# Cannibalism and conflict in *Formica* ants

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## Academic dissertation

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HELSINKI 2014

A wise man said

wisdom is better than silver or gold

# Contents

# Cannibalism and conflict in *Formica* ants

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

**I** Schultner, E, Oettler, J & Helanterä, H: The role of brood in Hymenopteran societies - manuscript.

**II** Schultner, E, Gardner, A, Karhunen, M & Helanterä, H: Ant larvae as players in social conflict: relatedness and individual identity mediate cannibalism intensity - The American Naturalist, in press.

**III** Schultner, E, d'Ettorre, P & Helanterä, H. 2013: Social conflict in ant larvae: egg cannibalism occurs mainly in males and larvae prefer alien eggs - Behavioral Ecology 24 (6): 1306-1311.

**IV** Schultner, E, Morandin, C, Helanterä, H & Oettler, J: Egg cannibalism links to expression of key nutrient-sensing genes in ant larvae - manuscript under review.

**V** Schultner, E, Saramäki, J & Helanterä, H: Network analysis reveals complex genetic substructure in ant supercolonies - manuscript.

# Contributions

| | I | II | III | IV | V |
|---|---|---|---|---|---|
| Original idea | ES | ES, HH | ES, HH | ES, JO | HH |
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| Data collection | | ES | ES, PdE | | ES |
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# Abstract

In complex societies like ant colonies individuals cooperate in the aim of maximizing offspring production. But cooperation is only flawless from afar. In fact, because adults can differ in their relatedness to brood they often have contrasting inclusive fitness interests, which may lead to outbreaks of social conflict. Although conflicts in ant colonies typically arise over offspring production, the role of offspring as actors in social conflict has received little attention. The primary aim of this thesis was to investigate the role of larvae in ant societies, with particular emphasis on selfish larval behavior in the form of egg cannibalism.

This thesis demonstrates that *Formica* ant larvae readily engage in egg cannibalism. Egg consumption allows larvae to increase survival and positively affects the expression of key growth-related genes. Levels of cannibalism across species decrease when relatedness between larvae and eggs is high, which suggests that cannibalism is a selfish trait that can underlie social control. Cannibalism appears to be plastic in *F. aquilonia*, where levels increase when larvae are presented with foreign eggs compared to sibling eggs. In addition, cannibalism intensity is highly dependent on larvae sex and size across eight species. I conclude that ant larvae are far from powerless. Instead, cannibalism may allow larvae to influence important determinants of individual fitness such as caste fate or size. By consuming eggs, larvae may furthermore affect overall colony fitness. For the first time, this thesis identifies larvae as individuals with selfish interests that have the power to act in social conflict, thus adding a new dimension to our understanding of colony dynamics in social insects.

In order to understand how relatedness between individuals potentially impacts conflict in ant societies on a larger scale, this thesis furthermore focuses on the genetic network of native wood ant populations. The societies of these ants consist of many interconnected nests with hundreds of reproductive queens, where individuals move freely between nests and cooperate across nest boundaries. The combination of high queen numbers and free mixing of individuals results in extremely low relatedness within these so-called supercolonies. Here, cooperative worker behavior appears maladaptive because it may aid random individuals instead of relatives. I use network analysis to test for spatial and temporal variation in genetic structure, in order to provide a comprehensive picture of genetic substructure in native wood ant populations. I find that relatedness within supercolonies is low but positive when viewed on a population level, which may be due to limited dispersal range of individuals and ecological factors such as nest site limitation and competition against conspecifics. Genetic network analysis thus provides novel evidence that ant supercolonies can exhibit fine-scale genetic substructure, which may explain the maintenance of cooperation in these low-relatedness societies.

Overall, these results offer a new perspective on the stability of cooperation in ant societies, and will hopefully contribute to our understanding of the evolutionary forces governing the balance between cooperation and conflict in other complex social systems.

# Summary

## Introduction

## Social evolution

All life is social. Cells first arose when separate replicators (genes) assembled into groups and formed compartmentalized genomes (Maynard Smith and Szathmary 1995). Symbiotic fusion between prokaryotic cell types resulted in formation of the first unicellular eukaryotes (Margulis 1970) and multicellular organisms like plants and animals arose through cooperation between eukaryotic cells (Bonner 1998). Maternal and paternal genes interact after an egg is fertilized (Haig 2000), and birds and insects display social behavior when they care for their young (Clutton-Brock 1991). In ant colonies and amoeba aggregates, individuals cooperate much like the cells of an organism (Bonner 2009; Hölldobler and Wilson 2009; Queller and Strassmann 2009) and even across species, individuals engage in intricate social relationships (Boucher 1985; Foster and Wenseleers 2006).

Social interactions between genes, genomes, and individuals can shape an organism's environment throughout its life. In the adult stage, social behavior plays a crucial role during mating and individuals engage in interactions with members of their own or other species when competing for habitats and resources (West-Eberhard 1983). During development, interactions between parental genomes can drive trait expression and heritability (Uller 2008) and social contact between offspring and their parents and siblings shapes key parameters like resource allocation (Trivers 1974; Godfray 1995; Forbes 2011).

### Social behavior

The importance of social environment for individual life history and fitness is best illustrated by taking a closer look at social behavior in an evolutionary context. Social actions can be classified into four categories – broad sense cooperation, altruism, selfishness, and spite - depending on their fitness effects (in terms of number of offspring) for both actor and recipient (Hamilton 1964; Alexander 1974) (Figure 1). Broad sense cooperation is defined as a social behavior that increases the number of offspring produced both by the actor and recipient of the action. Individuals that sacrifice their own reproduction completely in order to help others reproduce exhibit altruistic behavior (Hamilton 1972; West et al. 2007). Conversely, individuals behave selfishly when they act to increase their own fitness at the cost of others and exhibit spite when neither actor nor recipient benefits in fitness terms (Hamilton 1970; Foster et al. 2001; Gardner and West 2004; Gardner and West 2006).

| | | EFFECT ON RECIPIENT | |
| --- | --- | --- | --- |
| | | + | − |
| EFFECT ON ACTOR | + | BROAD SENSE COOPERATION | SELFISHNESS |
| | − | ALTRUISM | SPITE |

Figure 1: Classification of social behavior

## Inclusive fitness theory

The evolution of broad-sense cooperation and selfish behavior can be explained with Darwin's theory of natural selection, which posits that individuals (and genes: Dawkins 1976) are under selection to maximize their fitness. In contrast, the existence of altruism was long considered an evolutionary puzzle. Put simply, how can individuals evolve that never pass their genes onto the next generation? W.D. Hamilton (1964a) provided an answer to this paradox by introducing the concept of inclusive fitness. In his seminal paper, Hamilton formalized inclusive fitness theory (also known as kin selection theory, Maynard Smith 1964) and showed that reproductive altruism can evolve if the fitness benefit $b$ to the recipient outweighs the loss in fitness $c$ to the donor, weighted by the relatedness $r$ between recipient and donor (Hamilton's rule: $b * r > c$). In other words, a helping individual can gain fitness indirectly (in terms of genes passed onto the next generation) by aiding the reproduction of a relative because both share copies of the same genes. The more closely related two individuals are, the higher the indirect fitness gains for the helper. Across taxa, positive relatedness within groups is thought to arise because individuals preferentially associate with relatives (kin discrimination), or because individual dispersal is limited (Hamilton 1964).

The clear predictions of inclusive fitness theory concerning the role of relatedness in promoting cooperative behavior have proven straightforward to test in a diversity of organisms. From these studies, kin selection theory has received vast empirical support confirming that relatedness between social partners is of importance on both a behavioral and evolutionary scale (Wade 1980; Queller and Strassmann 1998; Bourke 2011a). For example, the larvae of many marine invertebrates form colonies by fusing. While larvae readily fuse with parents and siblings, somatic fusion between unrelated individuals is rare (Grosberg 1988). The complex societies of social insects are thought to have evolved from simple family units (Hughes et al. 2008) and groups of cooperatively breeding birds are typically comprised of kin (Hatchwell 2009, but see Clutton-Brock 2002). Similarly, mean relatedness is high in cooperatively breeding mammals (Briga et al. 2012).

## Social conflict

At the same time, these studies have highlighted that the evolutionary stability of social groups can be susceptible to invasion by selfish cheaters - that is by individuals who benefit from the cooperative acts of others but do not contribute cooperative behavior themselves (Ghoul et al. 2014) – for instance in social amoeba (Strassmann et al. 2000; Kuzdzal-Fick et al. 2010), rhizobia (Kiers et al. 2003; Oono et al. 2011) and Cape honeybees (Roth et al. 2014). Members of cooperative units may still attempt to follow selfish interests because individuals in social groups, while often related, are never genetically identical. Instead, individuals can encounter all kinds of social partners throughout their lives, including closely related individuals like parents and offspring and unrelated competitors and mates. Variation in relatedness between partners causes individuals to differ in their inclusive fitness interests, and this in turn may affect how they act toward social partners.

One major consequence of differences in the reproductive optima of individuals or groups of individuals within social groups is social conflict. In family groups for example, offspring and parents typically differ in their preferred parental investment (Trivers 1974; Harper 1986) because offspring are related to themselves by a factor of 1, while parents (in diploid species) are related to each offspring by 0.5. While each individual offspring thus prefers maximal investment in itself versus its siblings, parents favor equal investment in all offspring. In birds this leads chicks to compete over parental resources by begging. Chicks that beg at higher intensities are fed more often (Smith and Montgomerie 1991;

Ottosson et al. 1997), which can result in higher growth rates (Price 1998) and may ultimately influence adult fitness (Martín-Gálvez et al. 2011). Each chick is therefore under selection to increase its share of resources, often to the detriment of its nestmates (Johnstone 2004). In line with inclusive fitness theory, the intensity of such sibling competition can depend on the genetic relatedness between chicks (Briskie et al. 1994).

*Power*

Genetic relatedness thus not only plays a key role in promoting cooperation, but it is also a crucial factor in determining the potential for social conflict within groups (Bourke 2011b), for instance in social insect colonies (Ratnieks et al. 2006). However, whether potential conflict becomes actual conflict (*sensu* Ratnieks and Reeve 1992) depends largely on the power of individuals to follow their own interests (Beekman and Ratnieks 2003). Power as the ability to do or act is affected by several factors, including physical presence, strength, access to resources and access to information. Developing individuals for instance follow their own interests when they compete for resources with siblings or nestmates, and display power through begging, establishment of dominance hierarchies or, more drastically, through fatal aggression (Mock and Parker 1997). Sibling rivalry reaches extreme levels in sharks for example, where the first hatched embryo consumes its siblings within the womb (Gilmore et al. 2005). The power of parents can also influence the outcome of within-family conflict, for instance when birds use mouth color to assess the hunger status of their offspring chicks and adjust feeding frequency accordingly (Götmark and Ahlström 1997; Kilner 1997).

In some cases, clear power asymmetries between individuals can lead to rapid resolution of conflicts. One example is when dominant reproductives exert physical control over the reproduction of subordinates, for instance through increased aggression in groups of cooperatively breeding vertebrates (Creel et al. 1997a; Creel et al. 1997b) or cannibalism of subordinate eggs in ants (Monnin and Peeters 1997). Often however, conflicting parties are caught in an evolutionary arms race where each party has partial power, but neither reaches its predicted fitness optimum (Ratnieks et al. 2006). Evolutionary theory suggests that conflict levels can reach an evolutionary stable state when power traits are costly (Harper 1986; Royle et al. 2002). This is most likely the case in parent-offspring conflicts, where costly offspring signaling (e.g. in birds: Kilner 2001; Moreno-Rueda and Redondo 2011) can penalize misinterpretation of signals by parents, thus selecting for signaling honesty (Godfray 1995). When conflict is costly to the social group as a whole, this can furthermore select for complex traits like the policing behavior of social insect workers (Ratnieks 1988; Frank 1995). Power as access to information, and especially the ability to discriminate kin from non-kin, can be especially important in determining the balance between cooperation and conflict in social groups because it allows individuals to preferentially direct help toward relatives (Hamilton 1964), even when overall group relatedness is relatively low or highly variable (Cornwallis et al. 2009).

# Social Hymenoptera

Studying conflict potential and power in social systems can shed light on processes inherent to major transitions in evolution by helping to identify factors that drive and/or constrain social evolutionary processes. Social Hymenoptera (ants, bees and wasps) are unique models for this purpose. Their complex societies function because individuals interact in a large social network, constantly signaling their status, warning of predators or discriminating intruders, sharing information about the needs of colony and adjusting their behavior to social cues (Wilson 1971). Unlike other evolutionary transitions that occurred

on a microscopic scale, the transition from individuality in social Hymenoptera colonies involves large individuals who can be easily observed, and their behavioral complexity offers a wide variety of social behaviors to study. In addition, social Hymenoptera exhibit a range of social structures, from small family-like colonies with a single mother queen (e.g. bumblebees, Michener 1974) to huge networks of interconnected colonies containing several hundred queens each (e.g. many invasive ant species, Tsutsui & Suarez, 2003), thus offering a fitting framework to test the predictions of inclusive fitness theory.

## Altruism in insect societies

Societies of ants, bees, and wasps are characterized by the reproductive division of labor between fertile queens and (facultatively) sterile female workers (Wilson 1971). Virgin queens (gynes) mate and found colonies after which they specialize on egg production while their offspring workers cooperatively forage, defend the nest and rear new workers and sexuals. Males are thought to play only a small role in colony life because they die soon after mating (but see Boomsma et al. 2005; Shik et al. 2013). In advanced eusocial Hymenoptera, workers are morphologically constrained in their reproductive options, for instance because they lack a functional spermatheca (e.g. honeybees: Gotoh et al. 2012; most ants: Hölldobler and Wilson 1990; Gobin et al. 2008).

Whether a female larva develops into a reproductive queen or a sterile worker is decided during larval development. Several mechanisms have been identified that play a role in female caste determination, including genetic effects (Heinze and Buschinger 1989; Helms Cahan et al. 2002; Schwander and Keller 2008), maternal effects (Schwander et al. 2008; Libbrecht et al. 2013), nutrient-mediated predisposition of eggs (Bier 1952; Gösswald and Bier 1953), or – in most cases - the nutritional environment (Wheeler 1986). Food quality and quantity in particular seem to play a role in triggering queen development (e.g. in honeybees: Michener 1974; Ishay et al. 1976; Kamakura 2011; wasps: Gadagkar et al. 1991; Karsai and Hunt 2002; ants: Hölldobler and Wilson 1990; Smith et al. 2008), by acting on processes involved in juvenile hormone regulation (Penick et al. 2012b), DNA-methylation (Kucharski et al. 2008) and TOR-pathway signaling (Wolschin et al. 2011).
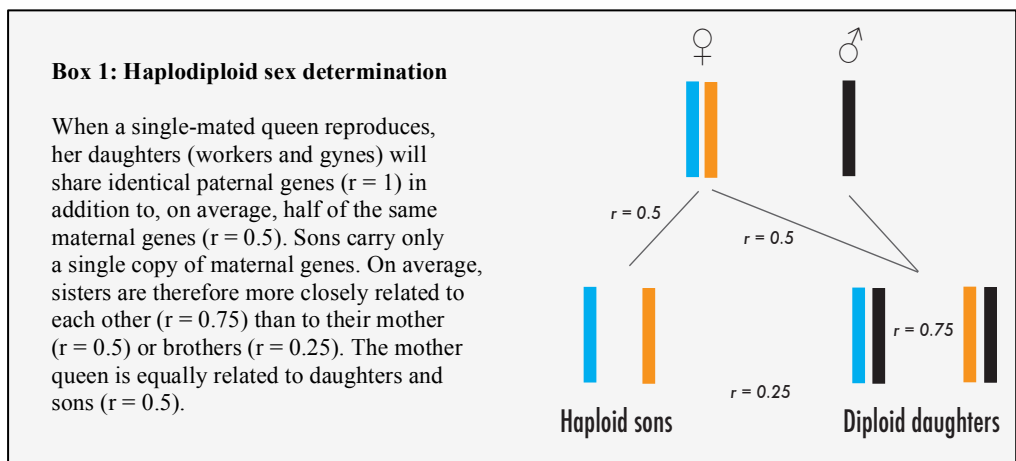
Nutritional environment is mainly controlled by workers, who forage, distribute food within the colony and directly feed the larvae (Cassill and Tschinkel 1995). Queens can also influence larvae nutrition, for instance through pheromonal control of worker feeding behavior (Vargo and Passera 1991; Vargo 1998). Both workers and queens thus partially control caste fate of developing female larvae, and use their power to optimize gyne rearing according to their fitness interests (Rosset and Chapuisat 2006; Helanterä and Ratnieks 2009). At the same time, individual larvae may attempt to gain control over their caste fate, for instance by begging for increased provisions (Creemers et al. 2003; Kaptein et al. 2005; den Boer and Duchateau 2006). Winning the race for queen is especially lucrative because it gives individuals the chance to become founders of new colonies. When parties of interest (i.e. queens, workers, larvae) disagree about optimal investment in individual larvae, conflict over caste determination can ensue (Bourke and Ratnieks 1999).

## Conflict within colonies

The developmental process of female caste determination and its consequences for both individuals (i.e. becoming a queen or a worker) and the colony (e.g. in terms of number of gynes reared) illustrates the intricate network of interests acting in a colony. More generally, it underlines the importance of development for individual fitness and highlights the constraints associated with becoming a worker. With limited direct reproductive options - in many species, workers can still produce male eggs (Wenseleers and Ratnieks 2006;

Helanterä and Sundström 2007) - workers are under selection to maximize their indirect fitness by rearing related brood. But because individuals within colonies are usually not genetically identical, their inclusive fitness interests do not necessarily overlap.

The potential for conflict between individuals varies with their relatedness (Ratnieks et al. 2006) and ants have proven especially powerful models for testing inclusive fitness predictions because of the extreme variation in the social structure of their colonies (Bourke and Franks 1995a). The kin structure of colonies depends mainly on queen number, which can vary both within and between species (e.g. in *Formica* ants: Pamilo 1982; Rosengren and Pamilo 1983; Rosengren, Sundström, and Fortelius 1993; Sundström 1993; Hannonen, Helanterä, and Sundström 2004; Pamilo et al. 2005; Bargum, Helanterä, and Sundström 2007; Seppä et al. 2009). In addition, variation in relatedness between nestmates arises due to haplodiploid sex determination, which leads to relatedness asymmetries between groups of individuals within colonies (Box 1). In single-queen colonies, these relatedness asymmetries can cause conflict between the queen and her workers over the sex of brood: because workers are more closely related to sisters than brothers (Box 1), they prefer higher investment in new queens, while the queen prefers equal investment in both sexes (Trivers and Hare 1976). Workers can attempt to reach their fitness optimum by preferentially feeding female brood (Hammond et al. 2002) or selectively destroying males (Keller et al. 1996; Passera and Aron 1996; Sundström et al. 1996). Inclusive fitness interests can also lead workers to attempt to produce male eggs (Wenseleers and Ratnieks 2006a) or police other egg-laying workers (Ratnieks 1988; Ratnieks and Visscher 1989; Wenseleers and Ratnieks 2006b).



**Box 1: Haplodiploid sex determination**

When a single-mated queen reproduces, her daughters (workers and gynes) will share identical paternal genes ($r = 1$) in addition to, on average, half of the same maternal genes ($r = 0.5$). Sons carry only a single copy of maternal genes. On average, sisters are therefore more closely related to each other ($r = 0.75$) than to their mother ($r = 0.5$) or brothers ($r = 0.25$). The mother queen is equally related to daughters and sons ($r = 0.5$).

Haploid sons          Diploid daughters

Several conflicts of interest over determinants of individual (larva, worker, queen) and colony fitness (e.g. sexual production, brood sex ratios) have been shown to play a role in social insect colonies (Ratnieks and Reeve 1992; Bourke and Franks 1995a; Tsuji 1996; Bourke and Ratnieks 1999; Ratnieks 2001; Reuter and Keller 2001; Sundström and Boomsma 2001; Hammond and Keller 2004). These studies have not only revealed that relatedness plays a substantial role in determining conflict potential, but have demonstrated that adults use a variety of power mechanisms to follow their respective inclusive fitness interests (Beekman et al. 2003; Beekman and Ratnieks 2003). The study of power in the form of access to information, and in particular the ability to assess identity, kinship or status of social partners has proven especially fruitful to understanding social interactions and conflicts.

Ants use chemical information to discriminate nestmates from non-nestmates for example (van Zweden and d'Ettorre 2010), which is essential in maintaining the integrity and organization of colonies in many species (Hölldobler and Wilson 1990). Across Hymenoptera species, queens use chemical signals to prevent worker reproduction and/or signal their fertility (Van Oystaeyen et al. 2014) while workers may use sex-specific odor profiles of eggs or larvae to identify males in the conflict over sex allocation (Carlin 1988; Nonacs and Carlin 1990; Aron et al. 1995; Sundström et al. 1996). Variation in odor profiles among individuals can also arise due to genetic variability and environment (e.g. in ants, van Zweden et al. 2010; Nehring et al. 2011), and can be a major factor in determining the potential for kin-preferential behavior within colonies (Ratnieks and Reeve 1992; Boomsma et al. 2003). Overall, the diverse power of individuals and the ease with which genetic relatedness can be measured in social Hymenoptera colonies makes them particularly good models for studying the role of inclusive fitness constraints in social conflict.

## The role of larvae in social conflict

To date most studies of evolutionary conflict have focused on the behavior of adult queens and workers. This is because they share expensive stakes in brood production – queens because they reproduce directly and workers because they sacrifice their own reproduction to assist in cooperative brood rearing. However, conflict studies among adult females are limited because workers and queens are already constrained in their respective roles, notably leaving workers with limited reproductive options (Wenseleers et al. 2004). This makes it difficult to compare possible fitness gains for queens and workers.

In contrast, the brood itself is most often totipotent, and all individuals potentially gain similar pay-offs later in life – females for instance by attaining queen caste or males by maximizing ejaculate quality. Even more so, developing individuals are at the center of both individual and colony-level selective processes. They embody future generations of sexuals and workers with individual fitness interests and at the same time represent the combined current reproductive investment of all the members in a colony. Overall, developing individuals are therefore central to the evolution of both cooperation and conflict within colonies.

Remarkably, developing individuals as a distinct party of interest have been largely neglected in studies of social Hymenoptera. In fact, larvae are often thought to possess little power, i.e. ability to act according to their own fitness interests (Beekman and Ratnieks 2003), because of their low mobility and overall dependence on workers. In contrast to many insects where offspring are either left to fend for themselves or social interactions are limited to short-term parental care (e.g. egg guarding in cockroaches, see review by Wong et al. 2013), development in social Hymenoptera occurs in closed environments that are strictly controlled by adults. This frees larvae of the need to forage and avoid predation, and may explain their apparent loss of power. Still, past work has demonstrated that brood can take an active role in colony interactions, for instance as food processors (Hunt and Nalepa 1994; Cassill et al. 2005) or producers of cues that affect worker behavior (Bigley and Vinson 1975; Pankiw et al. 1998). Recent studies furthermore indicate that developing individuals are capable of processing information which can impact their development (Suryanarayanan et al. 2011) and behavior (Schultner et al. 2013). This raises the question whether larvae are a powerful party of interest to reckon with after all.

*Egg cannibalism as the ultimate selfish behavior*

For individual larvae, having the power to act according to selfish interests can come with high fitness pay-offs. In social Hymenoptera, this is especially true for female larvae if they are capable of influencing whether to develop into a queen or worker (Bourke and Ratnieks 1999). Across insects, power over development can take different forms. One of the best examples is when offspring selfishly regulate their food intake. The quantity and quality of nutrition is an important factor implicated in development (Scriber and Slansky Jr 1981), and has been linked to a diversity of fitness-related traits such as developmental rate (Shafiei et al. 2001), body size (Chapman 1998; Davidowitz et al. 2003), reproductive success (Delisle and Hardy 1997) and male mating performance (Engels and Sauer 2007). In social Hymenoptera, nutrition has furthermore been shown to play an important role in female reproductive caste determination (Wheeler 1986).

Cannibalism is a particularly widespread way for developing individuals to actively increase food intake (Crespi 1992; Dickison 1992; Bilde and Lubin 2001; Ohba et al. 2006; Schausberger 2007; Vijendravarma et al. 2013). Consuming conspecifics allows individuals to increase growth and/or developmental rates (Osawa 2002; Roy et al. 2007; Crossland et al. 2011), heighten survival (Roy et al. 2007; Vijendravarma et al. 2013), increase adult body size (Osawa 2002), and remove competitors (Wise 2006), thus giving them power over their development and overall fitness.

While beneficial to the cannibal, such behavior clearly impacts negatively upon cannibalized victims, making cannibalism especially suitable for testing how social context affects larval behavior. Specifically, the high inclusive fitness costs associated with eating close relatives makes cannibalism intensity particularly likely to be mediated by relatedness between cannibals and their victims (Polis 1981; Pfennig 1997). In spadefoot toads for instance cannibal tadpoles exhibit excellent discrimination abilities and consequently associate less often with sibling groups (Pfennig et al. 1993). Similarly, earwig nymphs preferentially cannibalize unrelated individuals (Dobler and Kölliker 2009).

The immediate effects of cannibalism on development and growth and its clear costs to the eaten victim make it an excellent trait for studying how inclusive fitness costs constrain selfishness. Ant societies provide the perfect framework for testing inclusive fitness predictions of selfish brood behavior because of large inter- and intraspecific variation in their colony kin structures (Bourke and Franks 1995a). By recognizing larvae as a distinct party of interest, studies of evolutionary conflict in brood can offer a new perspective on social interactions within colonies. In particular, they allow testing conflict predictions on totipotent individuals, thus avoiding the constraints imposed by studies on adults with established behavioral roles. In addition, unlike other conflicts in ants, brood conflict involves males as potential actors, who as adults are rarely assumed to play a role in colony interactions (a unique exception are the wingless males in the ant genus *Cardiocondyla*, Oettler et al. 2010). Studies of larvae can thus offer rare insight into the life of social Hymenoptera colonies, and overall contribute to a better understanding of the fine balance between cooperation and conflict in social organisms.

## Selfishness in ant supercolonies

A selfish behavior like cannibalism is predicted to evolve when the costs of selfishness are negligible. This is the case when relatedness is extremely low, like in the societies of supercolonial ants (Hölldobler and Wilson 1990). The nests of these species can contain

hundreds or even thousands of reproductive queens, and supercolonies consist of networks of interconnected nests in which individuals move freely between nests, cooperate across nest boundaries and show little or no aggression towards non-nestmates (Helanterä et al. 2009). Supercolonies typically originate from one or a few nests that can grow by adopting daughter queens and subsequently colonize new habitats by reproduction by budding, i.e. the founding of new nests by queens and workers that disperse from parental nests to new nesting sites on foot (Hölldobler and Wilson 1977; Keller 1991). The combination of high queen numbers and free mixing of workers, queens and brood between nests results in extremely low nestmate relatedness in supercolonial ants that is often indistinguishable from zero (Holzer et al. 2006a; Kümmerli and Keller 2007; Fournier et al. 2009).

In such low-relatedness societies, cooperative worker behavior appears maladaptive because it may aid random individuals instead of relatives. Evolutionary theory indeed predicts that such lineages represent evolutionary dead ends that fail to diversify and degrade eventually, for example because worker traits degrade due to lack of selection (Queller and Strassmann 1998; Linksvayer and Wade 2009) or because of positive selection on selfish strategies such as cannibalism (Rankin et al. 2007; Helanterä et al. 2009). Still, supercolonial organization has evolved multiple times in ants, and supercolonial ants are among the most successful of all insect taxa, often dominating entire habitats (Savolainen and Vepsäläinen 1988, 1989; Savolainen et al. 1989) and causing considerable damage as invasive species (Wetterer et al. 1999; O'Dowd et al. 2003; Wilson 2005).

This raises the question whether cooperative behavior will be weakened by the invasion of selfish mutants, ultimately leading to the evolutionary breakdown of these low-relatedness societies. Assessing a selfish behavior like cannibalism in varying relatedness settings is the first step in understanding how genetic relatedness determines individual behavior in these systems. As relatedness plays a decisive role in determining potential fitness benefits for workers in supercolonies, a further crucial step in understanding the maintenance of cooperation in these species is detailed assessment of the genetic network within supercolonies.

## Genetic network of ant supercolonies

Past studies on the genetic diversity between ant supercolonies have revealed that while overall relatedness within nests is low, supercolonies can be genetically differentiated when considered on a larger geographical scale (Pedersen et al. 2006; Drescher et al. 2007; Kümmerli and Keller 2007; van Zweden et al. 2007; Holzer et al. 2009; Drescher et al. 2010), which suggests that inter-supercolony competition plays a role in determining the genetic substructure of populations, and gives a first indication of the importance of choosing the relevant spatial scale when assessing genetic structure of ant supercolonies (Helanterä et al. 2009).

Fewer studies have addressed the genetic substructure within supercolonies and those that do have assessed genetic structure across nests using F-statistics, within-nest relatedness analyses and classical measures of genetic differentiation in space such as isolation by distance. While these have proven powerful in studies of simple family-structured colonies, and can suffice to infer weak patterns of overall genetic differentiation within supercolonies (e.g. in *Polyrachis* ants, van Zweden et al. 2007), they may fail to disentangle genetic patterns on a smaller scale (e.g. in *Formica* ants, Kümmerli & Keller, 2007). In addition, much like in conflict studies, most work on the genetic diversity of ant colonies has focused on worker genotypes, which may fail to reflect the genetic reality of colonies that can be shaped by processes like differential reproductive partitioning between worker- and gyne-

producing queens within colonies (Pamilo and Seppä 1994; Bargum and Sundström 2007). Reproductive skew among the many queens in nests of supercolonial ants can furthermore influence genetic substructure (Keller and Reeve 1994; Holzer et al. 2008).

Finally, the complex social interaction network in supercolonial ants - which includes exchange of queens, brood and workers between nests, adoption of queens by neighboring nests and formation of new nests by budding (Helanterä et al. 2009) – demands detailed assessment of relatedness structures among different groups of individuals. In particular, regular movement of workers between nests may affect both within- and between-nest relatedness. This is especially likely to be the case in temperate, hibernating species where nests within supercolonies are cut-off from each other during winter but undergo massive worker exchange in spring and summer (Elias et al. 2004). In some temperate species of the ant genus *Formica* for instance, nests begin producing sexual brood in spring (Bier 1952; Gösswald and Bier 1954) before worker movement commences, which suggests that workers may be able to direct aid towards relatives by staying in their natal nests to rear sexual brood before moving to another nest. So far however, most population genetic studies of ant supercolonies have been based on worker genotypes sampled at a single point in time.

Standard population genetics analysis methods are generally not equipped to deal with such complex data sets, which makes it necessary to apply more accurate tools. Network analysis is a well-developed tool broadly used in the social and behavioral sciences (e.g. network analysis of human communication: Onnela et al., 2007, and animal behavior: Wey et al. 2008) to identify links between different entities or "nodes", which can then be represented in a network based on the intensity of their interactions and other variables such as space or time (Wasserman and Faust 1994). As the largest cooperative networks in nature (e.g. in the supercolonies of invasive Argentine ants: Tsutsui et al. 2000, and native *Formica* ants: Ito 1971; Higashi 1979), ant supercolonies are optimal models for testing the power of network analysis in resolving fine-scale genetic structure between nests. In particular, comparison of networks calculated from genotypes of different classes of individuals within colonies provides information about the strength of specific networks in space and time, thus allowing inferences about the importance of processes like queen dispersal and adoption (Hölldobler and Wilson 1977; Keller 1991; Fortelius et al. 1993; Rosengren et al. 1993; Sundström 1997; Chapuisat and Keller 1999) and worker movement and nest fidelity (Rosengren 1971; Rosengren and Fortelius 1986; Rosengren and Fortelius 1987; Gordon et al. 1992; Heller et al. 2008; Ellis and Robinson 2014) in shaping the dynamics of genetic networks within supercolonies. Overall, network analysis provides a novel way of disentangling disparate forces that impact on genetic diversity and resulting inclusive fitness predictions, making it a powerful tool for ant population genetics studies. More generally, it offers a new perspective on relatedness variation and may contribute to explaining overall patterns of cooperation and conflict in social systems.

## Aims of the thesis

The overall aim of my thesis was to shed light on novel aspects of conflict in Hymenoptera societies.

I begin with a review of the functional and social role of larvae in the social Hymenoptera, in an attempt to draw attention to a developing stage that has been neglected in both

cooperation and conflict studies so far (I).

I use larval egg cannibalism as the ultimate selfish behavior to investigate conflict in ants from a new perspective, with the aim of assessing the crucial parameters of Hamilton's rule – benefits, costs and relatedness (II, III, IV).

I provide a theoretical framework for selfish larvae behavior by developing a mathematical model in order to formulate predictions as to how the inclusive fitness effects of cannibalism are mediated by nestmate relatedness and larval sex. I test the predictions of the model by measuring levels of egg cannibalism in eight ant species with varying relatedness (II).

I put special focus on selfish behavior in low-relatedness societies like ant supercolonies by investigating plasticity of cannibalism behavior in varying relatedness settings and exploring the role of chemical cues in larval discrimination behavior (III). I measure the benefits of cannibalism by comparing survival (III) and growth-related gene expression of cannibals and non-cannibals (IV).

I conclude with a detailed study of the genetic substructure of ant supercolonies in order to test predictions of how cooperation is maintained in low-relatedness societies (V).


# Material and Methods

This thesis attempts to contribute to our understanding of cooperation and conflict by taking a multifold approach. I therefore combine theoretical work with assays of behavior and behavioral plasticity, chemical cue analyses, gene expression studies and population genetics. An overview of the methods used is given below and more detailed descriptions can be found in the corresponding chapters.


## Study species

The ant genus *Formica* is extremely diverse and currently contains 175 described species (Dlussky 1967; Bolton 1995; Goropashnaya et al. 2012; World Catalogue of Ants, www.antweb.org). *Formica* ants are especially abundant in southern Finland, where they dominate a variety of ecosystems including forests, sandy beaches, peat bogs, forest clear-cuts and rocky coastline. In addition to their diverse ecology, *Formica* ants exhibit strong inter- and intraspecific variation in colony kin structure, mainly due to varying queen numbers (Pamilo 1982; Sundström 1993; Pamilo et al. 2005; Sundström et al. 2005; Bargum et al. 2007; Seppä et al. 2009). Furthermore, unlike other ant species (Sendova-Franks et al. 2004), *Formica* ants do not separate brood by developmental stage and larvae therefore have easy access to eggs. This makes them excellent models for studying the evolution and maintenance of a selfish behavior like cannibalism in social organisms, and in particular for understanding the role of relatedness as a mediating factor in social conflict.

I put special focus on the ant *Formica aquilonia* Yarrow 1955, one of the most prominent species within Finnish forest ecosystems. The biology of this species lends itself to a detailed study of the effects of relatedness on selfish behavior in social systems due to its supercolonial population structure. Supercolonies consist of interconnected nests that contain hundreds of reproductive queens each (Pamilo et al., 2005, this study). Relatedness within nests approaches zero (Pamilo et al. 2005; Sundström et al. 2005), creating the

potential for extreme levels of selfishness. A selfish behavior like larval egg cannibalism may therefore be particularly likely to play a role in *F. aquilonia* colonies.

# Genotyping

Genetic relatedness in nests of the study species was assessed by genotyping individuals at polymorphic microsatellite loci designed for *Formica* species and tested for successful cross-amplification in our focal species: FE13, FE16, FE19, FE21, FE42, FE51 (Gyllenstrand et al. 2002); FL12, FL20, FL21 (Chapuisat 1996) and FY4, FY7, FY13 (Hasegawa and Imai 2004) (II, III, V). I estimated mean nestmate relatedness based on population allele frequencies using the relatedness index r implemented in Relatedness 5.0.8 (Queller and Goodnight 1989).

In *Formica* ants, genotypes can be used to determine larvae sex because males develop from unfertilized, haploid eggs while females develop from fertilized, diploid eggs. Sex was determined by classifying larvae that were heterozygous at one or more loci as females, and individuals that were homozygous at six loci or more as males (II, III).

# Cannibalism behavior

## Cannibalism in a kin selection framework

Inclusive fitness theory predicts that individuals refrain from selfish behavior if the costs to recipients outweigh the benefits to the selfish actor, weighted by relatedness between recipient and actor. In addition, selfish behavior may be influenced by individual traits such as sex. The predicted effects of relatedness and larvae sex on cannibalism levels were investigated by modeling cannibalism in a kin-selection framework (Taylor and Frank 1996; Taylor et al. 2007) (II).

## Cannibalism in Formica ants

I tested the predictions of the model using behavioral assays to measure levels of nestmate egg cannibalism in larvae of eight *Formica* ant species: *F. aquilonia*, *F. cinerea*, *F. fennica*, *F. fusca*, *F. lemani*, *F. pressilabris*, *F. sanguinea*, *F. truncorum* (II). Whole nests (high relatedness species: *F. fusca*, *F. lemani*) or nest fragments (high relatedness species: *F. sanguinea*; low relatedness species: *F. aquilonia*, *F. cinerea*, *F. fennica*, *F. pressilabris*, *F. truncorum*) containing brood were collected around Tvärminne Zoological Station in southwestern Finland. From each lab nest, brood was removed within 5 days of collection and classed visually into (1) eggs (2) small larvae (3) medium-sized larvae or (4) large larvae, roughly representing the first 10 days of development. Eggs were placed in piles of five on fresh petri dishes and a single larva placed ventrally on top of each pile. The number of eggs a larva consumed was recorded once a day for two consecutive days. To assess how relatedness and sex affect cannibalism intensities, larvae from bioassays were genotyped to determine intra-brood relatedness and larvae sex (methods see above & II).

## Plasticity of cannibalism

To find out whether larvae can adjust cannibalism levels to different levels of intrabrood relatedness, I measured cannibalism intensity in *F. aquilonia* larvae that were presented with eggs of varying kinship (III). Larvae and eggs were obtained by isolating egg-laying queens collected from nests in two *F. aquilonia* supercolonies on individual petri dishes. Eggs were removed once or twice daily from the queen dishes and moved to clean petri dishes to track their maternal origin and age. Eggs were left to hatch in the dark at room

temperature. Within a few hours after hatching, each larva was moved onto a separate dish and placed ventrally on top of a pile of five fresh eggs (1-3 days age), which were obtained in the same way as larvae. Each larva was presented with a batch of five eggs representing four levels of kinship: (1) sibling: eggs from the same mother queen (n = 91 larvae), (2) nestmate: eggs from nestmate queens (n = 201), (3) colonymate: eggs from queens of a different nest within the same supercolony (n = 87) and (4) alien: eggs from queens of a different supercolony (n = 80). For each of these treatments, the number of eggs each larva had consumed was recorded once a day for two consecutive days.

I investigated the cues larvae may use to discriminate between eggs of different origins by testing for matriline- and supercolony-specific cues in the chemical surface compounds of eggs using gas chromatography – mass spectrometry (GC-MS) (III). Finally, I sexed larvae from bioassays using microsatellite genotyping (methods see above & III) in order to confirm the impact of larvae sex on cannibalism intensities in *F. aquilonia* (III).

### Benefits of cannibalism

A selfish behavior like cannibalism is predicted to evolve if it increases the net inclusive fitness gain of the actor. I measured the effects of cannibalism by comparing the survival of cannibal and non-cannibal larvae of the ant *F. aquilonia* (III).

In order to understand the proximate effects of cannibalism on larval development, I compared the expression of key growth-related genes in cannibal, non-cannibal and worker-fed control larvae of the ant *F. truncorum* using quantitative real-time PCR (qPCR) (IV). Cannibal and non-cannibal larvae were obtained by isolating single larvae on petri dishes. Each larva was then presented with one nestmate egg and kept in the dark at room temperature for 24 hours. Larvae that consumed the egg within 24 hours were labeled as cannibals (n = 5 small larvae and n = 5 large larvae) while larvae that refrained from consumption were labeled as non-cannibals (n = 5 small larvae and n = 5 large larvae). For the control treatment, single larvae were kept in nest fragments containing nest material, one nestmate queen, 10 nestmate workers that had ad libitum access to food and water. Control fragments were kept under natural light conditions at room temperature for 24 hours after which larvae were removed (n = 5 small larvae and n = 5 large larvae).

Total RNA was extracted from whole larvae samples, reverse-transcribed and levels of mRNA of the four growth-related genes *IRS (*CG5686), *mTor (*CG5092), *Tsc1* (CG6147) and *Slimfast* (CG11128) and two control genes *RP9 (*CG3395) and *RP49* (CG7939) (Morandin et al. 2014) were measured by qRT-PCR reactions performed in triplicate. Transcript quantification calculations were performed using the $2^{-\Delta Ct}$ method (Schmittgen and Livak 2008) and a Kruskal-Wallis test followed by Wilcoxon-Mann-Whitney tests for non-parametric data were used to test for the effect of treatment on gene expression levels.


# Genetic networks of native ant supercolonies

Extremely low relatedness predicts workers in supercolonial nests gain little in inclusive fitness. Still, supercolonial ants are among the most successful and ecologically dominant of all ant taxa (Tsutsui et al. 2000; Helanterä et al. 2009), which raises the question of how cooperation is maintained within supercolonies. Assessing the kin structure of ant supercolonies is essential to understanding how relatedness drives behavioral and evolutionary processes in these systems.

## Sampling and relatedness analyses

Nests in two *F. aquilonia* supercolonies about 15 km apart (supercolony LA (n = 21): 59.95°N/23.17°W; supercolony MY (n = 20): 59.99°N/23.23°W) were mapped using GPS and nests were sampled once in 2010 and twice in 2011. In both years, the early sampling (t1) took place in mid-April before the snow had thawed and nests were still cut off from one another. For early sampling, resident queens (i.e. mated, established queens) and workers from 8 nests in each of the supercolonies were collected in 2010 and from 13 (LA) and 12 (MY) nests in 2011. In 2011, the same nests were resampled in June when nests contained pupae and worker exchange among nests had occurred (pers. observations). During this late sampling (t2), adult workers and pupae (workers, males and gynes) were collected. As not all nests produced brood, the final data set contained t1 spring workers from 41 nests, t1 queens from 39 nests, t2 summer workers from 23 nests and t2 pupae from 8 nests.

Genetic relatedness was assessed using microsatellite genotyping (methods see above and V). Relatedness within and between different classes of individuals within nests was calculated separately for each supercolony in Relatedness 5.0.8 following Queller & Goodnight (1989). Within-nest relatedness calculated from supercolony-specific allele frequencies was compared with estimates obtained using allele frequencies from both supercolonies to test for an effect of spatial scale on relatedness estimates. I also tested for isolation by distance in each sampling group by comparing pairwise geographical distances (in m) and genetic distances between nests (based on mean nestmate relatedness) using Mantel tests (ade4 package in R).

## Network analysis

Networks of genetic structure within supercolonies were generated based on GPS location data of nests and relatedness estimates within and between nests for different groups: 1) queens 2) spring workers 3) summer workers 4) brood (LA only). Relatedness within and between nests was calculated following Queller and Goodnight (1989) and background allele frequencies were based on supercolony-specific estimates. Where male genotypes were available, they were weighed by ½ to account for haploidy. The statistical significance of pairwise relatedness links between nests was tested by comparing each network with an ensemble of reference networks calculated from 1000 random permutations of the data (V).

Correlations between networks were tested in order to assess whether genetic substructure in supercolonies varies depending on sample type. Specifically, I tested whether estimates of within-nest relatedness for one group are correlated with within-nest relatedness estimates for the other groups across all nests, as would be expected if groups were genetically similiar. I also compared between-nest relatedness for all combinations of groups to test whether the genetic substructure of networks calculated from different groups overlaps. Here, positive correlations would indicate that relatedness between nests is similar irrespective of sampling group, which could arise if groups overlap in their dispersal patterns.

# Results and Discussion

## The role of larvae in Hymenopteran societies

In my literature review (I), I attempted to fill a gap in our understanding of the role of brood in social Hymenopteran colonies. In particular, I aimed to illustrate why, despite the apparent lack of power and activity of larvae, development in social ants, bees and wasps constitutes a crucial life stage with important consequences for the individual as well as the colony. An extensive search of the literature revealed that larvae have evolved specialized morphologies and behaviors that serve complex interactions with nestmates (Wheeler 1918; Wilson and Hölldobler 1980; Hunt 1984; Masuko 1986; Hunt 1988; Dorow et al. 1990; Cassill et al. 2005; Penick et al. 2012a) and that they can play an important functional role within colonies (Figure 1). In addition, they possess the tools to send and receive signals (e.g Ishay and Landau 1972; Ishay and Schwartz 1973; Cummings et al. 1999; Casacci et al. 2013), thus communicating just like adult individuals. By highlighting the selfish interests of developing individuals, I demonstrated that development in social Hymenoptera has far-reaching consequences for lifetime fitness, and that developing individuals may therefore be under selection to engage in competition. Furthermore, I was able to show that larvae possess the power to act in social conflict, for instance by engaging in begging or cannibalism (e.g. Baroni Urbani 1991; Creemers et al. 2003; Kaptein et al. 2005; Rüger et al. 2007). Finally, my review addresses the constraints developing individuals face in these social systems, including the importance of colony kin structure, species-specific brood rearing strategies and the conflicting interests of different colony members. By compiling a detailed description of the role of developing individuals, I have provided a general resource for researchers interested in the complexity of social interactions in ant, bee and wasp societies. At the same time, my review raises new questions concerning the evolution and maintenance of offspring traits in social organisms and will hopefully inspire future research in this fascinating field.

**Figure 2: Schematic overview of the role of brood in Hymenopteran societies**

Brood morphology, physiology and behavior affect individual and colony-level traits. Colors show direct effects on different groups of individuals (purple: brood, green: queens, yellow: workers, white: whole colony). Dashed lines indicate traits that can affect several parties of interest and are potential sources of conflict within colonies.
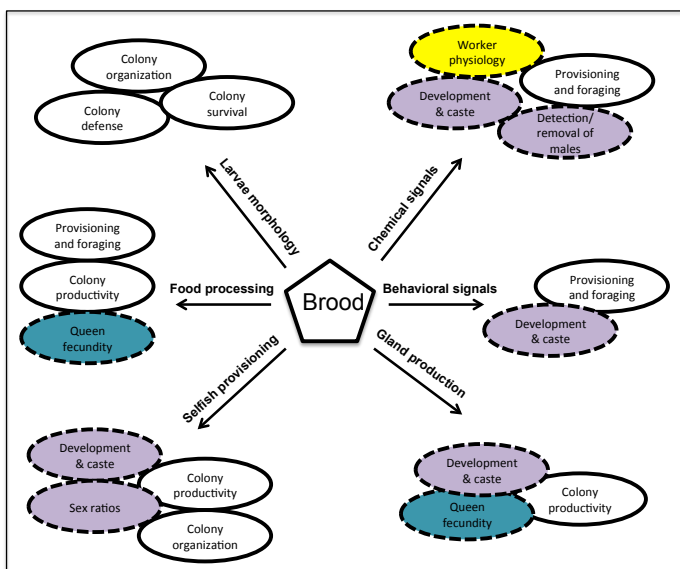
# Inclusive fitness constraints mediate cannibalism behavior

Modeling cannibalism in a kin selection framework predicted an overall increase in absolute levels of cannibalism with rising queen numbers (i.e. decreasing relatedness) for both male and female larvae (Figure 3A-B). Specifically, a linear increase in cannibalism was predicted for both sexes when queens are unrelated ($a = 0$). An increase in between-queen relatedness affected absolute levels of male and female cannibalism differentially, with females exhibiting a slightly steeper increase in cannibalism levels with rising queen numbers than males. Sex differences in cannibalism propensity arise because males are equally related to male and female nestmates, while females are more related to female than to male nestmates (II). In a nest with a single queen, there is thus more incentive for a male larva to cannibalize sibling eggs than for a female (Figure 3C).

When queen numbers increase relatedness drops overall, but this decrease is on average more drastic between female nestmates than between males and females and among males. As a result, relative levels of female cannibalism are predicted to increase. However, assuming no sex differences other than in relatedness coefficients, levels in females are predicted to be relatively higher only when nests contain at least two queens that are full sisters or an equal mix of full sister queens and unrelated queens, but not when queens are unrelated (Figure 3C).
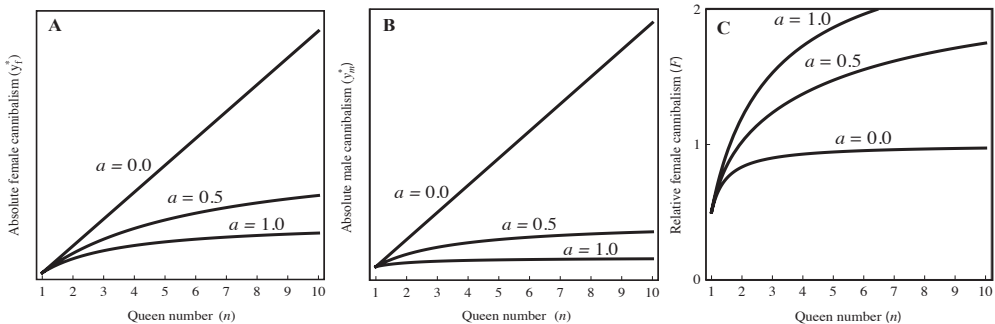


**Figure 3: Predicted effects of relatedness and sex on cannibalism**

Absolute cannibalism levels of female (A) and male (B) larvae are predicted to increase with decreasing relatedness, simulated by rising queen numbers and/or decreasing levels of consanguinity between queens. Lack of consanguinity strongly amplifies this effect ($a = 0$, queens unrelated; $a = 0.5$, equal mix of unrelated and full sister queens; $a = 1$, queens are full sisters). Differences in cannibalism levels between the sexes (C) are predicted to arise because of relatedness asymmetries between males and females. © The University of Chicago Press 2014

Sex-dependent differences in cannibalism propensity can be partly explained by relatedness asymmetries between males and females resulting from haplodiploid sex determination (Hamilton 1964). In particular, higher relatedness among females in single queen colonies may inhibit female larvae from engaging in selfish behavior. In addition, the model predicted that female:male cannibalism ratios can vary with sex-specific benefits and sex ratio (II). In particular, if males benefit more from cannibalism than females, this leads to relatively male-biased cannibalism (II).

# Egg cannibalism in *Formica* ants

Egg cannibalism occurred in all study species, albeit with varying intensity (Table 1). The overall proportion of cannibalistic larvae in the raw data ranged from 1.4% ± 1.4% in *F. fennica* to 19.4% ± 4.7% in *F. truncorum* (mean proportion ± 95% CI). The remaining six species showed intermediate levels of cannibalism (Table 1). The number of eggs eaten by larvae varied in a similar way, with only 0.3% ± 0.3% of eggs eaten by *F. fennica* larvae and 4.6% ± 1.3% eaten by *F. sanguinea* larvae (Table 1).

**Table 1: Larval egg cannibalism levels, intrabrood relatedness and sex ratios in *Formica* ants**

| Species | Number of nests | Number of replicates | Relatedness | Sex ratio | Eggs eaten (%) | Incidents (%) |
|---|---|---|---|---|---|---|
| *F. cinerea* | 3 | 70 | -0.06 ± 0.02 | 0.73 ± 0.05 | 2.3 ± 1.6 | 10.0 ± 7.0 |
| *F. truncorum* | 5 | 273 | 0.00 ± 0.01 | 0.50 ± 0.04 | 4.5 ± 1.1 | 19.4 ± 4.7 |
| *F. aquilonia* | 12 | 329 | 0.06 ± 0.02 | 0.37 ± 0.03 | 3.0 ± 0.8 | 12.5 ± 3.6 |
| *F. fennica* | 10 | 220 | 0.09 ± 0.02 | 0.31 ± 0.02 | 0.3 ± 0.3 | 1.4 ± 1.4 |
| *F. pressilabris* | 10 | 221 | 0.21 ± 0.02 | 0.98 ± 0.01 | 1.9 ± 0.8 | 9.5 ± 3.9 |
| *F. sanguinea* | 6 | 205 | 0.36 ± 0.02 | 0.63 ± 0.04 | 4.6 ± 1.3 | 18.5 ± 5.3 |
| *F. lemani* | 5 | 212 | 0.40 ± 0.01 | 0.18 ± 0.02 | 0.5 ± 0.4 | 2.4 ± 2.0 |
| *F. fusca* | 8 | 204 | 0.46 ± 0.03 | 0.41 ± 0.03 | 1.4 ± 0.7 | 5.9 ± 3.2 |

Note: Each replicate represents one larva. Sex ratios range from 0 (all females) to 1 (all males). Relatedness, sex ratio, proportion of eggs eaten and proportion of incidents are reported as means with 95% confidence intervals calculated from the raw data. Negative relatedness values indicate that nestmates are less related to each other than to a random individual from the population. © The University of Chicago Press 2014

In line with theoretical predictions, variation in levels of cannibalism across species was best explained by relatedness among brood and sex of cannibal larvae (General linear mixed model GLMM, factor: relatedness, z = -4.3, p = 0.003; factor: sex, z = 1.9, p < 0.001; II). Decreasing relatedness led to a significant increase in cannibalism (Figure 4A), as did increasingly male-biased sex ratios (Figure 4B). The interaction between relatedness and sex was also statistically significant (GLMM, factor: relatedness * sex interaction, z = 4.5, p = 0.005; II), indicating that male and female cannibalism levels respond differently to changes in relatedness. Specifically, for males high relatedness did not prevent cannibalism whereas females were only likely to be cannibalistic when relatedness was low (Figure 4C). Across species large larvae cannibalized significantly more than small larvae (GLMM, factor: size, p < 0.001; II), suggesting that in addition to sex, individual development stage determines cannibalism propensity.
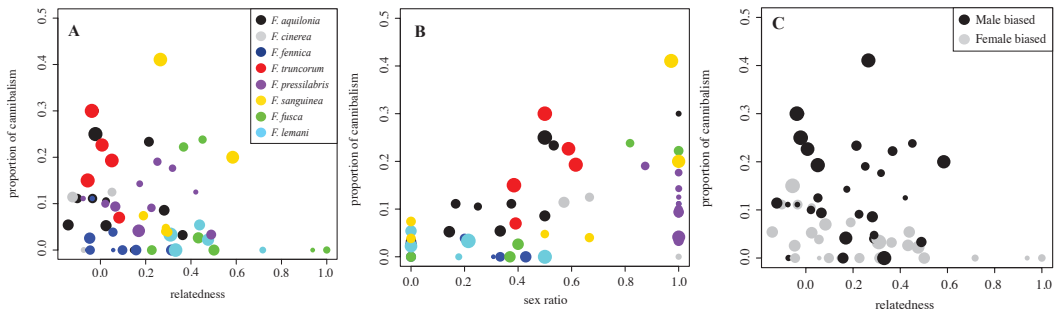
**Figure 4: Measured effects of relatedness and sex on cannibalism in *Formica* ants**

In line with the model's predictions, the proportion of cannibalism incidents in experiments increased significantly with decreasing relatedness (A). Cannibalism levels were significantly higher in nests with many male larvae, expressed as colony sex ratio where 0 = all females and 1 = all males (B). There was also a significant positive interaction between relatedness and sex ratio, which indicates that male and female cannibalism levels respond differently to changes in relatedness (C). Points represent the mean proportion of cannibalism incidents in different nests color-coded by species (A,B) and sex ratio (C). In (C) nests were classified as male or female biased depending on the sex ratio calculated from larvae genotypes ( > 0.5, male biased; < 0.5, female biased). Point size corresponds to the number of replicates for a particular nest (range 1 to 60, median 27). © The University of Chicago Press 2014

# Plasticity of cannibalism

Both model and data agree that larval egg cannibalism is mediated by kin selection. This implies that cannibalism is costly in inclusive fitness terms, confirming its suitability as a selfish trait. At the same time, this raises the question whether cannibalism levels represent an evolved response to average kin structures encountered by larvae or if larvae adjust levels plastically to the prevailing colony conditions. When *F. aquilonia* larvae were presented with eggs of varying kinship, they preferentially ate eggs from an alien supercolony compared to sibling eggs (Figure 5, III). Eggs from different supercolonies carried distinct odor profiles (Multivariate analysis of variance MANOVA, factor: supercolony, df = 1, F = 2.72, $R^2$ = 0.07, p = 0.016; III), and larvae may use this chemical information to discriminate among eggs. At the same time, failure to detect matriline-specific egg odors coupled with lack of discrimination between sibling, nestmate and colonymate eggs (III) suggests that larvae may be constrained in kin-preferential behavior within colonies due to uninformative cues, inability to discriminate at fine scales, or both.

A plastic increase in offspring cannibalism in response to increased genetic diversity has been found in other taxa (Polis 1981; Elgar and Crespi 1992; Pfennig and Collins 1993; Pfennig 1997; Bilde and Lubin 2001; Dobler and Kölliker 2009). In ants however, behavioral plasticity in response to local social structure is only known from adults, for example when workers adjust colony sex ratio according to the mating frequency of their mother queen (Sundström et al. 1996) or begin producing male eggs in orphaned queenless nests (Miller III and Ratnieks 2001; Helanterä and Sundström 2007). The present study reveals that chemical information use is not restricted to adults, but instead may also be a means of power for larvae.

**Figure 5: Cannibalism intensity varies with kinship between larvae and eggs in *F. aquilonia***

Mean proportion of cannibalism incidents (white) and number of eaten eggs (black) across treatments in *F. aquilonia* larvae. Bars indicate 95% C.I. intervals. Differences were tested for each response separately with a GLMM for binomially distributed data using treatment as fixed and colony as random variables, *: $p < 0.05$
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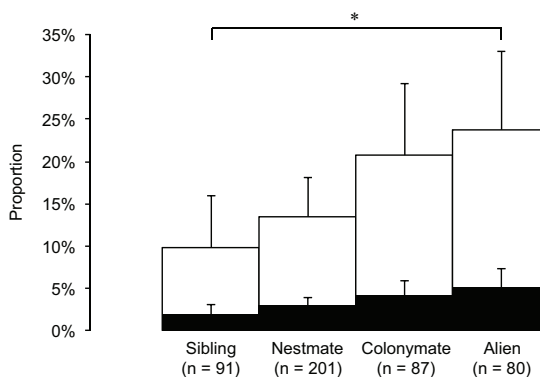


Thus relatedness variation creates potential for conflict among larvae, and they appear to possess the power to adjust their behavior to minimize inclusive fitness costs. However, they did not refrain completely from consuming eggs even when these were full siblings (III) or within-brood relatedness was high (II). Male *F. aquilonia* larvae for instance exhibited similarly high levels of cannibalism when exposed to sibling or nestmate eggs (III) and significantly higher levels than females overall across nests of different species (II, III). This indicates that larvae, and perhaps males in particular, can benefit from cannibalism in spite of inclusive fitness constraints.

Individuals differences in cannibalism propensity have been found in other taxa, where they have been shown to reflect unequal competitive abilities resulting from differences in size or mobility (e.g. in fruit flies, where second instar larvae collectively cannibalize the larger but immobile third instars: Vijendravarma et al. 2013) or variation in cannibalism benefits (e.g. in ladybirds, where cannibalism has relatively greater effects on male survival and adult body size: Osawa 2002). The model developed in Chapter II suggests that sex-specific benefits of cannibalism may also explain higher cannibalism levels in male ant larvae. In ants, males should be under selection to increase their nutrient intake if this leads to higher competitive ability or increased mating success (Shik et al. 2013), for example through improved sperm quality or quantity (Wiernasz et al. 2001) or larger adult body size (Davidson 1982; Wiernasz et al. 1995; Abell et al. 1999). Alternatively, males may cannibalize relatively more to compensate for their higher metabolic rate (Boomsma and Isaaks 1985) or to overcome nutritional restriction through workers that may arise in the course of queen-worker conflict over sex allocation (e.g. Trivers and Hare 1976; Craig 1980; Bourke and Franks 1995b). In broods comprised of males and worker-destined female larvae, differences in cannibalism levels between sexes could also arise as a result of lower average benefits to female cannibals (e.g. because cannibalism only carries indirect fitness benefits for worker-destined larvae). In *Formica* ants however, sexual brood is reared earlier in the season than worker brood (Gösswald 1951; Gösswald and Bier 1953; Bier 1954; Gösswald 1989), and female larvae from the sexual cohorts used in this study should thus all be under selection to develop into future queens (Dobata 2012).

## Benefits of cannibalism

Across animals egg cannibalism allows offspring to selfishly regulate nutrient intake, giving them power to influence survival (Roy et al. 2007), accelerate growth and

development rates (Crossland et al. 2011) and increase adult body size (Michaud and Grant 2004). In *Formica* ants, egg cannibalism significantly increased survival in *F. aquilonia* larvae (mean survival in days ± SE for cannibals: 2.94 ± 0.12 and non-cannibals: 2.45 ± 0.07; Wilcoxon-Mann-Whitney test, n = 167, W = 1642, p = 0.002; III). Cannibalism furthermore had significant effects on gene expression in *F. truncorum* larvae (IV). The expression of four genes that play an important role in nutrient-sensing and growth control in developing insects was consistently higher in 4-6 day old cannibal larvae compared to both non-cannibal and control larvae of the same age (Figure 6a): *IRS* (Kruskal-Wallis rank sum test, df = 2; $\chi^2$ = 10.5, p = 0.005), *mTor* ($\chi^2$ = 7.28, p = 0.026), *Tsc1* ($\chi^2$ = 7.58, p = 0.023) and *Slimfast* ($\chi^2$ = 8.34, p = 0.015). Egg cannibalism did not affect the expression of the same genes in younger larvae (Kruskal-Wallis rank sum test, df = 2; *IRS*: $\chi^2$ = 2.34, p = 0.310, *mTor*: $\chi^2$ = 2.18, p = 0.336, *Tsc1*: $\chi^2$ = 3.44, p = 0.179, *Slimfast*: $\chi^2$ = 2.34, p = 0.310; Figure 6b).

In insects, larval growth and development is regulated by molecular sensors, which respond to nutrient conditions (Martin and Hall 2005; Layalle et al. 2008; Hietakangas and Cohen 2009). Nutrient-dependent changes in the expression of key sensor-encoding genes like *IRS* and *mTor* can lead to pronounced differences among adult phenotypes (Böhni et al. 1999). In honeybees, nutrient-dependent upregulation of both *IRS* and *mTor* have been associated with queen development, while *IRS* knockdown larvae exhibit longer developmental times, reduced fresh weights and smaller ovaries (Wolschin et al. 2011). Similarly, *Tsc1* is upregulated in young queen-destined honeybee larvae compared to worker-destined larvae (Chen et al. 2012). In fat bodies of developing insects, *mTor* acts together with *Slimfast*, which encodes for an amino acid transporter. Experimentally induced downregulation of either *mTor* or *Slimfast* results in a growth defect in flies, similar to that seen in flies raised in poor nutritional conditions (Colombani et al. 2003). Overall, increased expression of *IRS*, *mTor*, *Tsc1* and *Slimfast* in 4-6 day old cannibal larvae therefore indicates that egg consumption initiates essential components of the nutrient-sensing network, confirming that eggs are a valuable food source. However, gene expression levels did not change in response to cannibalism in younger larvae, which suggests that timing of nutritional intake is a pivotal factor in determining the effect of cannibalism on development. Such age-related differences in responsiveness to cannibalism and its positive effects on nutrient-signaling may also explain why levels of cannibalism in *Formica* ants were consistently higher in old larvae compared to young larvae (II).
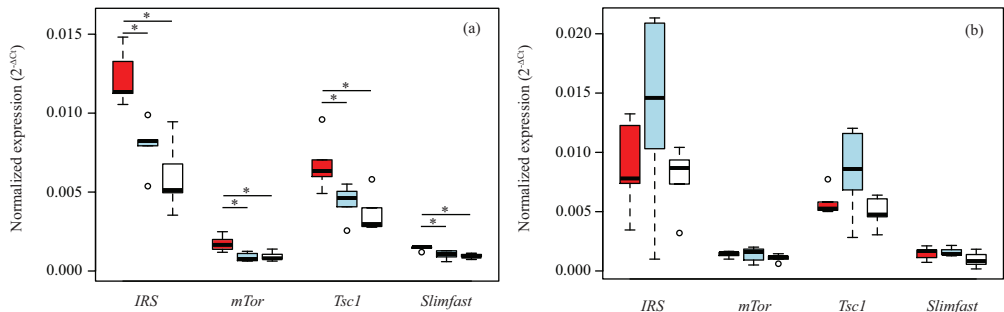


**Figure 6: Expression of nutrient-dependent signaling genes in ant larvae**

Expression of *IRS*, *mTor*, *Tsc1* and *Slimfast* in (a) 4-6 day old and (b) 1-3 day old *F. truncorum* larvae from cannibal (red), non-cannibal (blue) and control (white) treatments (n = 5 per treatment). Differences in expression levels were analyzed with Kruskal Wallis and Wilcoxon-Mann-Whitney tests and p-values corrected for multiple testing following Benjamini and Hochberg 1995. * p < 0.05.

# Genetic network of supercolonies varies in space and time

Overall relatedness within supercolonies was low when calculated from supercolony-specific allele frequencies, but confidence intervals did not overlap with zero in either supercolony (LA: 0.035 (0.020 – 0.05); MY: 0.028 (0.016 – 0.04)) (Table 2). When whole population allele frequencies were implemented, overall mean relatedness values increased to 0.185 in LA and 0.157 in MY (Table 2). When relatedness calculated from supercolony-specific allele frequencies was analyzed by group values were positive but low ranging from 0.014 – 0.072 and significantly higher than zero in four out of seven cases (Table 2). In LA, queens and spring workers exhibited positive relatedness while relatedness among summer workers and brood was not significantly higher than zero. In contrast, relatedness was positive in MY spring and summer workers, but not in queens (Table 2). Overall however, within-supercolony relatedness estimates calculated from different groups did not differ (Kruskal-Wallis rank sum test, LA: $X^2 = 0.883$, df = 3, p = 0.8295, MY: $X^2 = 3.6386$, df = 2, p = 0.1621).

**Table 2: Relatedness within and between different groups of individuals in two *F. aquilonia* supercolonies**

| Supercolony | Type | $n$ nests (individuals) | $r_{supercolony}$ (95% CI) | $r_{population}$ (95% CI) |
|---|---|---|---|---|
| LA | All | 21 (910) | 0.035 (0.02 - 0.05) | **0.185** (0.163 - 0.207) |
| | Queens | 20 (254) | 0.045 (0.0002 - 0.9) | |
| | Spring workers | 21 (336) | 0.043 (0.004 - 0.082) | |
| | Summer workers | 10 (160) | **0.014** (-0.024 - 0.052) | |
| | Brood | 8 (160) | **0.046** (-0.025 - 0.12) | |
| | Queens – spring workers | 20 (254, 320) | 0.042 (0.002 - 0.082) | |
| | Queens – summer workers | 10 (160, 160) | **0.023** (-0.022 - 0.068) | |
| | Queens - brood | 6 (96, 96) | 0.084 (0.034 - 0.137) | |
| | Spring workers - brood | 7 (112, 112) | 0.072 (0.003 - 0.141) | |
| | Summer workers - brood | 5 (80, 80) | 0.112 (0.053 - 0.171) | |
| | | | | |
| MY | All | 20 (746) | 0.028 (0.016 - 0.040) | **0.157** (0.134 - 0.179) |
| | Queens | 19 (202) | **0.014** (-0.014 - 0.041) | |
| | Spring workers | 20 (320) | 0.039 (0.002 - 0.076) | |
| | Summer workers | 12 (192) | 0.072 (0.013 - 0.131) | |
| | Queens – spring workers | 19 (202, 304) | **0.022** (-0.006 - 0.050) | |
| | Queens – summer workers | 11 (140, 176) | 0.060 (0.004 - 0.116) | |

Note: Values in bold have confidence intervals that overlap with zero.

Relatedness between different groups of individuals within nests ranged from 0.022 – 0.112, and was highest between LA summer workers and LA brood (Table 2). Relatedness between queens and summer workers in LA, and queens and spring workers in MY, was not significantly different from zero, but all other groups showed positive relatedness (Table 3). There was no significant variation in relatedness estimates between comparisons (Kruskal-Wallis rank sum test, LA: $X^2 = 7.7576$, df = 4, p = 0.1009; MY: $X^2 = 1.4007$, df = 1, p = 0.2366).

Isolation by distance analysis and network analysis provided evidence of genetic substructure within supercolonies, with nests in supercolony MY but not LA exhibiting significant isolation by distance (Mantel test; MY: all samples: r = 0.41 (95% CI: 0.24 – 0.59), p < 0.001; LA: all samples: r = 0.13 (-0.01 – 0.27), p = 0.06) (V). Network analysis of genetic structure within supercolonies furthermore revealed that genetic substructure changes depending on both the type of individual sampled (queens, workers, brood) and the sampling time point (Figure 7). Within-nest relatedness of different sample types was not correlated in either supercolony (V), providing further support that the genetic substructure varies among classes of individuals. Correlations between networks based on between-nest relatedness of queens, spring workers, summer workers and brood confirmed that networks do not overlap significantly in their structure in LA (V). In MY, relatedness between nests was weakly positively correlated for queens and spring workers (Pearson correlation $r = 0.16$, p = 0.03) and spring and summer workers (Pearson correlation $r = 0.26$, p = 0.03).

Shifts in genetic substructure between queen and worker networks may be partly explained by supercolony-specific habitat characteristics and differences in mobility between individuals. In supercolonial *Formica* ants, new colonies can be founded by queens entering the nests of related *Serviformica* species and hijacking their workforce (temporary social parasitism, Rosengren and Pamilo 1983) and through the dispersal of queens and workers on foot (reproduction by budding, Keller 1991). Once a nest or nests have been established in a new habitat, colonies usually reproduce by budding, as this carries far fewer risks than taking over heterospecific colonies as a social parasite (Hölldobler and Wilson 1990). Because of the limited dispersal range of walking individuals, *Formica* supercolonies can display high spatial genetic viscosity (Chapuisat et al. 1997; Holzer et al. 2006a; Holzer et al. 2009), which may be further promoted by ecological factors such as nest site limitation and competition against con- and/or heterospecifics (reviewed in Ellis and Robinson 2014).

Network analysis confirmed that supercolonies exhibit genetic structure on small spatial scales and visual comparison and correlation analyses revealed that genetic networks differ strongly between groups of individuals (Figure 7, V). Within-nest relatedness was not correlated for any combination of groups, indicating that different types of samples give contrasting information on population structure. This suggests that generalizing from one type of data (e.g. workers) has to be done with caution in studies of supercolonial species.

One factor that contributed substantially to changes in the genetic network of supercolonies was worker movement. In both supercolonies, spring and summer worker networks overlapped in two or fewer links, even though each separate network exhibited a minimum of 9 significant links (V). This shift in relatedness between workers of different nests shows that worker movement between nests can be sufficient to shuffle genotypes within the supercolony, even in the course of a single season. I hypothesized that workers rear related sexual brood in their natal nest before moving to other nests. While the data provides solid evidence for intense worker movement between nests, I did not find differences in

relatedness between brood and spring or summer workers, which indicates that temporal variation in genetic substructure cannot explain the maintenance of cooperation in these systems. Whether this is because relatedness is diluted by high numbers of reproducing queens within nests or because workers move between nests prior to hibernation remains to be shown.

Movement of individuals between nests in ant supercolonies has been suggested to mirror strategic redistribution of resources among functional units e.g. in multicellular organisms (Kennedy et al. 2014), thus supporting the idea that ant supercolonies are in a state of evolutionary transition from individuality (separate nests) to organismality (closed network of connected nests) (McShea 2001; McShea and Changizi 2003; Bourke 2011b; Pedersen 2012; Kennedy et al. 2014). However, to date few studies have actually investigated how transfer of individuals affects the substructure of these cooperative units. Study of *F. aquilonia* supercolonies confirms that worker movement can play a significant role in determining genetic substructure, indicating that redistribution of ants serves a functional purpose much like the sharing of resources between nests (Rosengren and Fortelius 1987; Gordon et al. 1992; Holway and Case 2000; Ellis and Robinson 2014). In particular, summer worker networks had fewer nests with significant links to other nests compared to spring worker networks, which could indicate that workers are preferentially recruited to certain nests within the supercolony, for instance because of their proximity to crucial foraging sites (Rosengren and Fortelius 1987) or suitability for brood rearing (Buczkowski and Bennett 2008). Alternatively, fewer significant relatedness links in summer networks may reflect homogenization of genetic substructure through increased movement of workers among all the nests within the supercolony.

Genetic network analysis provides novel evidence that native wood ant supercolonies exhibit fine-scale genetic substructure that varies between different groups of individuals. This raises new questions about the formation, evolution and persistence of supercolonial population structure. Studies on supercolonial wood ant species suggests that supercolonies are comprised of smaller genetically differentiated subunits that arise through budding and limited dispersal (Chapuisat et al. 1997; Pamilo et al. 2005; Holzer et al. 2009). In *F. aquilonia*, the genetic network of queens, workers and brood cannot be explained by population viscosity alone however, which indicates that other processes play a central role in determining distribution of individuals. It will therefore prove particularly useful to apply network analysis to other characteristics of substructure in ant supercolonies, like transitions in the network of resource flow between nests across seasons or patterns of supercolony expansion. In particular, more detailed comparisons of worker networks will help provide information about the direction and intensity of worker movement, which may help resolve questions about the functionality of worker movement (Ellis and Robinson 2014), and in particular whether workers are resources that are transferred from one nest to another when needed (Kennedy et al. 2014). Analyzing behavioral interaction networks within supercolonies will furthermore help elucidate patterns of cooperation among individuals, while study of competitive interaction networks between supercolonies promises to shed light on processes determining population genetic structure. Together, such studies will shed light on the potential transition from individuality to organismality in supercolonial ants and contribute to explaining overall patterns of social cohesion in low-relatedness societies.
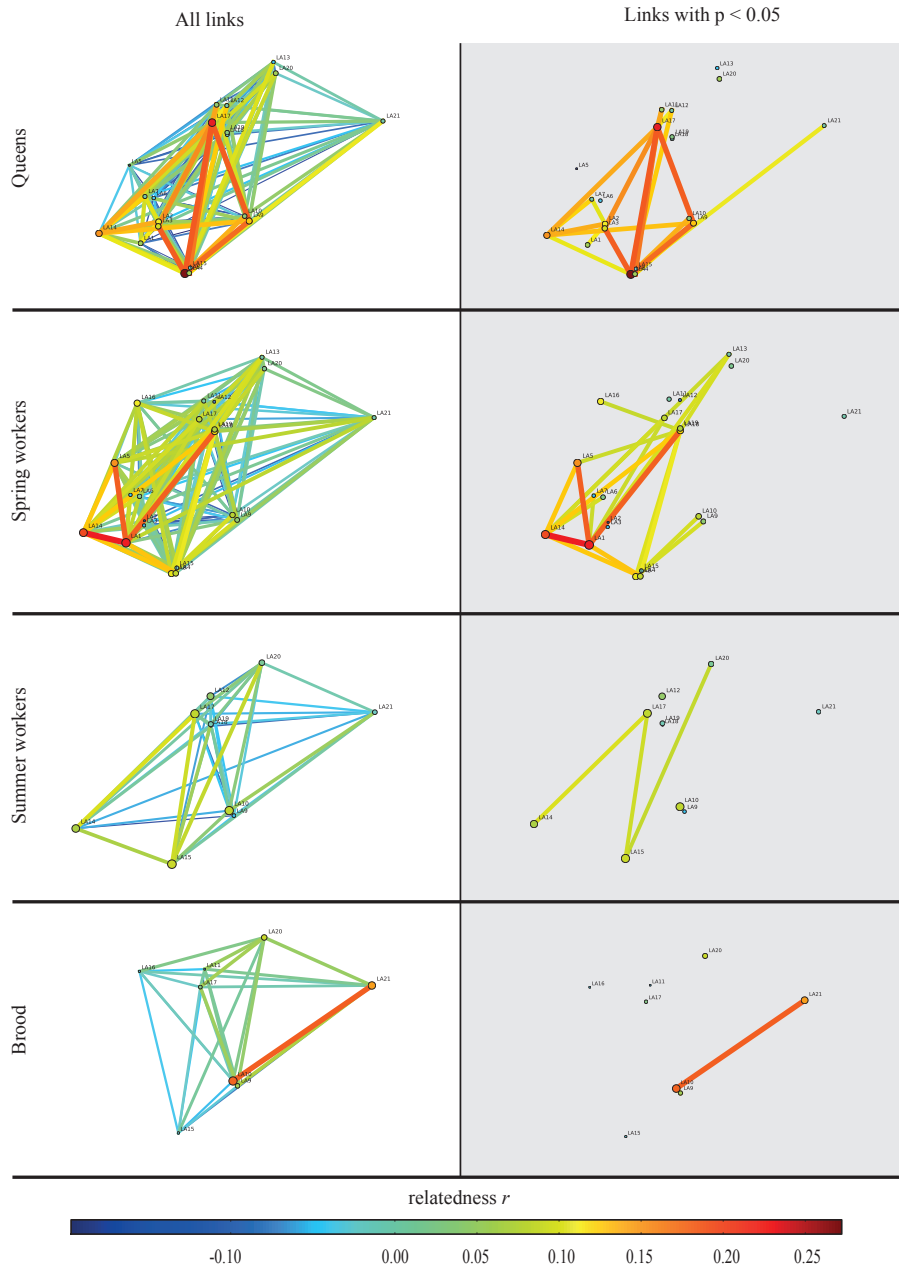
**Figure 7: Genetic networks in a *Formica aquilonia* supercolony**

Networks of genetic structure in the LA supercolony generated from GPS location data of nests and relatedness estimates within and between nests for different groups: 1) queens 2) spring workers 3) summer workers 4) brood. Networks with all relatedness links are given (left) and only with links that were significantly higher than those calculated from random reference models (right). Differences in numbers of nests per network are due to variation in sampling. The color of dots and links reflects mean relatedness within and between nests, respectively.

# Conclusions and perspectives

The main focus of this thesis lies in the role of brood as a distinct social caste in Hymenopteran societies. Within societies, brood can be structurally adapted to fulfill cooperative colony tasks. At the same time, each developing individual is under selection to maximize its direct and/or inclusive fitness, making larvae potential actors in social conflict. This thesis explores the role of ant larvae as actors in colony conflict by investigating larval egg cannibalism behavior, a prime example of a selfish behavior that has been widely studied in other organisms. Importantly, it contributes novel theory and empirical data to the study of evolutionary conflict by providing the first test of inclusive fitness predictions in ant larvae. Its focus on *Formica* ants and their wide range of kin structures allows a comprehensive analysis of conflict across relatedness levels and confirms that larvae, much like adults, are constrained in their selfishness by kin selective forces. Within species, cannibalism behavior remains plastic however, indicating that larval selfishness is mediated by other factors as well. The benefits associated with cannibalism such as increased survival and up-regulation of key growth-related genes may explain the overall widespread occurrence of cannibalism in *Formica* ant larvae. The strong male bias in cannibalism across species suggests that the benefits of cannibalism may even outweigh the inclusive fitness costs for male larvae, shedding new light on the social role of male ants.

While the results of this thesis provide a first picture of the causes and constraints of among brood conflict, they also raise many new questions. Exploring the effects of cannibalism on larval development, growth and ultimately adult fitness is an especially exciting avenue for future research. Here, studies focusing on the potential link between cannibalism and female caste could help answer open questions about the power of developing females in the conflict over caste determination (Bourke and Ratnieks 1999; Ratnieks 2001; Strassmann et al. 2002; Beekman and Ratnieks 2003; Wenseleers et al. 2003; Rüger et al. 2007). The clear male bias in cannibalism furthermore calls for studies investigating the effects of cannibalism on male fitness, for instance by comparing fitness-related traits like body size (Davidson 1982; Crozier and Page Jr 1985; Wiernasz et al. 1995; Abell et al. 1999; Wiernasz et al. 2001) or wing symmetry (Jaffé and Moritz 2010) in cannibals and non-cannibals. As little is known about male development in ants, studies of the overall effect of nutritional levels on male larval development will prove especially useful in assessing the importance of selfish provisioning.

Just as important as the benefits of social behavior are its costs. This thesis gives a tentative estimate of the inclusive fitness costs associated with cannibalism, however it is likely that other constraints play a role in mediating this selfish behavior. For example, size constraints are known to mediate cannibalism across taxa, with larger individuals typically consuming their smaller counterparts (Polis 1981; Dong and Polis 1992; Iida 2003). Similarly, young *Formica* ant larvae may cannibalize significantly less often than older larvae because of differences in size, and especially because young larvae are not larger than eggs (pers. observations). Alternatively, lower benefits for young larvae – as suggested by gene expression comparisons – could explain why young larvae refrain from cannibalism. Comparison of cannibalism levels across relatedness levels in *F. aquilonia* furthermore indicates that larvae may be constrained in their ability to adjust cannibalism levels to fine-scale changes in kinship.

In social insects, colony-level costs of individual selfishness are thought to select against the evolution of kin-informative cues (Ratnieks 1991; Keller 1997) and only a few studies have found such cues in ants (Nehring et al. 2011; Helanterä et al. 2013). In line with this, evidence for kin-preferential behavior is limited (van Zweden et al. 2010, but see Hannonen and Sundström 2003 for an example in *Formica fusca*), even though patterns resembling nepotism may arise through passive processes (for example in *F. exsecta*, Holzer et al. 2006b). Whether larvae are prevented from behaving nepotistically because of a lack of kin-informative cues (as seems to be the case in adult *F. truncorum* workers, Boomsma et al. 2003) or their inability to discriminate fine scale odor variation merits further study, and especially investigations of odor perception in larvae promise novel insight on larvae power and its costs.

Assessment of the cost of cannibalism on the colony-level is one important aspect that could not be addressed within the scope of this thesis. In ants, selective forces acting on the colony as a whole can play a major part in shaping individual behavior. One example is when workers destroy eggs laid by other workers (so-called policing) because this is less costly to the colony - in terms of productivity - than supporting egg-laying freeriders (Ratnieks and Wenseleers 2005; Ratnieks et al. 2006; Wenseleers and Ratnieks 2006b). Cannibalism could prove similarly costly to the colony if it favors the production of excess selfish queen larvae over workers (for an example of selfish queen determination and its costs in bees see Ratnieks 2001), or leads to a general decrease in colony output in terms of worker and sexual production. In this case, workers should be under selection to prevent costly cannibalism (Carlin 1988; Baroni Urbani 1991). Evidence for selection on worker behavior to prevent costly cannibalism comes from studies of brood sorting behavior, which reveal that workers of some species put considerable effort into separating larvae from eggs (Franks and Sendova-Franks 1992; Sendova-Franks et al. 2004). In *Hypoponera* ants, where larvae have been shown to cannibalize other larvae, workers rapidly separate larvae brought in close contact during experiments (Rüger et al. 2007), confirming that worker behavior is adaptively shaped by brood conflict in these species.

In low-relatedness societies like the supercolonies of *F. aquilonia* ants on the other hand, high levels of selfishness and/or lack of social control of behaviors like cannibalism could contribute to long-term disintegration of cooperation (Helanterä et al. 2009), especially if such conflict occurs on a local scale. However, analysis of genetic substructure in *F. aquilonia* populations revealed that relatedness within nests can in fact be positive on a population scale, i.e. a population of multiple separate supercolonies, which lends support to the idea that supercolonies are clusters of nests with locally-elevated relatedness levels (Chapuisat et al. 1997; Kümmerli and Keller 2007; Holzer et al. 2009). Indeed, ant supercolonies often originate from a single founding colony and can have strict boundaries (Holzer et al. 2006a; Helanterä 2009). Still, queens may move across supercolonies and attempt adoption in foreign nests (Holzer et al. 2008). In nature, larval discrimination of alien eggs may therefore play a role when queens enter foreign nests. That *F. aquilonia* larvae did not discriminate between sibling, nestmate and supercolony mate eggs, but preferentially consumed eggs from an alien population backs behavioral evidence from other supercolonial species showing that supercolonies indeed may compete on a large spatial scale (Drescher et al. 2010). The network analysis approach introduced in this thesis will prove especially useful for analyzing such competitive interaction networks between supercolonies, and help elucidate general patterns of behavioral interactions and cooperation among individuals within these low-relatedness societies.

In the future, it will furthermore be interesting to assess plasticity in cannibalism in response to the genetic and/or odor diversity of eggs in other taxa. Comparative studies of larval selfishness in distinct populations of socially polymorphic species for instance could help confirm whether larvae respond plastically to variation in kin structure, or if cannibalism levels are an evolved response to average kin structures within species. Preliminary data on differences in cannibalism levels in single-queen versus multiple-queen colonies of the ant *F. truncorum* not included in this thesis shows that average cannibalism levels are five times higher in larvae from multiple-queen nests, which suggests that larval behavior is highly plastic in response to social structure (Schultner, unpublished data). Validating these results across ant genera, and especially in species that exhibit both supercolonial and family-structured colony organization (i.e. some invasive ant species, Suarez et al. 1999), will contribute substantially to our understanding of the role of genetic relatedness and species-specific biology in determining overall levels of selfishness within societies, and help identify general principles of social group maintenance.

Overall, this thesis provides a first framework for the study of selfish larvae behavior in Hymenopteran societies. Yet larvae power is not restricted to selfish provisioning through cannibalism, as revealed by the review of larvae literature in Chapter I. For example, ant larvae have been shown to solicit increased provisions from workers by displaying begging behavior (Creemers et al. 2003; Kaptein et al. 2005). Evidence also exists for other forms of offspring signaling, including the brood pheromones of honeybee larvae which are known to modulate worker foraging and provisioning behavior (Le Conte et al. 1995; Pankiw et al. 1998; Le Conte et al. 2001; Pankiw 2007) and physiology (Arnold et al. 1994; Mohammedi et al. 1996; Le Conte et al. 2001; Smedal et al. 2009). In queen-less honeybee colonies, workers are more likely to choose female larvae that exhibit high pheromone concentrations as future queens (Le Conte et al. 1994), which underlines that Hymenopteran brood pheromones can be a means of power for offspring to advertise their quality.

Analysis of the causes and constraints acting on the evolution of begging and other potentially selfish larval behaviors is therefore the obvious next step in the study of brood conflict in insect societies. Studies of offspring signal production and response, and in particular detailed exploration of the costs and benefits of signaling will help elucidate the impact of individual and colony-level selection on the evolution of larval behavior. Examining the potential effects of kinship on the variation in signaling within and between species will provide additional insight into evolutionary constraints on selfishness in social organisms. Together with the findings of this thesis, such studies promise to offer a new perspective on the evolution and stability of cooperation in social insect societies, and will pave the way toward a better understanding of the social forces that shape life on earth.

# Acknowledgements

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