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Review Article

Supergenes as drivers of ant evolution

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Abstract

Ants show striking diversity in social organization, raising major questions on the proximate and ultimate causes of such variation. The shift from one-queen (= monogyne) societies to multi-queen (= polygyne) societies has long been viewed as a phenotypically plastic response to ecological and social conditions. In contrast to this view, in five independent ant lineages, alternative forms of colony social organization are controlled by supergenes. Supergenes are large groups of linked genes determining compound adaptive phenotypes, like colour morphs, ecotypes, or social forms. In the best-studied socially polymorphic ant species, *Solenopsis invicta* and *Formica selysi*, a supergene variant (= haplotype) is exclusively found in multi-queen colonies and does not recombine with the alternative haplotype. How did such supergenes spread? Supergenes might be favoured by natural selection because they link co-adapted alleles that are beneficial in one social form. The absence of recombination ensures that these alleles are transmitted together and prevents maladaptive combinations between alleles. However, supergenes can also spread selfishly, by distorting Mendelian transmission in their favour. Non-recombining regions are indeed prone to harbour selfish genetic elements, which are typically formed by tight linkage of a killer gene, or toxin, and a rescue gene, or antidote. Strikingly, the social supergene haplotypes associated with multi-queen colonies of ants selfishly favour their own transmission by causing gene drive through distinct mechanisms. In *S. invicta*, the "polygyne" haplotype causes a green-beard effect: Workers that carry this haplotype kill queens that lack it. In *F. selysi*, the "polygyne" haplotype is a maternal-effect killer: Offspring of mothers that carry this selfish haplotype fail to hatch when they do not inherit a copy of the haplotype. Overall, the ants' social supergenes induce unexpected combinations of adaptive and detrimental effects across levels of biological organization. On the one hand, they coordinate individual and colony-level traits, such as queen number, mating system, and dispersal. On the other hand, supergenes cause selfish gene drive and lethal effects due to accumulation of deleterious mutations. The evolutionary dynamics of these large groups of linked genes is therefore particularly complex, and explaining their long-term maintenance is challenging. I argue that social supergenes can be drivers of ant evolution because they spread selfishly and show lethal effects. Therefore, an understanding of the unusual properties of the underlying genetic system is needed to explain variation in colony queen number.

Key words: Social polymorphism, social structure, queen number, chromosomal inversions, behavioural genetics, genomics, Hymenoptera, Formicidae, review.

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Overview

The number of breeders in ant societies was traditionally considered a phenotypically plastic trait. Genomic studies have overturned this view: In five independent ant lineages, intra-specific variation in colony social organization is controlled by **supergenes** (Box 1 – glossary). This means that whether a single or multiple queens reproduce in each colony depends on a **genetic polymorphism** at a large group of linked genes. In the first part of the review, I present a personal view of the history of the field, from early enzymatic studies of fire ants to recent genome-wide association studies. After reviewing the two best studied socially polymorphic ant species, *Solenopsis invicta* and *Formica selysi*, I present novel findings in other ant lineages, and speculate on how widespread supergenes may be. Some of the more technical aspects and challenges of investigating ant social supergenes are covered in boxes.

In the second part of the review, I discuss how social supergenes evolve and why understanding the mechanisms by which they are maintained is challenging. A striking novel finding is that the ant supergenes have not only

Box 1: Glossary.

Alternative social forms: discrete phenotypic variation in social organization, occurring within the same species. In ants, the "monogyne form" has a single functional queen per colony, while the "polygyne form" has multiple queens reproducing in each colony. Alternative social forms differ in a suite of morphological, life-history, and behavioural traits, including queen body size, colony size, colony sex allocation, queen dispersal, and worker discrimination. Also referred to as variation in social organization or social structure.

Gene drive: a phenomenon whereby a genetic element biases inheritance in its favour. The "driven" element increases in frequency in the population until fixation (at which point drive ceases) or until it reaches a polymorphic equilibrium. See also Selfish genetic elements, this box.

Genetic polymorphism: genetically determined phenotypic variation. Sometimes used to refer to any type of genetic variation, irrespective of phenotypic effects.

Haplotype: a block of linked genes that are co-inherited. Supergenes have large haplotypes, due to an absence of recombination over an extended segment of a chromosome.

Inversion: a structural mutation occurring when a chromosome segment breaks and is reinserted in the inverted orientation.

Overdominance: a condition in which the fitness of heterozygotes is higher than that of homozygotes. Also described as heterozygote advantage. Associative overdominance occurs when the heterozygote advantage at neutral loci is due to selection on linked loci, for example when linked loci harbour recessive deleterious mutations.

Phenotypic plasticity: phenotypic variation due to environmental or social factors. Because of phenotypic plasticity, the same genotype produces various phenotypes in different environments, which constitute the "reaction norm" of the genotype.

Selfish genetic elements: elements that distort the laws of Mendelian transmission in their favour. They cause gene drive, either by being transmitted to a higher than 1:1 proportion of gametes (= meiotic drive) or by reducing the fitness of alternative genotypes at later developmental stage (= post-segregation distortion; includes toxin-antidote systems and green-beard systems).

Single-nucleotide polymorphism (SNP): DNA variation at a single nucleotide in the genome sequence (= point mutation).

Supergene: a group of tightly linked genes controlling discrete multi-trait phenotypes. Recombination between alternative supergene haplotypes is suppressed, usually because of inversions. The genetic polymorphism is maintained by balancing selection.

captured co-adapted loci controlling **alternative social forms**, but they also harbour **selfish genetic elements**. The mutant supergenes that contain inversions and underlie multi-queen colonies selfishly favour their own transmission by causing a green-beard effect in *Solenopsis invicta* and maternal-effect killing in *Formica selysi*. Such selfish **gene drive** strongly affects the dynamics of the genetic system. In particular, a recent model shows that the selfish behaviour of the *F. selysi* supergene destabilizes the polymorphism. I conclude that supergenes play an active role in driving ant evolution because their unconventional genetic properties influence the spread and maintenance of alternative forms of social organization.

Social organization in ants: an amazing diversity

Ants exhibit a remarkable diversity in social organization. Across species, the median number of individuals per social group ranges from only nine in *Thaumatomyrmex atrox*, a specialist hunter of millipedes, to over 22,000,000 in the army ant, *Dorylus wilverthi* (DORNHAUS & al. 2012). The number of breeders per group also varies greatly: from two (a female and her mate) to several thousand per nest, and many more when considering supercolonies that comprise numerous nests (HÖLLDOBLER & WILSON 1990, CROZIER & PAMILO 1996, HELANTERÄ 2022). This variation in social organization appears puzzling at first, because reproductive altruism by social-insect workers relies on kin selection (HAMILTON 1964a), and the presence of multiple breeders decreases the degree of relatedness between workers and the brood they rear.

In the social Hymenoptera (ants, bees, and wasps), the ancestral colony type in which reproductive altruism and eusociality evolved consisted of one singly-mated reproductive female and her daughters (HUGHES & al. 2008a, b, BOOMSMA & GAWNE 2018). Life-long monogamy means high intra-colony relatedness, which facilitated the evolution of reproductive altruism by kin selection (BOOMSMA 2009). Today, in about half of the ant species, the basic social unit still consists of simple families, typically a single, once-mated queen and her offspring (BOURKE & FRANKS 1995). Ant societies with multiple breeders in the same colony are common, but evolutionarily derived. These more complex societies evolved after reproductive division of labour between the queen and worker caste had been firmly established.

The shifts to polyandry (multiple mating by the queen) and polygyny (multiple queens in the same colony) require special explanations that combine kin selection with ecological and social benefits (Hamilton 1964b, 1972, Bourke & Franks 1995, Keller 1995, Crozier & Pamilo 1996). Polyandry and polygyny are associated with multiple potential benefits, including higher colony genetic diversity and larger colony size (Schmid-Hempel & Crozier 1999, Oldroyd & Fewell 2007, Hughes & al. 2008b, Helanterä & al. 2009, Bourke 2011, Wiernasz & Cole 2022). Polygyny is also associated with alternative dispersal: Queens have the option to stay in their mother nest or disperse with workers to establish novel colonies, which provides a competitive advantage in harsh or saturated habitats (Nonacs 1988, Keller 1995, Pedersen & Boomsma 1999). Such ecological and demographic benefits, coupled to constraints restraining worker reproduction, likely contribute to explaining the evolution of polygyny in ants (Helanterä & al. 2009, Bourke 2011). Yet, the long-term persistence of worker reproductive altruism in societies with very high queen number and near-zero relatedness remains puzzling (Chapuisat & al. 1997, Queller & Strassmann 1998, Helanterä 2022). More generally, understanding the proximate and ultimate causes of variation in colony queen number is an interesting evolutionary question because polygyny is a group-level trait resulting from behavioural interactions among multiple individuals (e.g., queen dispersal, worker acceptance), and the benefits of polygyny are not obvious.

Social organization: a phenotypically plastic trait?

Variation in colony queen number has long been thought to result from **phenotypic plasticity** (Box 1 – glossary), with colonies recruiting additional queens in response to harsh ecological conditions and increased intraspecific competition (Herbers 1993, Bourke & Franks 1995, Schradin & al. 2018). The hypothesis that colony queen number is phenotypically plastic is simple and attractive. In many social species, group membership is indeed optional – individuals can join or leave at will, depending on breeding opportunities. More generally, many behavioural traits are condition-dependent and follow rules like "in case A, do X, in case B, do Y". Cost-benefit models at the core of behavioural ecology predict that social traits depend on decision rules that maximize inclusive fitness according to the environmental and social context. In line with this view, the "habitat saturation hypothesis" proposed that queen number depended on nest-site availability (Herbers 1993, Pedersen & Boomsma 1999). In short, monogyne colonies are selected to recruit daughter queens as additional breeders when intraspecific competition is high and opportunities for independent colony founding are restricted (Nonacs 1988, Keller 1995).

Hence, for many years, colony social organization was by default considered to be flexible and condition-dependent. Even in species where social organization was later found to be under strict genetic control, such as the fire ant, *Solenopsis invicta,* and the Alpine silver ant, *Formica selysi*, early reports discussed "phenotypic plasticity and cultural transmission" (Keller & Ross 1993a) or "shifts in colony social organization" (Purcell & Chapuisat 2013). The idea that social organization is phenotypically plastic is also reflected in the concept of "facultative polygyny", which applies to species where most colonies are monogynous, while some colonies re-adopt mated daughter queens back into existing nests (e.g., Boomsma & al. 2014). Note that this terminology becomes misleading if variation in colony social organization is due to a **genetic polymorphism**. To illustrate this point, eye colour is not facultative.

As with most behavioural traits, variation in colony queen number might be phenotypically plastic, influenced by genetic polymorphisms, or the result of gene-environment interactions. Across species, factors influencing social organization might fall on a continuum from complete environmental determination to complete genetic determination, with any intermediate combinations. To understand the evolution of social organization in ants, one needs to examine if there are genetic polymorphisms influencing colony queen number, and not just assume by default that the trait is plastic or facultative.

The fire ant magic well

"*The bee's life is like a magic well: the more you draw from it, the more it fills with water"*

Karl von Frisch, in Bees: Their vision, chemical senses and language (first published 1950)

The fire ant's life is another magic well. While screening enzyme variants for population genetic studies in *Solenopsis invicta*, Ken Ross discovered a genetic marker associated with colony social organization (Ross 1992). The enzyme phosphoglucomutase-3 (*pgm-3*) had two alleles, and genotype frequencies deviated strongly from Hardy-Weinberg expectations in the polygyne form. Strikingly, *pgm3-aa* was completely absent from polygyne queens, while it was the most common genotype in monogyne queens (Ross 1992). Ten other polymorphic enzymes showed no significant deviation from Hardy-Weinberg equilibrium. Ken Ross discussed the effect of selection on this single genetic element regulating reproductive competition.

I was starting my PhD at that time, and this result struck me as a tour de force, and an exceptionally lucky finding. Allozymes are few – a survey of allozyme variation did not allow for a proper genome screening but a mere picking (Box 2 – the rise of genomics). Finding an association between an allozyme and an interesting social phenotype seemed improbable. Moreover, most researchers would have simply discarded a marker behaving strangely, focussing instead on boring, neutral markers (Keller 2007). Ken Ross did not. Instead, he tapped into the magic well.

Laurent Keller, who was then a post-doctoral researcher at Harvard University, has a special gift for detecting and interpreting unconventional genetic data (Keller 2007). So, Laurent Keller soon joined forces with Ken Ross. Together, they investigated experimentally how polygyne workers killed *pgm3-aa* queens, which are, counterintuitively, the larger and more fertile queens (Keller & Ross

Box 2: The rise of genomics.

Early genetic studies of ants were based on few markers with low genetic variation. Typically, up to 100 enzymes could be screened by protein electrophoresis, of which only a handful were polymorphic (e.g., Pamilo & al. 1978, Shoemaker & al. 1992). These allozyme markers were widely used from the 60s to the 90s, which provided important insights into social structure and population genetic structure. Protein electrophoresis also led to the discovery of the first genetic markers associated with social organization, in the fire ant, *Solenopsis invicta* (see section below, The fire ant magic well). Over the years, the fire ant research has provided a wealth of major findings on the genetic basis of social traits (reviewed in Ross & Keller 1995a, Gotzek & Ross 2007, 2009). Yet, because the first markers detected in *S. invicta* were not associated with social organization in other ant genera, the fire ant was considered an exception.

From the 1990s onwards, the next generation of genetic markers were microsatellites (Estoup & al. 1993, Queller & al. 1993). These short tandem repeats, which can be amplified by PCR, became the workhorses for socio-genetic research on ants. They were more polymorphic than allozymes and provided detailed information on colony queen number, mate number, intra-colony genetic relatedness, ploidy, dispersal patterns, and population genetic structure. But the low number of markers used, typically 5 - 20, did not allow for a proper search of the genetic basis of traits.

Scanning genomes to detect genetic variation associated with traits became feasible with high-throughput sequencing technologies. Typically, restriction-site associated DNA sequencing provides sequence information on a fraction of the genome, yielding thousands of genetic markers (Parchman & al. 2012). With so many markers and the possibility to sequence many individuals at low cost, one can perform genome-wide association studies and population genomic studies in non-model organisms. As the output of sequencing technologies continues to rise, whole-genome sequencing will soon become standard in socio-genomic research on ants, with more reference genomes being assembled (Nygaard & Wurm 2015, Favreau & al. 2018). This is an exciting time. The power of genomics provides access to wide-ranging genetic variation and allows researchers to dissect the genetic basis of complex traits.

1993b). Population genetic studies in the introduced and native ranges confirmed that the *pgm-3* genotype was consistently associated with reproductive roles, and under strong selection in the polygyne form (Ross & al. 1996).

This was just the beginning. Ken Ross discovered a second marker, general protein 9 (*gp-9*), by performing starch gel electrophoresis and non-specific staining (Ross 1997). This second protein showed even stronger association with colony social organization. Queens in monogyne colonies have the *BB* genotype at *gp-9*, queens in polygyne colonies have the *Bb* genotype, while the *bb* genotype is usually lethal in queens (Fig. 1A). Hence, a "Mendelian element" containing *gp-9, pgm-3,* and closely linked genes, controlled social organization in fire ants.

Over three decades, the magic well served as launching base for the careers of several cohorts of talented PhD students and Postdocs (DeWayne Shoemaker, Mike Goodisman, Michael Krieger, John Wang, Yannick Wurm, Brendan Hunt, to name just a few), and provided a steady supply of exciting results. For example, gene flow between social forms is largely restricted, and mediated by monogyne males mating to polygyne queens (Shoemaker & Ross 1996). *Gp-9* encodes an odorant-binding protein, and the sequence shows signs of positive selection (Krieger & Ross 2002). In addition to inducing polygyny, the *gp-9 b* allele is associated with a "green-beard effect", whereby *b*-carrying workers recognize and exclusively accept *b*-carrying queens as additional breeders (Keller & Ross 1998, Ross & Keller 2002, Trible & Ross 2016). "Green beards" are genetic elements that code for a conspicuous cue and direct altruistic behaviour towards individuals that carry the cue (Hamilton 1964b, Dawkins 1976). Interestingly, when reflecting on the theoretical possibility of the green-beard effect, Hamilton wrote: "at simplest we need to postulate something like a **supergene** affecting (a) some perceptible feature of the organism, (b) the perception of that feature, and (c) the social response consequent upon what was perceived" (Hamilton 1964b).

Hamilton's intuition was correct: These were too many effects for a single gene! In fact, *gp-9* lies in a large non-recombining supergene (Wang & al. 2013). By genotyping male offspring of *Bb* queens at thousands of **single-nucleotide polymorphism (SNP)** markers, Wang & al. (2013) showed that recombination was suppressed in a very large genomic region (13 Mb) containing approximately 600 genes. The *Sb* supergene **haplotype**, which contains *gp-9 b*, affects colony social organization, queen phenotype, and worker acceptance of queens (Fig. 1A , Ross & Keller 1998, Gotzek & Ross 2007, Lawson & al. 2012, Wang & al. 2013, Trible & Ross 2016). Polygyne colonies contain some proportion of females that are heterozygous at the supergene (*SbSB*), while monogyne colonies exclusively contain homozygous (*SBSB*) females. The *SbSb* genotype is lethal in females, at least in the introduced population (Hallar & al. 2007). Overall, in *Solenopsis invicta*, a large "social supergene" is perfectly associated with colony social organization (Fig. 1A).

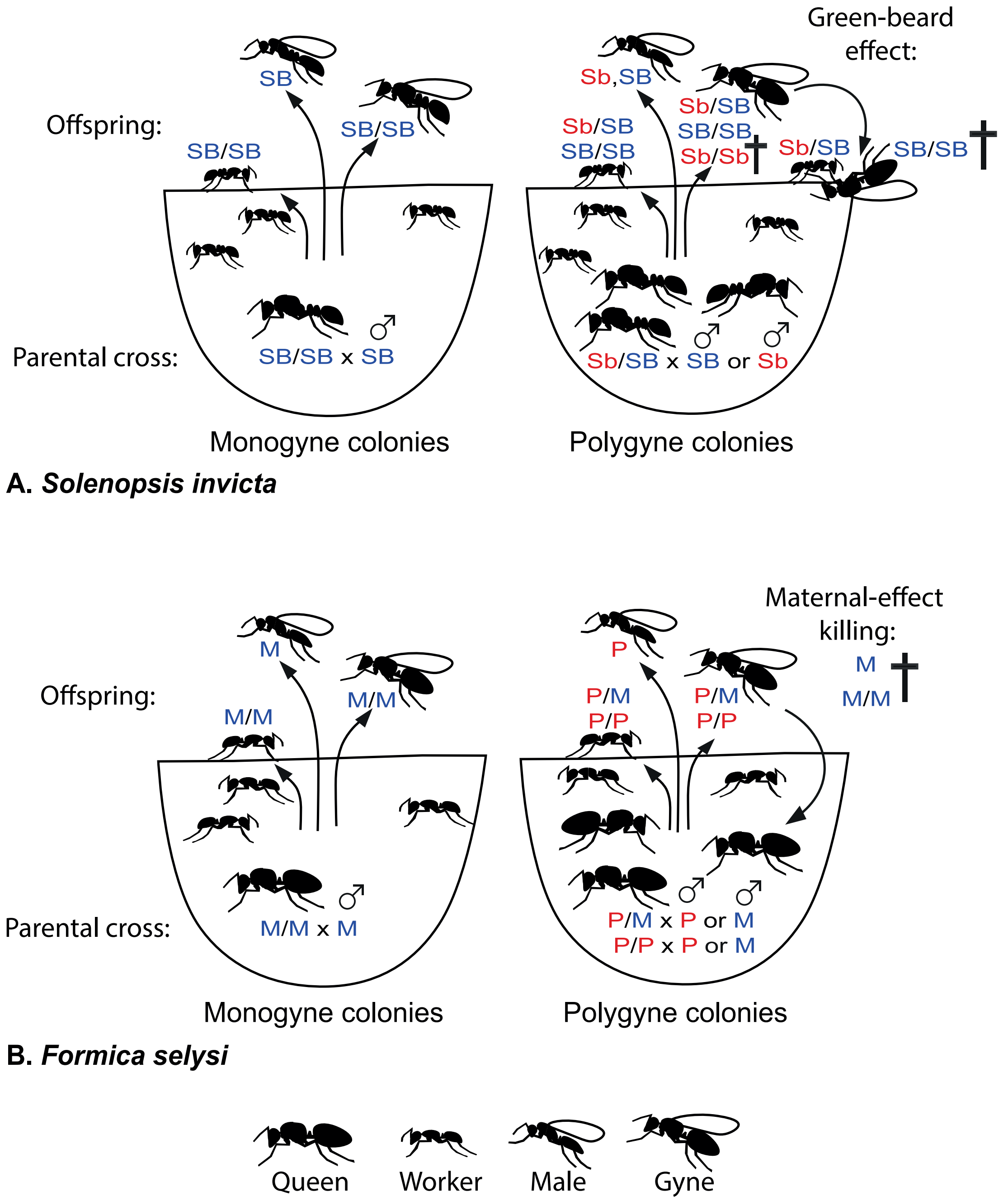

Fig. 1: Supergene control of social organization and supergene drive in the two best studied species with social supergenes, *Solenopsis invicta* (A) and *Formica selysi* (B).

(A) In the fire ant, *Solenopsis invicta*, all individuals living in monogyne colonies carry exclusively the *SB* supergene haplotype (left side). *SBSB* workers accept only one queen (their mother, also *SBSB*). The social supergene haplotype *Sb* is found in polygyne colonies – the presence of *Sb* is necessary and sufficient to make the colony polygyne (right side). The *Sb* haplotype is driven by a green-beard effect: *SbSB* workers recognize and kill *SBSB* queens but accept multiple *SbSB* queens. Hence, the supergene haplotype specific to multi-queen colonies, *Sb,* favours its own transmission because *Sb*-carrying workers kill queens that lack *Sb*. The *SbSb* genotype is usually lethal in females.

(B) In the Alpine silver ant, *Formica selysi*, all individuals living in monogyne colonies carry exclusively the *M* supergene haplotype (left side). In contrast, queens and workers established in polygyne colonies always have at least one copy of the *P* haplotype, being either *PM* or *PP* (right side). The *P* haplotype distorts Mendelian transmission ratios by maternal-effect killing: All eggs from *MP* mothers that do not inherit *P* die during development, so that no *M* male or *MM* female is produced by polygyne colonies. The supergene haplotype specific to multi-queen colonies, *P*, favours its own transmission by causing the death of all offspring that do not inherit *P*. See main text for references.

Box 3: Searching for social supergenes.

The methods to detect supergenes are well-established. However, identifying a supergene is not incidental, and one needs to perform a deliberate search. The search starts with a genome-wide association study (GWAS), whereby researchers look for statistically significant associations between thousands of genetic markers distributed across the genome and a phenotypic trait of interest (Uffelmann & al. 2021). In medical research, traits of interest are typically diseases. In ecology and evolution, any trait can be investigated. Traits can be dichotomous or quantitative.

To perform a GWAS, one needs a large sample of individuals with the phenotype of interest. In the case of ant social organization, the best strategy is to collect haploid males produced by independent monogyne and polygyne colonies. Restriction-site associated DNA sequencing (RAD-sequencing) or whole-genome sequencing of these males provides tens to hundreds of thousands of single-nucleotide polymorphisms (SNPs). One then looks for robust associations between these genetic variants and the social organization of colonies.

The next step is to examine if these SNP markers associated with social organization are linked. If a high-quality genome assembly is available, one can simply map the markers on the assembled genome, and examine if the markers that are differentiated between the monogyne and polygyne forms cluster on the same chromosome (e.g., Gutiérrez-Valencia & al. 2021). With steadily decreasing costs and improving methods for long-read sequencing and *de novo* genome assembly, this direct approach shall become widely available. The male-haploid, female-diploid sex determination of the social Hymenoptera offers a major advantage for haplotype identification. Indeed, male genotyping provides phased **haplotypes**, which greatly facilitates the identification and study of supergenes. To reach a chromosome-level assembly and examine if linked SNPs are associated with alternative social organization (revealing a social supergene haplotype), one can also construct a recombination map by genotyping male offspring from a single queen, which returns direct estimates of recombination. Moreover, if heterozygous queens are available, researchers can confirm the absence of recombination between the "monogyne" and "polygyne" haplotypes, by genotyping numerous offspring from these queens. This powerful approach has been used to identify supergene haplotypes associated with alternative social organization in fire ants and silver ants (Wang & al. 2013, Purcell & al. 2014).

Once the supergene has been characterized, one can design cheap and quick assays to distinguish diagnostic markers for each haplotype (SNPs that have fixed alternative alleles on each haplotype; e.g., Purcell & al. 2014, Fontcuberta & al. 2021). Using quantitative polymerase chain reaction (qPCR) or polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays, researchers can then infer the supergene genotype across many individuals coming from monogyne and polygyne colonies, to confirm that the supergene is associated with alternative social organization.

In summary, to detect supergenes, researchers need to collect appropriate samples of an interesting target species, prior to genome sequencing and bioinformatic analyses. Large enough numbers of individuals showing alternative phenotypes are needed, and their sequences must be mapped onto an assembled genome, or used to construct a recombination map. Once these logistic constraints have been overcome, the search for supergenes is relatively straightforward.

In retrospect, the original discovery by Ken Ross was not sheer luck. About half a chromosome, representing more than 2.2% of the entire fire ant genome, belongs to the social supergene and is associated with social organization. Therefore, when Ken Ross carefully screened for many markers in the early nineties, the odds were that one of these markers would be linked to social organization!

An independent social supergene in *Formica selysi*

When I started to investigate the social organization of Alpine silver ants, *F. selysi*, at the turn of the millennium, my working hypothesis was that colony queen number was a phenotypically plastic trait. Like *Solenopsis invicta, F. selysi* has **alternative social forms**, one with monogyne colonies in which a single queen monopolizes reproduction, and the other forming polygyne colonies in which multiple queens share reproduction (Chapuisat & al. 2004, Purcell & Chapuisat 2013). *Formica selysi* colonies are found in open grassy, sandy and stony areas by rivers in the Alpine region, in mosaic habitats (Fig. 2, Zahnd & al. 2021, Fontcuberta & al. 2022). Laboratory experiments suggested that monogyne colonies could become polygyne by accepting additional queens (Meunier & al. 2011). Conversely, polygyne colonies might become monogyne by losing queens (Purcell & Chapuisat 2013) or might give rise to monogyne colonies by producing queens that establish novel colonies independently (Reber & al. 2010). The fact that queens and males originating from polygyne and monogyne colonies interbred successfully in the laboratory (Reber & al. 2010), coupled with the absence of genetic differentiation between monogyne and polygyne individuals at microsatellite markers (Chapuisat & al. 2004, Purcell & Chapuisat 2013, Purcell & al.

Fig. 2: The Alpine silver ant, *Formica selysi*, is a pioneer species occupying riverine habitat in mountainous regions. *Formica selysi* thrives in sand banks, rocky plains, islands, and steppes by big rivers and alpine torrents. The patchy and transient habitat might favour alternative dispersal strategies. Photo credit: Timothée Brütsch (A), Michel Chapuisat (B).

2014, 2015), further indicated that the two social forms belonged to the same gene pool.

My early view was naïve. A lack of genetic differentiation between social forms at a moderate number of genetic markers is no proof that social organization is determined by the environment. Indeed, if a genetic polymorphism influences a trait, one does not expect genetic differentiation at most of the genome, but only at the causal gene(s) and closely linked loci. Similarly, correlations between environmental conditions and social organization do not mean that the trait is plastic, as such association may be caused by selection for genes underlying the trait. Finally, a cross-fostering experiment revealed that queen size was heritable and differed between social forms, which suggested that a genetic polymorphism affected colony social organization (Meunier & Chapuisat 2009).

A targeted search (Box 3 – searching for social supergenes) revealed that a large **supergene** controls alternative colony social organization in the Alpine silver ant (Purcell & al. 2014). Like in *Solenopsis invicta*, one supergene haplotype, *P*, is exclusively found in polygyne colonies (Fig. 1B). The presence of this haplotype is necessary and sufficient to induce a shift in social organization. However, the *Formica selysi* supergene shows no synteny and no homology in gene content with the *S. invicta* supergene (Purcell & al. 2014). Hence, each supergene evolved independently, and their similar supergene architecture points at a fundamental role of recombination suppression in driving the evolution of polygyny.

The discovery that social supergenes control intraspecific social polymorphism in independent ant lineages overturned the widespread belief that social organization was phenotypically plastic. In such systems, variation in colony social structure (i.e., belonging to the monogyne or polygyne social form) is due to a **genetic polymorphism** – like eye-colour in humans, or sex in mammals. This strict genetic control of social organization raises novel questions on the origin, evolution, and maintenance of genetic variation underlying social traits. In short, the research questions shifted from seeking ecological or social factors that flexibly influence colony queen number to understanding how the genetic polymorphism is balanced, and how alternative phenotypes are produced.

More social supergenes are being discovered

Recent and ongoing research revealed that **supergenes** control alternative social organization in three other ant lineages (Tab. 1). Hence, "social supergenes" – groups of linked loci associated with intraspecific variation in social organization – have been discovered successively in the fire ant, *Solenopsis invicta* (Fig. 1A, Box 1, Wang & al. 2013), the Alpine silver ant, *Formica selysi* (Fig. 1B, Purcell & al. 2014), *Leptothorax acervorum* (Braim 2015, John 2022), *Pogonomyrmex californicus* (Errbii & al. 2022), and *Cataglyphis niger* (Lajmi & al. 2022, Tab. 1).

The five independent social supergenes (Tab. 1) show commonalities and differences (reviewed in Kay & al. 2022). They all span large non-recombining genomic regions (8 - 12 Mb) and contain many genes (≈ 400 - 750). The *Solenopsis* supergene (Yan & al. 2020) and the *Formica* supergene (Brelsford & al. 2020) harbour three large **inversions**. The *Formica* supergene is associated with intra-population variation in social organization (monogyne and polygyne colonies occur within the same populations; Purcell & al. 2014, Avril & al. 2019, Zahnd & al. 2021), while the *Solenopsis, Leptothorax,* and *Pogonomyrmex* supergenes occur in separate monogyne and polygyne populations (Gotzek & Ross 2007, Braim 2015, Errbii & al. 2022, John 2022). Moreover, the *Pogonomyrmex* supergene seems to act in concert with other, unlinked genomic regions (Errbii & al. 2022). The five social supergenes differ widely in their putative age. The oldest supergene likely originated in the ancestor of all *Formica* species, as much as 30 million years ago (Brelsford & al. 2020, Purcell & al. 2021). In contrast, the youngest supergene, found in *Pogonomyrmex californicus*, seems less than 200,000 years old (Errbii & al. 2022).

In fire ants, a supergene is associated with social organization in at least six species (Wang & al. 2013, Yan & al. 2020, Stolle & al. 2022). In the genus *Formica*,

Tab. 1: Supergenes in ants and bees.

Species	Phenotype	Supergene drive	Supergene shared by other species of the genus	Main references
Ants				
Solenopsis invicta	Social organization: monogyne/polygyne	Yes, green-beard effect	Yes, ≥ 6 species	(Wang & al. 2013, Trible & Ross 2016, Yan & al. 2020)
Formica selysi	Social organization: monogyne/polygyne	Yes, maternal-effect killing	Yes, ≥ 7 species	(Purcell & al. 2014, Avril & al. 2020, Brelsford & al. 2020, Purcell & al. 2021)
Formica glacialis	Colony sex allocation: male/female sex ratio	?	?	(Lagunas-Robles & al. 2021)
Leptothorax acervorum	Social organization: monogyne/polygyne	?	?	(Braim 2015, John 2022)
Pogonomyrmex californicus	Social organization: monogyne/polygyne	?	?	(Errbii & al. 2022)
Cataglyphis niger	Social organization: monogyne/polygyne	?	?	(Lajmi & al. 2022)
Bees				
Apis mellifera	Ecotype: high/low elevation	?	?	(Wallberg & al. 2017).

another supergene controls social organization in seven polymorphic species (Purcell & al. 2014, Brelsford & al. 2020, Lagunas-Robles & al. 2021, McGuire & al. 2022), and is shared by widely divergent clades within the genus (Purcell & al. 2021). Overall, it seems likely that many more, if not all, socially polymorphic ant species harbour social supergenes, which amounts to a rough estimate of 260 candidate species (Kay & al. 2022). The potential role of supergenes in controlling between-species variation in social organization (e.g., Pamilo & al. 1997, Sundström & al. 2005) is less clear. In particular, we do not know yet if supergenes explain social variation across monomorphic species that are exclusively monogyne or polygyne, respectively.

Beside colony queen number, many other complex traits involving coordinated changes in behavioural and morphological traits might also be controlled by supergenes in the social insects. Two cases have already been documented, for colony sex allocation in ants and ecotypes in honeybees (Tab. 1). *Formica glacialis* has two adjacent supergenes, one associated with colony social organization, and the other with alternative patterns of colony sex allocation (Lagunas-Robles & al. 2021). In many ant species, colonies specialize in producing either males or females (Meunier & al. 2008), and that such split sex ratios are commonly controlled by supergenes is an exciting hypothesis that deserves further investigations. In the honeybee, *Apis mellifera*, two haplotypes have been detected in high-altitude populations, suggesting that these non-recombining blocks play a role in adaptation to altitude (Wallberg & al. 2017). Supergenes might also be involved in alternative dispersal morphs (e.g., microgynes / macrogynes), alternative male morphs, genetic caste determination, social hybridogenesis, and workerless social parasites (Linksvayer & al. 2013, Darras & al. 2014, Lacy & al. 2019). Social insects are rich in interesting behaviours, colony-level coordinated traits, and unusual reproductive systems. The magic well is not about to run dry.

The evolution of supergenes

Supergenes are large groups of linked genes that underlie compound adaptive alternative phenotypes (Schwander & al. 2014, Thompson & Jiggins 2014). Supergenes are widespread across the tree of life and control spectacular phenotypic variations, such as mimetic colour morphs, mating morphs, ecotypes, or sexes (Lowry & Willis 2010, Joron & al. 2011, Charlesworth 2016, Küpper & al. 2016, Lamichhaney & al. 2016, Tuttle & al. 2016, Llaurens & al. 2017, Gutiérrez-Valencia & al. 2021). Supergenes are characterized by the absence of recombination between alternative haplotypes. Explaining the origin of supergenes, and how recombination has been suppressed between loci controlling compound, co-adapted traits, is challenging.

Supergenes usually originate from one or several chromosomal inversions (Kirkpatrick 2010, Llaurens & al. 2017, Faria & al. 2019, Durmaz & al. 2020). When a chromosomal segment is broken and re-inserted in the reverse orientation, a novel **haplotype** is formed. Recombination between the ancestral haplotype and the mutant inverted haplotype is completely suppressed, except for very rare cases of double cross-over or gene conversion (Kirkpatrick 2010, Gutiérrez-Valencia & al. 2021). Therefore, the non-recombining supergene haplotype becomes inherited as a single Mendelian element and follows its own evolutionary trajectory. Apart from inversions, recombination can also be suppressed by other types of structural changes (duplications, deletions) and by epigenetic mechanisms (Sun & al. 2017, Gutiérrez-Valencia & al. 2021, Kim & al. 2022). Once inversion(s) or other mechanisms have locked neighbouring genes together in one haplotype, two questions arise: (1) What causes the rare non-recombining haplotype to spread in the popula-

Box 4: The power of comparative genomics.

Cross-species comparisons of whole-genome sequences provide a prime tool to study the origin, evolution, and maintenance of supergenes (Brelsford & al. 2020, Hunt 2020, Yan & al. 2020). Social supergenes evolved independently in several ant lineages (Purcell & al. 2014, Tab. 1), and are shared among multiple species within each of these lineages (Brelsford & al. 2020, Yan & al. 2020, Purcell & al. 2021). This is a powerful setup for phylogenomic analyses, which can reveal when the supergene arose in ancestors of existing ant lineages, whether it has been conserved in socially polymorphic species through multiple speciation events, and whether it has crossed species boundaries by introgression.

The *Formica* supergene is very ancient. It controls polymorphism in at least seven socially polymorphic *Formica* species separated by 20 - 40 million years of independent evolution (Brelsford & al. 2020, Lagunas-Robles & al. 2021, McGuire & al. 2022). Moreover, the supergene is widely shared among many *Formica* species from divergent lineages (Brelsford & al. 2020, Purcell & al. 2021). Hence, the ancestral *Formica* supergene has been conserved throughout the *Formica* phylogeny, through multiple speciation events. Although the *Formica* supergene itself is very large and spans hundreds of genes, only a small set of highly conserved trans-species **single nucleotide polymorphisms** (SNPs) associated with alternative social organization are shared by most species, a clear sign of strong balancing selection (Brelsford & al. 2020, Purcell & al. 2021). These conserved trans-species polymorphisms cluster in small disjunct regions distributed across the supergene (Brelsford & al. 2020). This unusual pattern of genetic diversity suggests that rare recombination (double cross-over) or gene conversion events have eroded differentiation between haplotypes in 99% of this ancient supergene, leaving only small, highly differentiated regions where natural selection has conserved polymorphisms across species (Brelsford & al. 2020). Importantly, these conserved polymorphisms point at candidate genes controlling social organization (Brelsford & al. 2020, Purcell & al. 2021). Strikingly, 22 conserved SNPs are in the gene *Knockout*, a transcription factor involved in motor neuron development in *Drosophila*.

Another supergene is shared by six species belonging to the genus *Solenopsis* (Yan & al. 2020). The six species harbour the same three inversions, which have been acquired sequentially, less than one million years ago (Cohen & Privman 2020, Yan & al. 2020, Helleu & al. 2022, Stolle & al. 2022). Importantly, the fire ant supergene evolved recently in one species and then repeatedly crossed species boundaries (Helleu & al. 2022, Stolle & al. 2022). Such adaptive introgression of supergenes has also been documented in butterflies (Jay & al. 2018, Edelman & al. 2019). The evolution of supergenes by introgression can explain one of the most intriguing features of supergenes, namely how coadapted alleles became linked. Indeed, alternative haplotypes would first evolve in separate populations or species, progressively accumulating diverging, co-adapted alleles, as well as structural variations preventing recombination. When two species hybridize, a haplotype that is already packed with co-adapted alleles can spread to another species through introgression.

By comparing alternative supergene haplotypes within the same species, one can further decipher the evolutionary fate of non-recombining regions. In *Solenopsis invicta*, the *Sb* haplotype, associated with multi-queen colonies, has reduced genetic diversity but has accumulated allelic differences at odour-binding proteins (Pracana & al. 2017a, b). The standard model for sex-chromosome evolution is that non-recombining chromosomes degenerate and shrink (e.g., the y-chromosome in mammals, Bachtrog 2013). In contrast, the non-recombining haplotypes in social supergenes expanded in size, by accumulating transposable elements and gene duplicates (Stolle & al. 2018, Fontana & al. 2020, Purcell & al. 2021). Genes with multiple copies on the *Sb* haplotype of *S. invicta* include enzymes involved in cuticular hydrocarbon synthesis, as well as, again, odour-binding proteins (Stolle & al. 2018, Fontana & al. 2020).

tion? and (2) What prevents each haplotype from reaching fixation, that is, what maintains the polymorphism? Below, I discuss the two questions in turn.

How do supergenes spread?

Co-adapted gene complex

The original, adaptive scenario for the evolution of supergenes is that the mutant haplotype captured alleles that are co-adapted (Dobzhansky 1970, Schwander & al. 2014, Thompson & Jiggins 2014, Durmaz & al. 2020, Gutiérrez-Valencia & al. 2021). If multiple alternative alleles are more favourable in one morph than the other (i.e., there is antagonistic selection depending on ecotype, social form, sex, etc.), recombination suppression prevents the formation of maladaptive intermediates, and the mutant haplotype spreads by natural selection. For sex chromosomes, the standard model is that selection linked a sex determining locus under balancing selection to a sexually antagonistic allele (Charlesworth 2016, Gutiérrez-Valencia & al. 2021). Selection may then progressively suppress recombination between more loci controlling antagonistic phenotypes, so that the supergene increases in size. A role of antagonistic selection in the evolution and maintenance of supergenes is attractive conceptually, and has been documented in several cases, like mimetic butterflies (Joron & al. 2011, Chouteau & al. 2017, Jay & al. 2022) and sex chromosomes in guppies (Wright & al. 2017).

Selection for linked co-adapted alleles likely favoured the evolution of supergenes controlling social forms in ants because the social forms differ in multiple integrated phenotypic traits. In the two best studied socially polymorphic ants, *Solenopsis invicta* and *Formica selysi*, the monogyne and polygyne social forms differ in many independent morphological, behavioural, and life-history traits (Ross & Keller 1995a, Rosset & Chapuisat 2007, Kay & al. 2022). For *F. selysi*, these traits include queen and worker body size (Schwander & al. 2005, Rosset & Chapuisat 2007, Meunier & Chapuisat 2009), colony size (Rosset & Chapuisat 2007), colony density (Rosset & Chapuisat 2007, Zahnd & al. 2021), colony sex allocation, and dispersal (Rosset & Chapuisat 2006, Fontcuberta & al. 2021). The non-recombining regions of the *S. invicta* and *F. selysi* supergenes therefore seem to fit the original definition of supergenes as they likely comprise numerous linked loci that control discrete phenotypes differing in multiple co-adapted traits (Dobzhansky 1970, Thompson & Jiggins 2014). Yet, the regulatory elements or genes controlling social organization or antagonistic traits remain poorly understood (Brelsford & al. 2020, Martinez-Ruiz & al. 2020, Purcell & al. 2021). Overall, the hypothesis that the ant social supergenes captured co-adapted alleles with antagonistic effects in alternative social forms needs further testing.

Selfish drive

Another, non-mutually exclusive hypothesis is that non-recombining haplotypes spread selfishly. **Gene drive** arises when a distorter locus, or killer, becomes linked to an insensitive allele at a responder locus, or rescuer (Burt & Trivers 2006, Werren 2011). The two loci must be co-inherited to spread in the population: If unlinked to the rescuer, the killer would become a suicidal gene that wipes itself out of the population. The loss of recombination permanently links the killer to the rescuer. For this very reason, non-recombining supergene haplotypes tend to accumulate **selfish genetic elements** that distort the laws of Mendelian inheritance (Avril & al. 2020). The supergene haplotype then hitchhikes along the selfish genetic element, which spreads in the population through gene drive.

Mechanistically, transmission-ratio distortion can be caused by meiotic drive, whereby one allele is transmitted to more gametes than expected, or by various post-segregation distorters, such as green-beards and maternal-effect killers (Burt & Trivers 2006, Werren 2011). Examples of driving supergenes include the *t*-haplotype in mice (Lyon 2003), the *Segregation Distorter* supergene in fruit flies (Wong & Holman 2020) and a centromere causing female meiotic drive in *Mimulus guttatus* (Finseth & al. 2022).

It is striking that the two best studied social supergenes in ants selfishly distort transmission ratios in their favour, through different mechanisms (Fig. 1). In fire ants, the *Sb* haplotype associated with multi-queen colonies causes a green-beard effect (Fig. 1A, Keller & Ross 1998, Ross & Keller 2002, Huang & Wang 2014, Trible & Ross 2016). Specifically, *SbSB* workers kill *SBSB* queens but accept *SbSB* queens as supernumerary breeders. Hence, *Sb*-carrying workers recognize and only accept queens that carry *Sb,* while they execute queens that lack it. This behaviour depends on a threshold proportion of *SbSB* workers in the social group (Ross & Keller 2002) and on the odour of queens (Trible & Ross 2016, Zeng & al. 2022). The green-beard effect has important consequences: It favours the spread of *Sb* and contributes to the association between supergene genotype and colony social organization (monogyne colonies lack *Sb*, while polygyne colonies possess it). On top of the green-beard effect, the *Sb* supergene haplotype is also associated with unusual transmission ratios (Ross & Shoemaker 2018). In single-queen progenies, *Sb* deviates significantly from the expected Mendelian proportion, in both directions. This over-dispersed transmission pattern shows no clear drive, and likely reflects a stalemate situation between multiple meiotic drivers and suppressors.

In Alpine silver ants, the *P* haplotype of the social supergene acts as a maternal-effect killer – a selfish genetic element that distorts the laws of Mendelian inheritance by causing the death of progeny that did not inherit this element (Fig. 1B, Avril & al. 2020). Our detailed analyses of colony social structure documented that all ants in monogyne colonies have the *MM* (diploid females) or *M* (haploid males) genotype. In contrast, all ants produced by polygyne colonies carry at least one *P* haplotype: Queens and workers have the *PP* and *PM* genotypes, and males the *P* haplotype (Fig. 1B, Purcell & al. 2014, Avril & al. 2019, 2020, Fontcuberta & al. 2021). The complete absence of *MM* females and *M* males was surprising as 51.3% of the queens breeding in polygyne colonies were heterozygous at the supergene, and 22.7% of these *MP* queens had mated with *M* males (Avril & al. 2019).

We designed controlled breeding experiments to investigate the occurrence and mechanism of transmission-ratio distortion by the supergene (Avril & al. 2020). These experiments revealed that heterozygous queens laid *M* and *P* eggs in Mendelian proportions. Hence, there was no meiotic drive. The distortion occurred independently of the presence and genotype of caring workers, which ruled out green-beard effects. Eggs from heterozygous queens started to develop normally, but the ones that did not inherit the *P* haplotype failed to hatch into larvae (Avril & al. 2020). Thus, *MP* females never produce adult *MM* daughters nor *M* sons (Fig. 1B). The *P* haplotype causes complete gene drive, by inducing developmental arrest for up to half of the offspring from heterozygous mothers. It distorts Mendelian transmission in such a way that all individuals produced by polygyne colonies carry at least one *P* haplotype (Fig. 1B). By acting as a maternal-effect

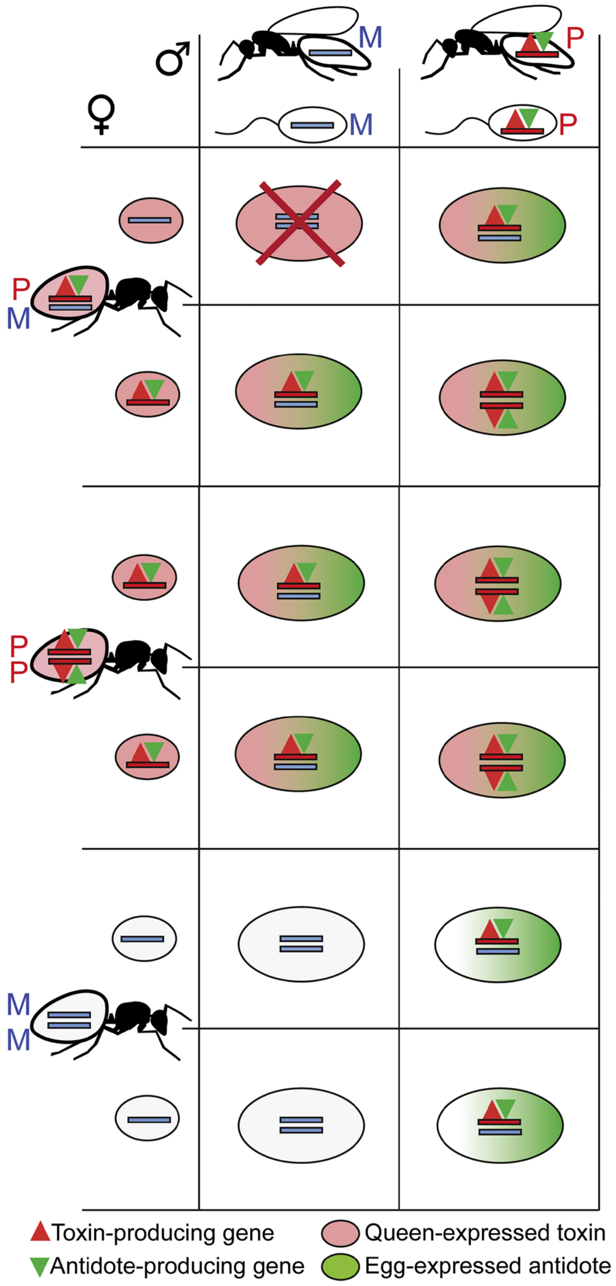

Fig. 3: Model for a putative toxin-antidote system causing maternal-effect killing in *Formica selysi*. This hypothetical model shows the expected outcomes for each parental cross. A toxin gene on the *P* haplotype is maternally expressed, the toxin is transmitted to the eggs, and a zygotically-expressed (or insensitive) antidote on *P* rescues the embryo. The red cross marks the maternal-effect killing effect: Offspring from heterozygous (*MP*) mothers stop developing when they do not inherit *P*, while all offspring from other crosses develop normally.

killer, the *P* haplotype, specific to multi-queen colonies, selfishly favours its own transmission to the detriment of the alternative haplotype.

Mechanistically, the *P* haplotype must contain a maternally-expressed toxin transmitted to the eggs and a zygotically-expressed (or insensitive) antidote that rescues the developing embryo, so that offspring from *MP* queens die if they did not inherit the toxin-antidote genetic element (Fig. 3, Burt & Trivers 2006, Burt & Crisanti 2018, Burga & al. 2020). Note that the toxin might be any type of regulator or effector with a lethal action on a responder locus, and the antidote an insensitive allele at this responder locus. So far, very few selfish toxin-antidote elements have been discovered in animals, but they might be more common than previously thought (Burga & al. 2020). The most famous toxin-antidote element is *Medea*, for "maternal-effect dominant embryonic arrest", discovered long ago in the flour beetle, *Tribolium castaneum* (Beeman & al. 1992, Beeman & Friesen 1999, Lorenzen & al. 2008). Toxin-antidote elements have also been well-characterized in *Caenorhabditis elegans* (Seidel & al. 2011, Ben-David & al. 2017) and in two distantly related *Caenorhabditis* species, with an exciting suggestion that linked, antagonistic toxin-antidote elements on alternative haplotypes are under balancing selection (Ben-David & al. 2021). In such a case, if a mother is heterozygous, with one toxin-antidote element on a haplotype and another linked toxin-antidote element on the alternative haplotype, only her heterozygous offspring will survive, and a genetic polymorphism can persist. This is similar to associative **overdominance** leading to balanced lethal systems, which are typically unstable (Berdan & al. 2022). Whether some supergenes contain antagonistic toxin-antidote elements on alternative haplotypes deserves further investigation.

The *Formica selysi* drive system might be idiosyncratic or shared by other *Formica* species. The presence of *MM* individuals in polygyne colonies of another socially polymorphic species, *Formica neoclara* (McGuire & al. 2022), suggests that the drive does not occur in all species of the genus, either because it has been lost in some lineages, or because it evolved recently in the *F. selysi* lineage.

More generally, for both *Formica selysi* and *Solenopsis invicta*, we do not yet know whether the selfish drive was involved in the origin of supergenes, when the first inversion occurred, or whether the first inversion was associated with polygyny and the drive was acquired secondarily (Huang & Wang 2014). Deciphering the genomic position of drive systems and genes for social organization, respectively, and dating the order of inversions could solve this question (Kay & al. 2022). Comparative analyses across *Formica* and *Solenopsis* species can further reveal if drive systems were basal to the evolution of supergenes (and have been lost in some species), or if the selfish genetic elements got inserted into already established supergenes influencing social organization.

What maintains the polymorphism?

Many supergenes persist over long evolutionary periods, raising the question of which forces lead to such balanced polymorphism (Llaurens & al. 2017, Wellenreuther & Bernatchez 2018, Faria & al. 2019). The *Formica*

Box 5: From genotype to phenotype: lots of work ahead.

Genome-wide association studies provide single-nucleotide polymorphism (SNP) markers that are physically linked to genetic polymorphisms controlling a trait of interest, but most of these SNPs are not directly causal to the phenotypic variation. Because social supergenes are very large (8 - 12 Mb), contain many linked genes (≈ 400 - 750, reviewed in KAY & al. 2022), and show pronounced differences between haplotypes (e.g., in length, gene duplication, and repetitive elements), identifying the functional variants causing alternative social organization is challenging. These variants might be different genes, isoforms of the same gene, or alternative splice variants. They might be structural genes, regulatory genes (e.g., transcription factors), or non-coding regulatory elements (e.g., enhancers, promoters). Hence, once supergenes have been identified, characterizing the genes, functional pathways, and molecular cascades leading from genotype to phenotype and generating a dimorphic social organization is still a long way to go.

The genetic control of social organization might occur by various mechanisms. First, the supergene might include several genes with major effects. Second, the supergene might contain a master regulator, for example a transcription factor influencing a downstream cascade of gene expression. These downstream genes might be located outside of the supergene and shared between social forms. Third, a bi-stable outcome might emerge from the interactions of many genes, with multiple feedback loops regulating the dimorphic expression of antagonistic pathways.

Research on vertebrate sex-determination beautifully illustrates the diversity and complexity of possible mechanisms (CAPEL 2017). Early models proposed a hierarchical molecular cascade, with a master regulator at the top of the cascade activating conserved downstream elements interacting in a linear chain. The mechanisms at the top of the cascade turned out to be remarkably diverse, while the downstream elements interact in non-linear networks. Recent research revealed that antagonist male and female pathways are expressed in both sexes, albeit differentially. One or a few major-effect loci might tilt the balance and canalize the expression network, or many genes with allelic variants might contribute to a threshold decision process (CAPEL 2017). In the latter model, sex-determination results from a "parliamentary decision" by many genes. The antagonism between the male and female pathways, along with multiple feedback loops, results in a sexually dimorphic outcome. For socially polymorphic ants, we do not yet know whether the canalization into monogyne or polygyne colonies, respectively, is due to one major-effect locus affecting queen and worker behaviour, either directly or by regulating the expression of many other genes, or to many genes with allelic variants.

To further characterize how social supergenes function, researchers can rely on a comparative approach to identify genetic elements that (1) differ between alternative supergene haplotypes ("monogyne" or "polygyne"), (2) are differentially expressed, and (3) have been conserved across the ant phylogeny. Differential-gene-expression analyses revealed that the supergene genotype and social form affect the expression of many genes, with an upregulation of multiple genes belonging to the supergene, allelic-biased expression, and trans-regulatory effects on genes elsewhere in the genome (NIPITWATTANAPHON & al. 2013, ARSENAULT & al. 2020, MARTINEZ-RUIZ & al. 2020). The most conserved trans-species polymorphisms point at candidate genes for social organization (BRELSFORD & al. 2020, PURCELL & al. 2021). The gold-standard techniques to functionally validate such candidate genes would be to use genome editing technology (particularly *Crispr-Cas9*), to remove, add, or alter specific sections of the genome, or *RNA* interference, to suppress the expression of specific genes (TRIBLE & al. 2017, FAVREAU & al. 2018). Yet, proving that a specific candidate genetic element affects social organization remains a daunting task because colonies take several years to develop, and the colony social phenotype (queen number) might be altered by laboratory conditions. One way out of the problem would be to identify a behavioural proxy for colony queen number (e.g., recognition of some specific odour cue, TRIBLE & ROSS 2016, ZENG & al. 2022).

Interesting evolutionary questions can also be tackled by studying the social phenotypes. Behavioural and field studies will help to further understand how queens with alternative supergene genotype establish and maintain colonies with distinct social organization, monogyne or polygyne. Dispersal, mating, and colony founding by queens and males of alternative social forms (SHOEMAKER & ROSS 1996, ROSS & SHOEMAKER 1997, DE GASPERIN & al. 2020, BLACHER & al. 2021, DE GASPERIN & al. 2021, FONTCUBERTA & al. 2021), as well as queen acceptance by workers (SUNDSTRÖM 1997, KELLER & ROSS 1998, ROSS & KELLER 1998, MEUNIER & al. 2011), play key roles in generating alternative social organization.

supergene is about 30 million years old, which makes it one of the oldest autosomal supergenes (BRELSFORD & al. 2020). The age of the *Solenopsis* supergene is still debated, with estimates ranging between 390,000 and 1.6 million years (WANG & al. 2013, COHEN & PRIVMAN 2020, YAN & al. 2020, STOLLE & al. 2022). Such persistence of polymorphic social supergenes becomes particularly intriguing when considering that mutant haplotypes associated with polygyny cause gene drive (Tafreshi & al. 2022). Indeed, in absence of counter-acting forces, the driven or positively selected haplotype should go to fixation (reach 100% frequency in the population), at which point drive ceases, due to the absence of polymorphism. The long-term evolutionary maintenance of a genetic polymorphism requires a stable internal polymorphic equilibrium (both genetic variants coexist in stable proportion), while each variant must be able to invade a population that would have fixed the alternative variant (Tafreshi & al. 2022). This is non-trivial.

Mechanisms leading to balanced polymorphisms include various forms of negative frequency-dependent selection, temporally or spatially varying selection, and **overdominance** (heterozygote advantage). Overdominance is likely to be a key factor for the maintenance of many supergenes (Llaurens & al. 2017, Faria & al. 2019). Overdominance arises if the mutant haplotype produces an adaptive phenotype in heterozygotes but is detrimental in homozygotes. Lower fitness of homozygotes is expected, because the non-recombining haplotype tends to accumulate recessive deleterious mutations over time (Schwander & al. 2014, Llaurens & al. 2017, Faria & al. 2019, Jay & al. 2021, Villoutreix & al. 2021) and because inversion breakpoints occasionally disrupt vital genes (e.g., Küpper & al. 2016).

Overdominance probably occurs in fire ants: The *Sb* supergene haplotype, which produces the more competitive polygyne form, is lethal in homozygous females, at least in the introduced range of *Solenopsis invicta* (Ross & Keller 1995a, Hallar & al. 2007). In *Formica* ants, the *PP* genotype is common in *Formica selysi*, but extremally rare across many other species of the genus (Brelsford & al. 2020, Purcell & al. 2021). In both lineages, the driving haplotype has accumulated recessive deleterious mutations, which may prevent it from going to fixation. However, the role and magnitude of overdominance in ants is difficult to estimate, because ant colonies are perennial and social forms differ in many life-history traits, making it challenging to assess the lifetime fitness of queens living in monogyne versus polygyne colonies (Keller 1993, Tafreshi & al. 2022).

Selection varying through time and space is likely to play some role in maintaining the ant social polymorphism (Ross & Keller 1995a, Zahnd & al. 2021, Kay & al. 2022). Typically, the monogyne form is better at long-range dispersal by flight and independent colony founding, and can thus colonize novel, less-densely populated, disconnected habitat. In contrast, the polygyne form tends to show restricted dispersal and establishes colonies by budding, a process whereby queens and workers walk away from their natal nest to establish another nest in the vicinity. The polygyne form therefore thrives in old, saturated, and continuous habitat, where it tends to outcompete the monogyne form (Pedersen & Boomsma 1999). In line with the hypothesis that social forms have alternative dispersal and competitive abilities, a detailed field survey revealed that monogyne and polygyne colonies of *Formica selysi* tend to occupy distinct micro-habitat patches, with more polygyne colonies in older habitat (Zahnd & al. 2021). It should however be noted that polygyne queens are also able to found colonies independently, and that the *P* haplotype can disperse through polygyne males mating with monogyne queens (Reber & al. 2010, De Gasperin & al. 2020, Blacher & al. 2021, De Gasperin & al. 2021, Fontcuberta & al. 2021). Although temporally or spatially varying selection contributes to explain the local distribution and short-term coexistence of alternative social forms, it seems unlikely to balance the polymorphism over millions of years, without social forms diverging into separate species or evolving conditional dispersal strategies that outcompete the alternative form.

The selfish drive by supergenes affects the dynamics of the genetic system. Population genetic models developed for *Formica selysi* show that the drive produced by maternal-effect killing tends to destabilize the social polymorphism (Tafreshi & al. 2022). This is because it prevents rare monogyne colonies from invading a population fixed for polygyne colonies. A stable polymorphic equilibrium can however be reached under a restricted set of conditions, involving high rates of assortative mating by social form and large fitness differences among supergene genotypes (Tafreshi & al. 2022). Assortative mating by social form has been documented in the field (Avril & al. 2019, Fontcuberta & al. 2021). Hence, a major condition necessary for a stable polymorphism is met in this species.

Non-random mating with respect to social forms has interesting consequences for gene flow. Indeed, gene flow mostly occurs through males, and from the monogyne to the polygyne social form, in *Solenopsis invicta* (Fig. 1A, Ross & Keller 1995b, Shoemaker & Ross 1996) and *Formica selysi* (Fig. 1B, Avril & al. 2019, Fontcuberta & al. 2021). The occasional mating of monogyne queens with polygyne males might also contribute to the spread of the polygyne form because monogyne queens mated to polygyne males carry sperm with the polygyny-associated supergene haplotype (*Sb* or *P*), which is dominant (Ross & Shoemaker 1997, Fontcuberta & al. 2021). Such females will produce heterozygous daughter workers and queens, and the colony will likely adopt additional queens from the polygyne social form (Blacher & al. 2021, De Gasperin & al. 2021). By hitchhiking within monogyne queens that have better dispersal and colony founding abilities than polygyne queens, the male-transmitted, polygyny-associated haplotype may propagate to new populations, contributing to spread the polygyne form.

Overall, social supergenes have complex evolutionary dynamics, influenced by positive selection, selfish drive, deleterious mutations, non-random mating, and asymmetric gene flow (Kay & al. 2022, Tafreshi & al. 2022). Social forms differ in dispersal, colonization, and competitive abilities, which likely contribute to their co-existence. However, explaining their long-term evolutionary stability remains challenging and the key factors balancing the genetic polymorphism have yet to be identified.

Supergenes as drivers of ant evolution

The selfish behaviour of social supergenes has interesting consequences for our understanding of the evolution and maintenance of alternative social organization in ants. Colony queen number is a group-level trait influenced by the behaviour of queens and workers. When the genetic basis of complex behavioural traits is unknown, researchers use what Alan Grafen coined the "phenotypic gambit" (Grafen 1984). In short, they neglect the details of the underlying genetic system and treat alternative behavioural strategies as if they had a simple genetic basis. In practice, it means counting the number of adult offspring associated with each strategy, and assuming a direct relationship between genetic variation, phenotypic variation, and fitness. The "phenotypic gambit" is a powerful shortcut. In many cases, working out the exact link between genotype and phenotype would be extremely difficult and tedious, while the "phenotypic gambit" provides answers that are approximately correct. However, on some occasions, the phenotypic gambit fails because the underlying genetic system matters, and one can't apprehend a phenotype without some understanding of the underlying genetics. Grafen (1984) mentioned a mutation in the haemoglobin gene, which causes sickle-cell anaemia in the homozygous state but protects heterozygotes from malaria. In that textbook case, the optimal phenotype (corresponding to the heterozygous genotype) can't invade because Mendelian segregation continuously produces less fit homozygotes. Hence, the genetic system matters to understand the distribution of phenotypes in the population.

The two ant social supergenes that have been well characterized so far, in *Solenopsis invicta* and *Formica selysi*, are clear cases where the "phenotypic gambit" fails because the genetic mechanisms underlying social organization play a direct role in the spread and maintenance of alternative social forms. Supergenes can thus be active drivers of ant evolution, rather than passive responders to natural selection. Strikingly, two independent supergene haplotypes found in multi-queen colonies are driven by selfish genetic elements, causing a green-beard effect in fire ants (Keller & Ross 1998, Trible & Ross 2016) and maternal-effect killing in Alpine silver ants (Avril & al. 2020). Moreover, these haplotypes have other detrimental effects. In both cases, the selfish behaviour of one supergene haplotype promotes a cooperative, adaptive colony-level phenotype, in which reproduction is shared by multiple queens. Therefore, understanding the underlying genetic system becomes central to explaining the persistence of alternative forms of social organization in ants. Sometimes, genetics matters!

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