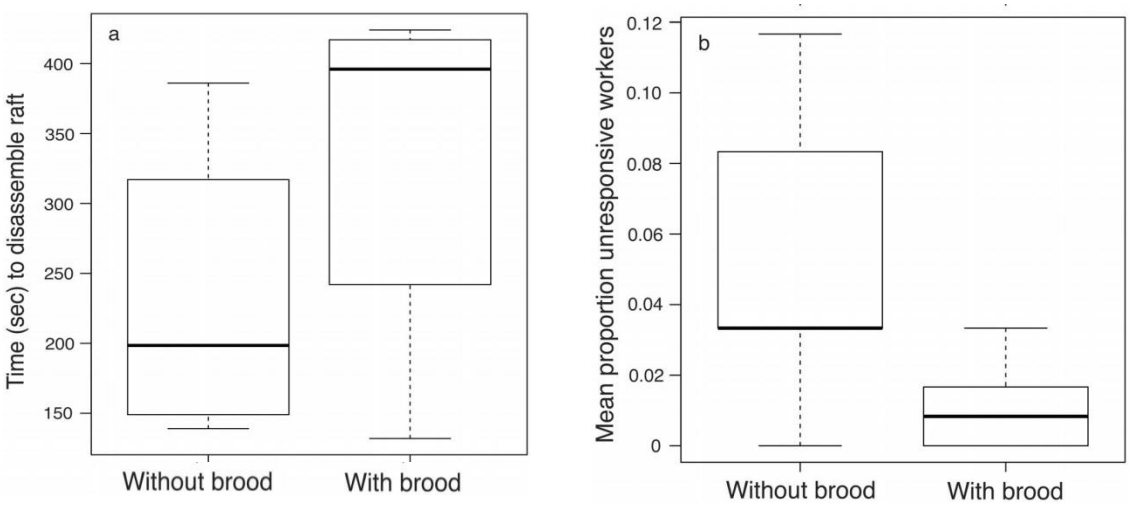

Figure 2: Comparison of recovery of rafts with and without brood: time to disassemble raft (a) and proportion of unresponsive workers after 60 minute recovery period (b).

Discussion

Some ants have evolved a remarkable ability to self-assemble into rafts in response to floods. The formation of rafts is a progressive and coordinated process, resulting in a collective structure with a well-defined geometry. Strikingly, workers place brood on the base of the raft and use them as a floating platform. We expected *F. selysi* ants to protect particularly vulnerable or valuable members of their society by placing them in the center of their rafts. Indeed, queens consistently occupied rafts centers, out of the water and protected by workers on all sides. In contrast, ants placed larvae and pupae, both worker and sexual, on the raft base. This geometry did not result from constraints due to lack of workers; rafts generally consisted of three or four layers of workers, so placing brood in an internal position would have been possible if the workers holding brood in their mandibles occupied a higher layer.

We observed little mortality in our experiments, suggesting that the social dilemma facing rafting ants may have less severe consequences than we initially predicted. Contrary to our expectations, even workers and brood that stayed underwater for hours on the raft base exhibited very high survival rates. The reluctance of ants to raft combined with the protective placement of queens in the raft center, however, suggest that there may be other costs or dangers not accounted for in our experiments. Obvious costs of rafting include the risk of losing the nest, of colony fragmentation, and of being washed away to unsuitable habitat. Moreover, predation by fish or exposure to turbulent waters may cause higher mortality than measured in laboratory conditions. Consistent with the hypothesis of elevated risks, fire ants increase the venom in their stings while rafting (Haight, 2006). Finally, there are likely to be physiological costs associated with submersion in water, including oxygen deprivation, increased CO_2 levels, and possible thermal effects from cold water.

The collection and placement of brood on the raft base may serve multiple functions. First, brood items are more buoyant than adult workers, and thus serve as flotation devices. When submerged for an extended time, *F. selysi* workers become immobile, and require an hour or more to recover. Thus, workers from rafts with brood recover more quickly, on average, than workers from rafts without brood. This would likely be highly advantageous in the natural environment, where groups need to find cover quickly after reaching shore. Along the same lines, Adams et al. (2011) showed that *S. invicta* rafts are able to remain afloat longer when brood items are integrated.

Other essential functions of self-assembling into a single raft are to preserve the progeny, and to keep the colony together (Depickere et al., 2004, Sempo et al., 2006). Given that the brood

suffers little or no mortality and workers preferentially incorporate brood into the raft over other buoyant materials, we suggest that brood rescue and colony cohesion are the primary motivations to incorporate brood in the raft, while their buoyant properties explain their placement on the base.

The rafts in our study contained fewer ants and brood items than most natural colonies (see supporting information), but given the consistent and deliberate placement of brood and queens across our tests, we expect the functional geometry of rafts to scale up to full size colonies. Other measurements, such as mortality rate and raft recovery, may not scale linearly with raft size and the time spent rafting. Future tests investigating how self-assemblages such as ant rafts are affected by colony size would be of interest (Dornhaus et al., 2012). Moreover, a careful investigation of individual behavior as the raft forms would provide novel perspectives on how ants self-organize to form complex structures.

Ants from at least two phylogenetically independent species, *F. selysi* and *S. invicta*, use brood items as a floating platform when they raft. Brood placement in rafts is one of the few examples of hymenopteran societies actively exploiting the functional characteristics of their young, which are usually dependent on adults and only passively contribute to the colony, due to the complete metamorphosis of holometabolous insects. Other examples include weaver ants using silk produced by larvae to build sturdy nests (Wilson & Hölldobler, 1980), *Leptanilla japonica* brood providing nutrition to queens through a larval hemolymph tap (Masuko, 1989) and various forms of brood cannibalism (Chapuisat et al., 1997b, Bourke, 1991).

Overall, collective structures keep nest-mates together during emergencies. Within this function, groups can optimize the structural geometry, taking advantage of the properties of

different group members to minimize costs and maximize survival probability. Rafting ants seem to solve this optimization problem by placing brood on the base of the raft, thereby maintaining the colony integrity and constructing a more durable raft without imposing high costs on the brood.

Supporting Information

File S1 contains the files: Text S1 Description of the study species, *Formica selysi.* Text S2 Additional information about the experimental set up and rafting apparatus. Text S3 Information on rafting pilot studies. Text S4 Details about experimental methods. Text S5 Overview of results from the buoyancy experiment. Text S6 List of references cited in the supplementary information. Figure S1 Photo of erosion of *Formica selysi* habitat caused by a flood of the Rhône River. Figure S2 Side view of the experimental set up. Table S1 Results of buoyancy tests of workers, brood, and wood cylinders. Movie S1 60 workers with ten sexual larvae forming a raft, filmed from below and played at 64x speed. Workers place sexual larvae on the base of the raft. We replicated this raft configuration five times with similar results, but provided five sexual pupae instead of the ten larvae shown here to ensure that brood placement was not due solely to the workers' limited ability to manipulate these large brood items. Movie S2 60 workers with ten worker brood and ten wood cylinders, played at 64x speed. Workers place brood in a pile as the water level rises and form the raft above the brood. Wood cylinders are not actively collected, but some are incorporated around the perimeter of the raft after the group is afloat.

Acknowledgments

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Appendix 2

Ant workers exhibit specialization and memory during raft formation

Avril A., Purcell J., and Chapuisat M.

Published in The Science of Nature, 2016

Abstract

By working together, social insects achieve tasks that are beyond the reach of single individuals. A striking example of collective behaviour is self-assembly, a process in which individuals link their bodies together to form structures such as chains, ladders, walls or rafts. To get insight into how individual behavioural variation affects the formation of self-assemblages, we investigated the presence of task specialization and the role of past experience in the construction of ant rafts. We subjected groups of *Formica selysi* workers to two consecutive floods and monitored the position of individuals in rafts. Workers showed specialization in their positions when rafting, with the same individuals consistently occupying the top, middle, base or side position in the raft. The presence of brood modified workers' position and raft shape. Surprisingly, workers' experience in the first rafting trial with brood influenced their behaviour and raft shape in the subsequent trial without brood. Overall, this study sheds light on the importance of workers' specialization and memory in the formation of self-assemblages.

Introduction

Members of insect societies cooperate in sophisticated ways. By coordinating their actions and working collectively, workers manage to perform tasks that are beyond the reach of isolated individuals, such as transport large items (Detrain & Deneubourg, 2008). On occasion, social insects link their bodies together to construct adaptive structures termed self-assemblages (Anderson et al., 2002). Self-assemblages take a variety of functional forms, such as bridges, bivouacs, protective curtains or rafts (Anderson et al., 2002, Reid et al., 2015).

The proximate mechanisms and behavioural processes involved in the formation of self-assemblages are poorly known (Anderson et al., 2002). Most self-assemblages described so far require quick reactions from colony members (Peeters & De Greef, 2015, Reid et al., 2015). For instance, workers of the arboreal ant species *Azteca andreae* use an elaborate hunting technique: many workers self-assemble into "pulling chain" structures to capture very large prey items, which requires fast coordination to prevent prey from escaping (Dejean et al., 2010). Researchers hypothesize that simple behavioural rules under positive feedback exponentially attract workers to the first individuals involved and therefore ensure a rapid growth of the self-assemblage (Foster et al., 2014, Garnier et al., 2013, Lioni et al., 2001). For example, Anderson and colleagues (2002) suggested that a rule such as "run to the end of the chain and hang there" would be sufficient for *Eciton* ants to build structures such as chains or ladders. Hence, organised patterns arise from simple behavioural rules and individual decisions based on local information (Camazine et al., 2001).

Task specialization emerges when members of a social group consistently differ in their behavioural responses to a given input, and such division of labour is central to the organization

of social insects colonies (Holbrook et al., 2013, Ferrante et al., 2015, Cahan & Gardner-Morse, 2013). The specialization of individuals in performing particular tasks or roles tends to increase group efficiency (Oster & Wilson, 1978, Beshers & Fewell, 2001). In social insects, workers can display a wide array of specific tasks in colony defense, foraging or brood care (Rocha et al., 2014, Wilson, 1980). Factors determining task specialization are numerous and include worker morphology, age, previous experience, or genetic background (reviewed in Duarte et al., 2011). Extensive research has been carried out on task specialization and division of labor in insect societies (Smith et al., 2008). Yet, the role of task specialization in the construction of self-assemblages remains little explored, with only *Eciton* ants' bridges being investigated so far (Garnier et al., 2013, Franks, 1985).

Here, we investigate the presence of specialization and the role of past experience in the construction of rafts by the Alpine silver ant *Formica selysi*. This species is found primarily in floodplains, where colonies may respond to floods by forming a living raft and floating to safety (Lude et al., 1999, Purcell et al., 2014a). In a first experiment, we assess whether workers show specialization in the positions they occupy during the self-assembly of successive rafts. If workers uniformly follow a single set of assembly rules, we expect that they will be positioned at random in each raft. In contrast, if workers differ in their individual responses, they will consistently occupy similar positions in successive rafts. In a second experiment, we explore the effect of brood presence and prior experience by workers on raft assembly. Since brood are placed on the raft base (Purcell et al., 2014a) we expect rafts with brood to have a different configuration than rafts with only workers. We then test for an effect of prior experience on raft assembly by removing brood for a second rafting trial. Together, these experiments describe individual behavioural variation in the formation of self-assemblages.

Materials and Methods

Study system and apparatus

Formica selysi is a floodplain specialist living along rivers in the mountainous parts of central and southern Europe (Seifert, 2002). When the rivers flood, this species can form floating rafts in both field (Lude et al., 1999) and laboratory conditions (Purcell et al., 2014a). We collected workers and brood from 25 field colonies of *F. selysi*. All colonies come from a population located along the Rhône River, between Sierre and Susten in Valais, Switzerland (7°36'30" E, 4°18'30" N, altitude 565 m). Groups of workers from each field colony were used in only one experiment, with each worker group being subjected to two successive rafting trials.

We mimicked natural floods using the apparatus described by Purcell et al. (2014a). Briefly, we placed the ants on a raised watchglass mounted inside a plastic container and slowly increased the water level in the container. We recorded the positions of ants from above and below using two Logitech C905 webcams.

Experiment 1 - Worker specialization in rafts

We examined whether workers specialize on specific positions in raft assembly by subjecting the same groups of workers to two successive experimental floods and recording the positions of individuals in rafts. We formed 17 groups of 60 workers, with 20% of the workers marked with Lackmalstift® paint dots on the abdomen. Each marked worker had a unique combination of colours on both the ventral and dorsal faces. At least three days before the experiment, we returned the marked workers to their respective groups. We replaced marked individuals that died before the start of the experiment. After the experiment started, marked workers that died were

not replaced. When they were not participating in rafting trials, groups were kept in plastic boxes with *ad libitum* access to water and standard ant food (Meunier & Chapuisat, 2009). To initiate a rafting trial, we transferred each group to the watchglass of the apparatus and elicited raft formation by slowly increasing the water level. We considered groups to be rafting when the workers lost contact with the watchglass and were fully afloat. Three days after the first trial, we subjected the same groups to a second rafting trial.

Our analysis of worker positions in rafts began when the raft formed and began to float, and lasted for 30 minutes during each rafting bout. We recorded the amount of time spent by each focal worker at the *base*, *middle*, *top*, and *side* positions (**Figure 1**). We detected consistent inter-individual behavioural variation by comparing the proportion of time spent by workers in each of the four positions between the first and the second trials, as described in the statistical analysis section.

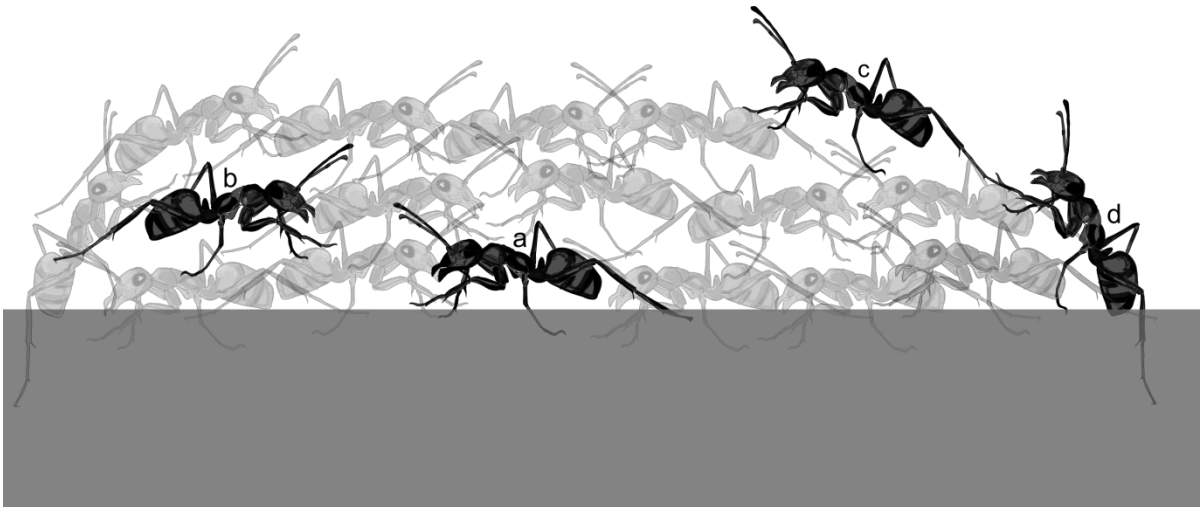

Figure 1. Schematic representation of an ant raft. Labelled workers depict base (a), middle (b), top (c), and side (d) positions.

Experiment 2 - Effects of brood presence and workers' prior experience on raft assembly

We explored the effect of brood on raft assembly. As in experiment 1, we subjected the ants to two successive floods and examined if workers show consistent positions across trials. However, in experiment 2, brood were added to the first raft trial and not to the second trial to further assess if individual position in the second trial depends on prior experience. In a previous study, we showed that brood is always placed at the bottom of the rafts in *F. selysi*, increasing the buoyancy of the rafts without any costs for brood survival (Purcell et al., 2014a). We supplemented eight groups of 60 workers with 10 nestmate pupae. We marked 20% of the workers with paint and elicited raft formation as described above. For this analysis, we scored a single rafting position for each rafting session and each marked worker. In the rare cases where the focal worker moved from one position to another during the rafting trial, we recorded the position that was occupied the longest. We assessed whether brood presence influenced raft configuration by comparing the proportion of workers in each position in rafts without brood and in rafts with brood, from the first trials of experiment 1 and 2, respectively.

To test if workers' prior experience influenced raft assembly, three days after the first rafting trial with pupae, we elicited a second raft with the same workers but without pupae. We scored the position of workers as described above. We compared the proportions of workers in each position in the first raft with brood and in the second raft without brood, from the first and second trials of experiment 2, respectively.

Statistical analysis

We used permutation tests to distinguish between the null hypothesis that there would be no relationship between the positions of individually-marked workers in the first and second flooding trial, and the alternate hypothesis, that marked workers would occupy the same position in successive rafts (robust gamma rank correlations with 10'000 permutations calculated in the R package "rococo", R project software version 3.0.0, R Development Core Team, 2015). This analysis examines whether the positions of the marked ants in the first trial are correlated with their positions in the second trial by comparing the correlation coefficient from the actual data with correlation coefficients obtained after permutating the identity of individually-marked workers. Therefore, this test accounts for unequal proportions of marked workers in each position within each experiment. To control for multiple comparisons, we adjusted the alpha with a Bonferroni correction. To evaluate the effect of brood presence on raft configuration, we compared the proportion of marked workers in each position between the first trials of experiments 1 and 2 (without or with brood, respectively) using a chi-square test on the absolute number of marked workers. Based on a previous experiment (Purcell et al., 2014a), we expected differences in the proportion of workers in each position in this initial raft. To test the effect of prior experience, we then compared the first and second trials of experiment 2 using a Wilcoxon signed rank test for paired data, in order to distinguish between our null hypothesis that the proportion of workers in each position would remain the same between trials, and our alternate hypothesis that the proportions would change in the absence of brood. We removed from our analyses marked workers that died between the two rafting trials.

Results

Experiment 1 - Worker specialization in raft assembly

Individual workers often occupied the same position in successive rafting trials. Specifically, workers that spent time on the top, middle, base or side of the first raft were significantly more likely to spend time in the same positions in the second raft (**Table 1**, **Figure 2a**). Along the same lines, workers were significantly less likely to occupy a different position in the second trial in most cases (**Table 1**). The only exception to this pattern was occupancy of the base and side positions, which were positively correlated across successive rafting trials (**Table 1**). Eighty percent of the marked workers stayed in the same position for the 30-minute rafting duration. The numbers of moves for the same individual workers were not correlated between the first and second trial (correlation coefficient $\gamma = 0.118$, $P = 0.48$), suggesting that moving within the raft is not a specialized behaviour. Together, these results point at consistent behavioural differences among workers in their positioning during self-assembly.

Table 1: Correlations between the positions of individual workers in the first and second raft (Experiment 1). Asterisks indicate significant robust gamma rank correlations with 10'000 permutations (* $P < 0.05$, ** $P < 0.01$, after Bonferroni correction). Positive and negative significant correlations are depicted in bold and italic, respectively.

Position in the second raft	Position in the first raft			
	Base	Middle	Top	Side
Base	**0.599****	*-0.526***	*-0.394**	**0.345****
Middle	*-0.545***	**0.532****	0.259	*-0.372***
Top	-0.337	-0.103	**0.377***	0.006
Side	**0.334****	*-0.364***	-0.192	**0.246***

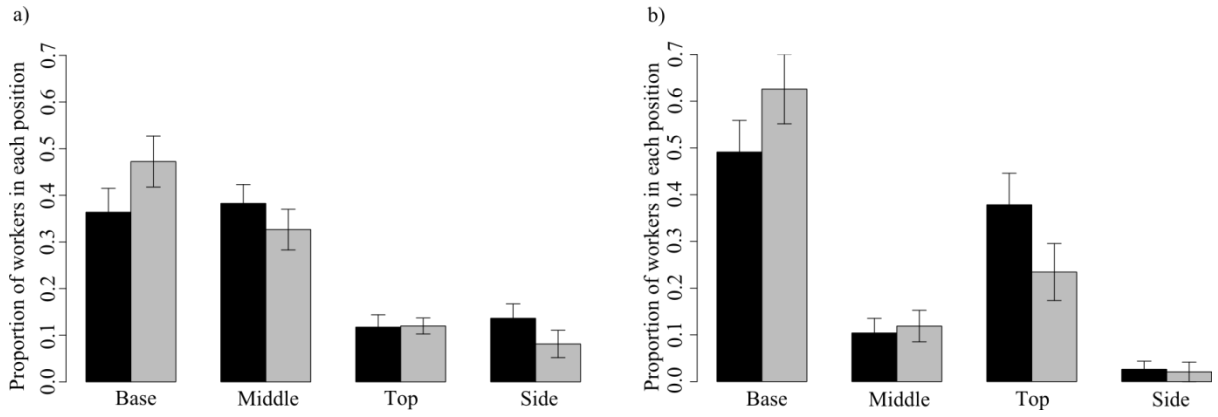

Figure 2: Proportion of workers in each position during rafting. Black bars represent first trials and grey bars second trials. Error bars indicate 95% confidence intervals. (a) Experiment 1. The groups of workers had no brood in the two successive trials (N = 17 colonies, n = 171 workers). (b) Experiment 2. The groups of workers had brood in the first trial (black bars) and no brood in the second trial (grey bars; N = eight colonies, n = 68 workers).

Experiment 2 - Effects of brood presence and workers' prior experience on raft assembly

The presence of brood influenced raft shape. Workers placed the brood at the base in all rafts, as previously documented (Purcell et al., 2014a). The configuration of the raft, measured as the proportion of marked workers in each position, differed between first rafts without brood and first rafts with brood, as observed in the first trials of experiments 1 and 2, respectively (**Figure 2**, chi-square test, $\chi^2_{(4)} = 33.52$, $P < 0.001$). Rafts with brood were flattened and contained fewer layers of workers than rafts without brood. As a result, rafts with brood had a larger proportion of workers occupying the base and top positions and a smaller proportion of workers occupying the middle and side positions, as compared to rafts without brood (**Figure 2**).

Workers' prior experience had an effect on raft assembly. Indeed, workers without brood in the second trials of the two experiments formed rafts that differed significantly in their configuration, depending on whether brood was present (second experiment) or not (first experiment) in the first

trial (**Figure 2a** and **2b**, grey bars, chi-square test, $\chi^2_{(4)} = 37.05$, $P < 0.001$). The effect of past experience with brood was strong. In the second experiment, the configuration of the second raft without brood did not differ significantly from the configuration of the first raft when brood was present (**Figure 2b**, Wilcoxon signed rank test, V = 3, $P = 1$).

Discussion

Workers often occupied the same raft positions in successive rafting trials, indicating inter-individual variation in assembly rules. Consistent inter-individual differences in decision rules have often been documented in animal societies (Jeanson & Weidenmuller, 2014). Such variation generates adaptive division of labour (Beshers & Fewell, 2001, Pruitt & Riechert, 2011) and improves collective decisions (Dussutour et al., 2009a, Jeanson et al., 2012). However, to our knowledge, specialization in the context of self-assemblages has only been demonstrated previously in *Eciton* ants, wherein morphologically distinct worker castes differ in their participation to bridge construction (Franks, 1985, Garnier et al., 2013). Our study is the first to demonstrate individual specialization during self-assembly in an ant species that lacks discreet morphological worker castes. Models of self-organisation consider that patterns emerge from simple behavioural rules on the basis of variation in local information (Couzin & Krause, 2003, Camazine et al., 2001). Integrating inter-individual differences in these models should improve our understanding of the formation and function of self-assemblages (Jeanson et al., 2012).

We do not yet know which factors determine the consistent position of workers in the raft. Different positions may be associated with differences in behavioural castes, age and/or body size

(Schwander et al., 2005). For example, workers on the base of the raft with brood may be nurses, while workers on top may be foragers. Foragers have a slightly larger body size than nurses in *F. selysi* (Schwander et al., 2005), and worker body size might also affect their placement in the raft. Along the same lines, the position of workers in bee swarms depended on their age, with younger bees tending to occupy the core of the swarm and older bees the mantle (Cully & Seeley, 2004). Consistencies in worker positions may also result from different personalities among workers. Personality, i.e. consistent behavioural strategies through time and situations, has been documented across families of social insects (Jandt et al., 2013). In addition, behavioural group composition was shown to influence collective behaviour in ants (Cronin, 2015, Hui & Pinter-Wollman, 2014, Modlmeier et al., 2014) and social spiders (Pruitt & Riechert, 2011). Further experiments involving different behavioural contexts are needed to determine whether consistency in worker position reflects individual personality, and whether variation in behavioural group composition affects raft formation.

The presence of brood influenced raft geometry and the positions of workers in the raft. Purcell et al. (2014a) demonstrated that brood was always placed on the base of the rafts. Larvae and pupae improved the buoyancy of the rafts, and brood survival was not impaired by rafting (Purcell et al., 2014a). Pupae and larvae are large items that have to be linked together by a number of workers at the base of the raft. These physical properties result in a flatter raft with a larger footprint. When brood is absent, rafts contain more layers and fewer individuals form the base. Therefore, having brood in the group constrains raft shape and workers' choice of positions, resulting in distinct raft configurations.

The presence of brood in a raft also influenced the configuration of subsequent rafts without brood. Specifically, the configuration of the second rafts, without brood, differed significantly between groups that had previously rafted with or without brood. Moreover, groups that rafted with brood in the first trial and without brood in the second trial assembled in rafts with similar configurations on both occasions. This result suggests that groups of workers have a form of memory, as their prior experience influences their behaviours in subsequent self-assemblages. Past experience affects how individuals behave in future interactions in many contexts (e.g. Grüter & Farina, 2009, Liang et al., 2010, Shah et al., 2010, Schwartz et al., 2007, Ravary et al., 2007), but to our knowledge memory had not been demonstrated in self-assemblages until now.

Workers rarely moved from one position to another when rafting in our experiment. This result contrasts with the high mobility of workers observed in much larger rafts of fire ants (Adams et al., 2011). A possible explanation is that worker behaviour during raft assembly varies with group size. In small rafts, workers would position themselves according to their individual specializations. When the number of workers reaches a certain threshold, workers would position themselves more randomly, ensuring a quick growth of the raft. In line with this argument, the behaviour of fire ant workers changed from linear to diffusive motion when the raft size increased (Mlot et al., 2012). In addition, an effect of group size on task specialization has been documented in multiple contexts. For instance, the complexity of the array of tasks performed increases at larger colony sizes in attine ants (Ferguson-Gow et al., 2014) and in *Pogonomyrmex californicus* (Holbrook et al., 2011). Moreover, the proportion of workers involved in different foraging tasks varies with colony size in *Lasius niger* (Mailleux et al., 2003). Further experiments involving variable raft size are needed to investigate whether behavioural rules during raft construction vary along with group size.

Together, these results shed light on the importance of inter-individual variation in collective behaviour. We demonstrated that workers assemble rafts according to their individual specialized position and past experience. The origin and adaptive value of individual variation in self-assemblages deserves further investigation.

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