REPRODUCTIVE PARTITIONING
IN THE POLYGYNOUS BLACK ANT
FORMICA FUSCA

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ABSTRACT

Individuals opt to breed cooperatively to increase their chances of successful propagation when resources are scarce or unpredictable. Yet, these same individuals are not genetically identical and come into conflict over limited resources they are to utilize for own reproduction. In spite of the predicted reproductive conflicts, many species, notably social insects, have evolved to become obligate cooperative breeders unable to propagate solitary. So how do individuals share reproduction? In social insects, reproduction and work is divided among the society members i.e. the queen(s) and workers. The evolution of a non-reproductive caste - workers, can be explained by kin selection theory, which holds that individuals gain fitness indirectly by helping their kin – queen, to reproduce. In many species of ants, however, colonies permanently contain multiple reproductive queens (polygyny). Queens compete for limited colony resources, which may lead to a conflict over personal reproduction and unequal reproductive shares. The reproduction by several queens also dilutes within-colony relatedness, which comes at a cost to worker indirect fitness. Hence, underneath the exemplary cooperation among colony individuals, both queens and workers, are predicted to act to enhance their own inclusive fitness.

The aim of my dissertation was to disentangle mechanisms underlying reproductive partitioning in a polygynous black ant, Formica fusca, within the framework of kin selection theory. I examined queen traits that likely impact queen fitness including timing of oviposition, queen presence during brood rearing, her fecundity, and viability and size of queen offspring, along with the underlying chemical communication, as well as workers ability to utilize and exploit the available information to manipulate queen reproduction to own fitness advantage. My thesis has revealed that both, queens and workers, have the means and ability to influence the outcome of reproductive competition by actively utilizing chemical cues present on ant cuticle to pursue selfish actions. At the same time, the results suggest that passive feedback mechanism between the queen reproductive output and worker behaviour, nevertheless, ensures alignment of fitness interests between both parties. Overall, my dissertation highlights that complex within-group interactions govern the reproductive partitioning in social insects and contributes to better understanding of how reproductive conflicts are resolved to ensure peaceful coexistence.
SUMMARY

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1. INTRODUCTION

1.1. Maximizing fitness in the group

Reproduction i.e. passing on genes to future generations is the most fundamental feature of life. Reproductive success of individuals will follow from their decisions shaped by physiological, environmental and evolutionary constraints on maximal reproduction (Stearns 1992). Whatever reproductive decision an individual takes must, however, increase its survivorship and reproductive success in comparison with an alternative option (Darwin 1859). Such reproductive decisions may concern, for example, whether to invest in reproduction versus growth, to reproduce in a single or multiple bouts, or the decision whether to breed solitarily or join a group. The advantages of cooperative breeding versus solitary breeding are often reflected in increased fitness of breeding individuals through a higher breeding output and/or better survival of the offspring (Pusey and Packer 1987; Creel and Creel 1991; Heinze and Oberstadt 2003; Russell et al. 2003). Moreover, fitness benefits may accrue if cooperative actions of individuals increase the production of related, but non-descendant offspring (Hamilton 1964). Nevertheless, group productivity does not necessarily translate to similar benefits for all group members and within-group reproductive conflicts arise whenever the cooperating individuals are not genetically identical (Hamilton 1964; Trivers and Hare 1976). This is because each individual is under selection to maximize its own inclusive fitness (Darwin 1859).

1.2. Social insects

Social insects represent the pinnacle of the continuum of cooperative breeding observed across diversity of taxa and include species, the individuals of which are unable to propagate without the help of others
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(Sherman et al. 1995). The remarkable level of cooperation and social organisation observed in social insects has led some biologists to liken the social insect colony to a “superorganism” in its own right (Wheeler 1911; Wilson and Sober 1989; Hölldobler and Wilson 2008). Reproduction in social insect colonies is divided between the reproductive queen(s) and the non-reproductive workers. The queen and worker castes are commonly morphologically distinct and have distinct tasks in the colony. Queens mate and lay eggs in some species well into thousands per day, while the workers are commonly unable to mate and reproduce sexually (Hölldobler and Wilson 1990). Instead, they care for the queen and her brood, and engage in colony maintenance, foraging and defense (Wilson 1971). The evolution of a non-reproductive worker caste can be explained by kin selection theory (Hamilton 1964), which holds that individuals can gain indirect fitness by helping their relatives i.e. queens to reproduce. If the indirect benefits of helping, weighed by relatedness, outweigh the cost of sacrificing one’s own reproduction, individuals are selected to help instead of reproducing directly.

However, as harmonious as these societies appear to be, they are rife with reproductive conflicts. Most notably, conflicts arise between queen(s) and workers over the allocation of resources between male and female offspring, or between the production of sexual brood (new males and queens) vs. colony maintenance (workers) (Bourke et al. 1995). Due to the haplodiploid sex-determination system of Hymenoptera, whereby females are produced from diploid eggs and males from haploid eggs, relatedness asymmetries arise between workers and queen-laid females and males. Workers are on average more closely related to their sisters (75%) than to their brothers (25%), unlike the queen who is equally related to both her offspring. Workers are hence predicted to bias the colony offspring production towards females (Trivers and Hare 1976). Moreover, the queen should favour higher investment into production of a worker than a sexual brood. This is because having more workers prolongs colony life and hence represents investment into the queen herself as opposed to investment into her offspring. Workers, on the other hand, should be indifferent as to their investment into parents versus siblings (Pamilo 1991). Moreover, workers in many species are able to produce haploid males parthenogenetically. Because workers are more closely related to their own offspring than to their brothers (queen-laid males) and nephews (worker-laid males), conflict arises between workers and the queen and among workers themselves over parentage of males (Bourke et al. 1995; Crozier and Pamilo 1996).
1.3. Polygyny in ants

In many ant species, several queens coexist and reproduce in a single colony (polygyny). Polygyny in mature colonies may be a consequence of cooperative colony founding (primary polygyny) in which several queens have survived to continue reproducing. More commonly, polygyny arises through the re-adoption of daughter queens back into their natal colony (secondary polygyny), which join their mothers in reproduction (Bourke et al. 1995). Polygyny, like cooperative breeding in general, is thought to have evolved in response to the high costs of individual dispersal (Bourke and Heinze 1994; Keller 1995). Benefits of polygyny include higher productivity (van der Meer et al. 1992; Heinze and Oberstadt 2003), and higher genetic diversity, which benefits the colony by increasing parasite resistance (Liersch and Schmid-Hempel 1998; Hughes and Boomsma 2004; Reber et al. 2008) and reducing the degree of inbreeding (Crozier and Fjerdingstad, 2001). However, polygyny has important consequences for the balance between cooperation and conflict within the colony (Ross and Keller 1995; Heinze and Keller 2000). With many queens present in a single nest, new reproductive conflicts arise such as: which queen gets to reproduce and to what extent (between queens), and whose brood should the workers rear and into what kind of offspring (among workers).

1.3.1. Consequences for queens

Each queen strives to become the dominant reproductive member of the colony, but because colony resources are limited queens must compete over their individual reproductive share (Medeiros et al. 1992; Sledge et al. 1996; Hannonen and Sundström 2002; Fournier et al. 2004). In addition, conflicts may arise between queens over the production of the most valuable brood (Pamilo and Seppä 1994; Bargum and Sundström 2007; Kümmerli and Keller 2007). Sexuals, i.e. future reproductives, confer much larger fitness benefits to their mothers than (largely) non-reproductive workers. Nevertheless, new workers are needed to ensure colony persistence and the survival of queens and their brood. In mature polygyne colonies queens rarely compete using physical aggression (Keller 1993). However, queens may differ in a number of intrinsic traits, such as age, condition, fecundity, and/or their ability to utilize resources, which may predispose some individuals to become reproductively dominant over others.
1.3.2. Consequences for workers

Simultaneous reproduction of several queens results in different matrilines of workers in the colony, and so reduces the average inclusive fitness payoffs the workers gain from rearing a queen’s brood. Under such circumstances, workers are predicted to assess the fitness payoffs currently available to them and respond appropriately to increase their indirect fitness. Workers could increase their fitness via selective antagonism and/or providing preferential care towards the queen(s) and brood to which they are most related (Hamilton 1972). Moreover, workers in many ant species have retained their ability to produce haploid males parthenogenetically. If relatedness in the colony is low, because many queens reproduce, workers should become selfish and start laying their own eggs. At the same time, low relatedness conditions should select for counteractive measures to prevent worker reproduction (i.e. worker policing) (Bourke 1988; Wenseleers et al. 2004b) and the potential of colony collapse.

1.4. Reproductive conflicts and access to information

Although reproductive conflicts among individuals may be predicted based on relatedness arguments (i.e. potential conflicts) they may not necessarily be detectable in their behaviour (i.e. actual conflicts) (Ratnieks and Reeve 1992). Such a discrepancy between potential and actual conflict is exemplified in the social wasp Polistes dominulus, the workers of which unconditionally accept an unrelated replacement queens and rear her brood. Moreover, workers do not reproduce and offset the zero indirect benefits from the replacement queen’s reproduction, even if they can lay eggs (Monnin et al. 2009). The lack of a predicted response may arise if individuals are unable to access information required to make decisions and/or are constrained to or otherwise prevented from responding appropriately upon the information received (Ratnieks et al. 2006).

Hence, the ability to access information about identity and the reproductive potential of other individuals is of crucial importance in order to enhance one’s own fitness. In social insects, such information is primarily mediated via hydrocarbons associated with the insect cuticle (CHCs) (Monnin 2006; Liebig 2010). In ants, CHCs are produced by queens and workers in postpharyngeal gland (Bagnères and Morgan 1991) and distributed among colony members either through direct contact (Boulay et al. 2000) and/or via nest material (Bos et al. 2011). CHCs inform nestmates about their
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reproductive status (Sledge et al. 2001; Dietemann et al. 2003), fecundity (Hannonen et al. 2002; Holman, Dreier, et al. 2010) and/or identity (Hannonen and Sundström 2003a; El-Showk et al. 2010). In polygynous species, queen pheromones appear to regulate the fecundity of other queens (Vargo 1992; Yamamoto and Matsuura 2011; Holman et al. 2012). The same chemical cues are also present on queen-laid eggs (Endler et al. 2004; Holman, Jørgensen, et al. 2010; Holman et al. 2012) and attract worker attention (Matsuura et al. 2010), which increases the likelihood of such eggs being reared.

The ability to discriminate kin from non-kin is necessary to enable nepotism, i.e. preferential helping to more related individuals. In social insects, however, kin discrimination has only rarely been reported (see Wenseleers 2007), most likely due to the erosion of precise enough cues to allow discrimination between close and more distant kin within a colony (van der Meer and Morel 1998; Dani et al. 2004; Boomsma and D’Ettorre 2013; Breed et al. 2015). This is thought to be a result of cue mixing due to selection favouring group cohesion (i.e. nestmate recognition) (Crozier and Dix 1979; Holman et al. 2013). Hence, individuals may be constrained in their ability to act selfishly. In addition to cue mixing, active intervention of individuals with the reproduction of others (e.g. aggression towards ovipositing individuals and/or their eggs) may prevent selfish actions and favour the evolution of self-restraint (Wenseleers et al. 2004a).

1.5. Enforcing reproductive interests

Given that all individuals aim to maximize their fitness in the nest, the outcome of competition will depend on the relative power that each party has over the reproduction of (other) queens (Beekman et al. 2003; Beekman and Ratnieks 2003). In other words, who controls the reproductive allocation in the ant colony: is it the queens, the workers or both? High reproductive capacity of the queen and her ability to produce new queens are indispensable to the colony persistence and to indirect fitness of workers. Moreover, ant queens are often larger than workers, and thus produce larger quantities of pheromone, and able to enforce their reproductive interests to gain the cooperation of workers (Holman et al. 2010; Matsuura et al. 2010) and influence the reproductive output of other queens (Yamamoto and Matsuura 2011; Holman et al. 2012). On the other hand, workers should be especially motivated to make up their reduced direct fitness (Hamilton 1964), and in doing so shape the outcome of competition among queens.
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They are superior in number to queen(s) and may enforce queen reproductive fate physically (Keller et al. 1989; Keller and Ross 1993; Chernenko et al. 2012). Workers do have ample opportunities to act selfishly, because queens and their brood are entirely dependent on them for care and survival. But do they have the means of doing so?

2. AIMS OF THESIS

In my PhD thesis, I studied the mechanisms underlying reproductive partitioning in multi-queen ant species with the aim to disentangle how queen reproductive shares are determined and who controls the reproductive allocation. I examined queen traits that are likely important in shaping queen reproductive output together with the chemical cues that advertise them as well as the ability of individuals, both queens and workers, to perceive and act upon the available information to enhance their own inclusive fitness.

In Chapter I, I investigated whether an early onset of oviposition relative to a nestmate queen and queen presence during brood rearing confers fitness benefits to a queen. Moreover, I examined whether worker reproduction is conditional on their relatedness to queens currently reproducing in the nest, when relatedness is inferred from the queen’s brood.

In Chapter II, I examined whether queen realized fecundity (i.e. her egg-laying rate), and queen cuticular chemistry, by which queen advertises her fecundity to nest mates, is dependent on the initial fecundity of the queen at the onset of oviposition and the density of workers associating with the queen.

Chapter III, I examined the presence of trade-offs between queen fecundity and offspring quality (hatching success and larvae size) in queens of disparate fecundities. Furthermore, this chapter investigates how oviposition timing and social environment (i.e. worker density) shape queen maternal investment, and ultimately affect queen fitness.

In Chapter IV, I investigated whether queens compete via fecundity or cannibalize each other’s eggs, and whether chemical cues on the cuticle of queens and/or their eggs are used by nestmate queens to discriminate kin and adjust the level of competition to enhance their own reproductive output.
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3. MATERIAL AND METHODS

3.1. Study species: *Formica fusca*

The black ant, *Formica fusca* (Linnaeus 1758) is a common ant species in Fenno-Scandinavian region (Collingwood 1979). *F. fusca* is a pioneer species, which often rapidly colonizes recently felled forest areas and bogs (Puntila et al. 1991; Czehowski et al. 2002). Colonies are relatively small and contain between 500-2000 workers that build their nests in rotten tree stumps (Savolainen and Vepsäläinen 1988). In Finland, polygyne *F. fusca* colonies exist alongside the monogyne (one-queen) colonies within the same populations (Bargum et al. 2007). Polygyne nest may contain anything up to 50 queens (personal observation) but most commonly between 2-3 queens co-reside (Hannonen et al. 2004).

Moderately high average relatedness among queens in polygyne nests ($r=0.39-0.64$; Hannonen et al. 2004; Bargum 2007) suggests that recruitment of daughter queens (i.e. secondary polygyny) in *F. fusca* is common. The reproductive life-span of individual queens appears to be short, since up to 35% of queens are replaced annually (Bargum et al. 2007). Nest mate queens are of similar size (Hannonen and Sundström 2003b), and coexist peacefully (Hannonen and Sundström 2002), yet differ in their fecundity at the onset of oviposition (Hannonen and Sundström 2002) as well as final reproductive shares (Hannonen and Sundström 2003b; Bargum and Sundström 2007). Queens communicate their fecundity using cuticular hydrocarbons and workers respond to these by preferentially associating with highly fecund queens (Hannonen et al. 2002). *F. fusca* workers show precise nestmate recognition capabilities (Helanterä et al. 2007; Helanterä and Sundström 2007; Chernenko et al. 2011), and more importantly in the context of this thesis, workers are able to distinguish queen-laid from worker-laid eggs (Helanterä and Sundström 2007) as well as between eggs laid by different queens (Hannonen and Sundström 2003a). Workers readily start laying male eggs upon colony orphaning (e.g. when queen dies) but they do also lay eggs in the presence of the queen (Helanterä and Sundström 2005).

Given the above characteristics, their plentitude and the ease with which they can be manipulated, polygyne colonies of *F. fusca* provide an ideal model system in which to investigate reproductive partitioning.
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3.2. Experimental work and behavioural assays

Experimental work provides the core of my thesis work. Entire colonies of *F. fusca* were collected in spring before the onset of oviposition, in the proximity of the Tvärminne Zoological Station (on Hanko peninsula, southern Finland). Polygyne colonies were used to establish worker fragments that would provide a nest for the queen(s) and/or brood, and enable manipulation (Chapters I-III), or used as a source of queens for behavioural assays in the absence of workers (Chapter IV).

To investigate the effect of oviposition timing on queen final reproductive share (Chapter I), I split each two-queen colony into two fragments of 100 workers. In a reciprocal design, I then introduced 50 eggs from both queens with a 5 day delay in between (i.e. on day 1 and 6) into both worker fragments. To investigate the effect of queen presence on her reproductive share (Chapter I), in addition to above, the design described was replicated with one of the nest mate queens being present in each worker fragment. The queen was caged in a box covered with mesh to prevent physical contact with the workers but allowing transfer of chemical cues. In both cases, workers were allowed to rear the brood to pupation at which point the brood was collected, and its caste (i.e. male, gyne or worker) and maternity (i.e. queen(s) or worker) was determined using genotyping, to define reproductive shares of queens and the extent of worker reproduction.

To investigate changes in fecundity and cuticular chemistry of queens kept at different worker densities (Chapter II), and the trade-offs between queen fecundity, offspring size and hatching success (Chapter III), I split each colony into fragments containing 5 and 50 workers to simulate low and high worker densities. One queen from each two-queen colony (or two from 4-queen colonies where available) was put in a low, and one in a high worker density fragment, and their egg-laying rate was recorded over the period of 24 days. At that point queens were freeze-killed and their cuticular hydrocarbons extracted for further analyses (Chapter II). The eggs collected from the low and high worker density fragments were reared on Petri dishes until hatching and newly emerged larvae were photographed, measured and sexed (Chapter III).

For the behavioural assays (Chapter IV), I used highly polygyne colonies from which queens were removed and their behaviour tested in the absence of workers. I recorded changes in queen egg-laying rate in response to a glass bead covered with a CHC extract from another nestmate queen.
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(simulating the presence of another queen) over 5 day period. Another set of queens, some of which were prevented from initiating oviposition, was presented with queen-laid eggs of different provenance and composition (i.e. own, nestmate or mixture of both) to assess the extent of egg cannibalism with respect to queen reproductive status and relatedness to eggs over 5 day period (Chapter IV).

3.3. Genotyping

To assess whether physiological and behavioural responses of queens and workers were mediated by their genetic relatedness to each other, I genotyped all the experimental queens and a subset of colony workers at the end of the experiments (Chapters I, II, III, IV). Since *F. fusca* workers can also reproduce, brood was genotyped to ascertain queen maternity (Chapters I, II, III) and to determine queen reproductive share when broods were mixed (Chapter I). Moreover, in Formica ants, males develop from haploid and females from diploid eggs, and brood sex could be determined based on the presence or absence of heterozygous loci in the offspring genotype (Chapter III). I used microsatellite markers developed for Formica species and successfully cross-amplified in *F. fusca*: FL12, FL20, FL21 (Chapuisat 1996); FE13, FE16, FE19, FE21, FE42, and FE51 (Gyllenstrand et al. 2002) and FY4, FY7, FY13 (Hasegawa and Imai 2004). PCR and allele calling protocols were optimized for each chapter, and are described in more detail therein. Average and pairwise relatedness (*r*) based on sample-wide allele frequencies was estimated using Relatedness (Queller and Goodnight 1989; Chapters I, II, III) or ML-relate (Kalinowski et al. 2006; Chapter IV).

3.4. Chemical analyses

To determine the link between queen chemical profile and her fecundity and/or the number of workers residing with the queen (Chapter II) I extracted the non-volatile compounds from the cuticle of dead queens. I then analyzed CHCs profiles using Gas Chromatography coupled with Mass Spectrometry (GC-MS) to qualify and quantify the different classes of CHCs detected on the queen cuticle (more detailed description in Chapter II). I then compared the CHC profiles between queens residing in different worker densities.
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4. RESULTS AND DISCUSSION

Initially I discuss the main findings of my thesis in the light of queen and worker fitness separately and provide a synthesis at the end.

4.1. Enhancing fitness - queens

4.1.1. Timing of oviposition and sexual production

The ability of a queen to commence oviposition early in the season relative to her nest mates may confer a fitness advantage, if workers preferentially rear early brood, or if it develops into sexual brood. Seasonality limits the time available for reproduction and in boreal regions, should favour the rapid onset of oviposition in spring. This may also be reflected by the fact that in boreal Formica ants, sexuals are usually reared from the first brood cohort of the breeding season (Collingwood 1979; Gösswald 1989). Taken together, the ecological incentives for queens to initiate oviposition early are high. In Chapter I, I show that, as expected, the queen that oviposits earliest gets more sexual pupae reared than the queen that oviposits six days later (results in Chapter I). Because sexual pupae represent a queen’s future reproductive offspring, the relative timing of oviposition is of crucial importance to polygyne F. fusca queens. In F. fusca, queens differ greatly in their fecundity and hence their ability to start laying eggs (early), but whether these differences are attributable to queen quality, age and/or differences in competitive ability, or results from preferential treatment by workers prior to, or shortly after, hibernation remains to be investigated in more detail.

4.1.2. Queen presence and reproductive share

Following nest establishment, an ant queen is entirely dependent on workers for her survival, reproduction and brood care. She should hence benefit by advertising her presence in the colony to gain worker attention for herself and her brood. This is especially important in species with high queen turnover, such as F. fusca (Bargum et al. 2007), where queen residence in the colony and her reproductive lifespan may be short and reproductive success unpredictable (Keller et al. 1989; Keller and Ross 1998). In colonies of social insects non-volatile hydrocarbons (CHCs) associated with the
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queen’s cuticle and her eggs appear to signal the presence of a reproductive queen (Ortius and Heinze 1999; Endler et al. 2004). Workers are expected to respond to the queen’s signal by associating with her and/her brood and thereby promoting their survival because it also increases the indirect fitness of the workers (Keller and Nonacs 1993). In Chapter I, I show that workers reared disproportionately more eggs into pupation of the queen present in the nest during brood rearing, than of the absent queen (Figure 1a). Moreover, the share of sexual brood that a queen gains by reproducing early (results in Chapter I) was larger when the queen was also present in the colony (Figure 1b), suggesting additive effects of queen presence and early reproduction on queen fitness. Because the queen present in the colony was kept in a cage during the experiment, the results confirm that chemical cues alone are sufficient to communicate queen presence. Moreover, workers appear to be able to perceive and combine the chemical information from different sources (queen cuticle/nest material and eggs) in order to target preferentially the brood of a present queen.

4.1.3. Competition via fecundity

Queens may influence each other’s reproductive share directly i.e. independently of workers. Queens can inhibit the fecundity of other nest mates with pheromones (Vargo 1992; Yamamoto and Matsuura 2011) or compete via increasing their own fecundity in the presence of a nest mate queen. Although each queen is predicted to act to increase her own direct fitness, increasing fecundity may be costly. I find no strong evidence in F. fusca that the presence of a queen (per see) inhibits or promotes fecundity of
her mature nest mates, as neither a consistent decrease nor an increase in queen fecundity was observed in queen associations compared to when a queen resided alone. Instead, the queen decreased her own fecundity when in the close proximity of a fecund and related nest mate, but increased it when the nest mate was unrelated (Figure 2). These results testify to the ability of queens to perceive kin-informative chemical cues from the cuticle of their nest mates and their ability to pursue indirect, in preference to direct, fitness benefits if queens are related (Hamilton 1964). Such facultative investment into direct reproduction based on nestmate relatedness has rarely been reported in queens of social insect (see Harradine et al. 2012) and suggests that increasing fecundity may be costly for the queens, contrary to general beliefs. Everything else being equal, this suggests that early ovipositing queens may be able to benefit in form of a larger reproductive share if co-residing with relatives, although confirmation would require brood rearing experiments.

4.1.4. Egg cannibalism and reproductive status

Egg cannibalism may provide queens with additional nutrition needed during oviposition and simultaneously serve as an effective tool to reduce the
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reproductive output of nest mates (Bourke 1991; 1994). For effective egg cannibalism to occur, however, queens should cannibalize before the onset of their own oviposition, unless they are able to distinguish their own eggs from those laid by nest mate queens. On the other hand, *F. fusca* queen eggs do contain queen-derived chemical cues (Helanterä et al. 2014) which appear not easily transferable between eggs, hence kin recognition should be possible (d’Ettorre et al. 2006). I tested for the presence and the extent of egg cannibalism among *F. fusca* queens in Chapter IV. Although egg cannibalism appears to be a rare behaviour in this species, non-reproductive queens i.e. queens that have not yet began to oviposit cannibalized more than reproductive queens (results in Chapter IV). If queens are unable to tell the eggs apart, this result is theoretically expected because the cost of cannibalizing one’s own eggs will be negligible to the queen that has not yet reproduced. The instances of cannibalism among reproductive queens was similar regardless of the relatedness of queen to the available eggs (results in Chapter IV), which indicates that queens are unable to tell the eggs apart or the costs of doing so are prohibitive. The first scenario is unlikely given queens can utilize and respond to the same cues from the queen cuticle (Chapter IV). However, once reproduction has been initiated, a trade-off likely arises between queen investment into oviposition and cannibalism, which could lead to a queen becoming insensitive to egg-derived cues.

4.1.5. Maternal investment and trade-offs

Life-history theory predicts that there is a trade-off between fecundity and offspring size (Smith and Fretwell 1974; Lloyd 1987). Given that *F. fusca* queens differ in their fecundity (Hannonen et al. 2002; Hannonen and Sundström 2002), such trade-offs should impact reproductive competition among queens, if offspring of some queens survive better or have higher future reproductive potential than others. Nevertheless, investment per offspring in the context of reproductive competition has rarely been considered in studies of social insects (see Kranz 2005). The reproductive trade-offs in *F. fusca* queens were the subject of study in Chapter III, where I examined egg hatching success and size of newly hatched larvae among queens of different fecundities. The results show that at any given time during oviposition, there is a trade-off between queen fecundity and the size of her newly hatched larvae and that larger larvae have higher hatching success (Figure 3a, results in Chapter III). However, results also show that early in oviposition queens are able to increase their fecundity without compromising egg-hatching success, whereas fecundity and hatching success
4.2. Enhancing fitness - workers

4.2.1. Worker density and queen fecundity

Queens in mature colonies are entirely dependent on workers for their survival and reproduction. A higher worker:queen ratio increases egg-laying rates of queens (Tschinkel 1988; Offenberg et al. 2012), which is likely a result of higher worker attention and/or nutrition provided to queen (Chen and Vinson 2000; Trettin et al. 2011; Abril and Gómez 2014). If the reproductive output of queens is positively correlated with the amount of care she receives, then workers have the means to influence their indirect fitness by providing care to queens selectively. I show in Chapter II that the egg-laying rate of all queens indeed increased when density of workers (i.e.
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their worker entourage) was high compared to low (Figure 4ab). Whether F. fusca workers preferentially associate with their mother as opposed to less related queens awaits further investigation. Nevertheless, workers may preferentially respond to queens of high fecundity because they are on average more likely to be related to them (Forsyth 1980; Reeve and Keller 2001) Alternatively, high fecundity of the queen may offset fitness losses due to lower kin value of the queen. F. fusca workers have previously been shown to preferentially associate with more fecund queens (Hannonen et al. 2002). Analyses of CHCs revealed that queens of high vs. low fecundity at the onset of the reproductive season differed in their CHC profiles even after three weeks of reproduction (results in Chapter II). This could provide a means for the workers to selectively favour queens of high reproductive potential. Moreover, queens that started to oviposit as highly fecund continued to lay more eggs than those with low fecundity but only if residing with a large worker entourage (Figure 4ab, Chapter II). This suggests that positive-feedback exists between queen fecundity and worker density. Moreover, worker density was also reflected in queen cuticular profile (results in Chapter II). Hence, queens signal their worker association, potentially providing workers with proximate bases for assessing queen future reproductive potential. Such feedback between queen physiology and chemistry could, in longer term, lead to the alignment of fitness interests between queens and workers, providing that egg quality is not traded-off against egg quantity.

4.2.2. Worker density and queen reproductive investment

Given that a queen trades-off the offspring number against offspring quality (Chapter III), worker benefits from increasing queen fecundity may not become realized, unless high worker density allows a queen to increase her total investment into her offspring. Results of Chapter III show precisely this - queens that increase their fecundity when with workers are also able to invest into larger larvae early in oviposition that does not come at cost to hatching success (Figure 5ab, results in Chapter III). This together with the observation that hatching success generally increases with offspring size (Chapter III), suggests that high density of workers allows queens to increase their total reproductive investment early in oviposition and so relaxes the trade-offs between offspring number, size and hatching success. Presuming a larger larvae are more likely to develop into sexual rather than worker offspring (Bier 1952; Gösswald and Bier 1953), such adjustments to reproductive investment early in oviposition should benefit both queens and
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Figure 4 – Queen fecundity i.e. her daily egg-laying rate (mean ± CI) between days 2-24 of the experiment in nests with queens of high a; n = 25) and low (b; n = 38) fecundity respectively split by worker treatment. Day 0 depicts queen initial fecundity and the vertical dashed (day 2) marks the queen transfer into separate worker treatments.

Figure 5 – The relationship between the size of newly hatched larvae (a) and hatching success (i.e. proportion of hatched larvae) (b), and the change in queen fecundity, early and late into oviposition. X-axis shows the change in queen fecundity with workers (realized – initial egg-laying rate; zero indicates no change).

workers. On the other hand, the queens that decrease their fecundity with workers also reduce their investment per offspring, which suggests that such queens suffer considerable losses in fitness. This should provide an incentive to queens to attract large worker entourage.
4.2.3. Indirect fitness returns and worker reproduction

Theory predicts that workers should only reproduce if the benefits of doing so outweigh the costs of caring for queen brood (Hamilton 1972; Bourke 1988). This presupposes that workers are able to assess the indirect benefits of rearing the queen brood i.e. their relatedness of queen and/or her brood. I show in Chapter I that the proportion of worker-produced males increased with decreasing average relatedness between nestmate queens reproducing in the nest (Figure 6; Chapter I). This testifies to workers being able to adjust their reproductive decisions facultatively in response to changing fitness payoffs (also see Foster and Ratnieks 2000). Moreover, given the experimental design (the absence of direct contact with queens), results suggest that workers based their decision on the CHCs from queen-laid eggs. Although CHC may not be easily transferable between eggs (d’Ettorre et al. 2006), worker response to average kin value may be expected as brood from different queens is reared communally in Formica ants (Sendova-Franks et al. 2004), and the individual egg recognition may be error-prone. Moreover, F. fusca the relatedness between queens is often very high and/or that of queens to workers low (e.g. results in Chapter II), rendering the fitness benefits from individual recognition of queens low. Such facultative worker reproduction should be especially beneficial in species, such as F. fusca, where continuous replacement of queens results in changing relatedness and indirect fitness benefits that ensue.

Figure 6 – Male production (number of males) as a function of queen-worker relatedness (n=14 colonies): queen-produced males (open circles, dashed line) and worker-produced males (closed circles, full line).
4.3. Synthesis

The combined results of my thesis clearly illustrate the fitness benefits for those *Formica fusca* queens that are highly fecund at the onset of colony oviposition. Queens that start reproducing early, gain larger share of the sexual brood because workers invest care preferentially into early eggs (Chapter I). Moreover, early in oviposition fecund queens are able to enhance their egg production without reducing offspring hatching success (Chapter III). In addition to this, results of Chapter IV suggest that by ovipositing early queens may be able to escape fecundity inhibition by related nest mates. So what makes a fecund queen? I show that queen fecundity is largely a function of worker density i.e. the size of the queen worker entourage during queen oviposition. Having a large worker entourage increases queen fecundity (Chapter II) and, early in oviposition, enables the queen to increase her total maternal investment (Chapter III). Conversely, having a low worker entourage has the opposite effect on queen oviposition. Moreover, an initially fecund queen with high worker entourage is able to maintain higher oviposition rate than queen with initially low fecundity. Such positive feedback between queen physiology and worker behaviour should benefit both the queen and workers alike, providing workers are related to queen, and could eventually lead to functional monogyny, with colony brood being produced by one, well-endowed queen. This is, however, not the case in *F. fusca*, in which reproduction in the colony is shared (Bargum and Sundström 2007)

High queen turnover in polygynous colonies of *F. fusca* (Bargum et al. 2007) calls for a mechanism of queen exchange. Changes to composition of queen caste likely take place once the brood pupates and new queens emerge. So who gets to reproduce in the spring and how do new queens enter into competition with resident queens? Earlier analyses of *F. fusca* brood revealed that queens partition sexual and worker reproduction in such a way that worker relatedness to sexual brood in spring increases (Bargum and Sundström 2007). This suggests a queen that produces the sexuals in spring is the mother of contemporary workers i.e. workers produced the previous summer. Given that new workers are produced every year, it is conceivable to assume that a large portion of the worker count prior to the onset of hibernation is composed of newly emerged individuals. To promote their future indirect benefits, these naïve workers should preferentially associate with their mother, if this promotes her condition and successful hibernation, and allows her to reproduce early the next spring. This could be assured by
preferentially associating with their mother, or more indirectly, with the most fecund queen (Hannonen et al. 2002). Here, the CHC signal of worker density on queen cuticle linked to queen previous reproductive success (Chapter II) could provide the means to facilitate queen-worker association. This mechanism does not preclude kin recognition of queens by workers, rather offers an alternative solution when direct cues to fecundity (i.e. eggs) are not available. Indeed, I show in Chapter I that workers are able to assess their indirect fitness gains from rearing queen brood based on chemical information on queen laid eggs and adjust the level of own reproduction to the amount of these gains i.e. their relatedness to reproducing queens.

Ensuing from the above, queens should compete for worker attention, and/or sexual production, which may lead to a low hatching success in eggs produced by low quality queen i.e. those unable to attract sufficiently large worker entourage early in oviposition (Chapter III). If increasing fecundity is costly to these queens (Chapter III), the late onset of oviposition may benefit them two-fold. Firstly, by cannibalizing eggs of their early reproducing nestmates (Chapter IV) queen may gain nutrition independent of worker provisioning (and hence worker entourage) which may advance her own oviposition (Hora et al. 2007). Secondly, starting oviposition later allows such queen to assess the level of indirect fitness gains from already reproducing queens and save resources by not entering into competition with fecund relatives (Chapter IV). This could allow such queens to invest into worker production late during colony oviposition, when competition for sexual production subsides. I show in Chapter I that by advertising her presence to workers even relatively later ovipositing queen may be able to gain a share of worker brood (Chapter I) and hence, enter into queen-queen competition for sexual production the next spring.

The ready changes in fecundity demonstrated by *F. fusca* queens in response to oviposition timing (Chapter III), worker density (Chapter II) and nest mate relatedness (Chapter IV) suggests a large environmental component to queen fecundity in this species. Queen phenotype and/or age may nevertheless underlie differences in queen quality and/or fecundity based on which workers form associations with new queens. There is some evidence that fecundity increases with queen age in *F. fusca* (unpublished results), and such correlation has been observed in other ant species (Heinze and Schrempf 2012; Heinze et al. 2013). Elucidating the genetic component to queen quality and/or fecundity would require breeding experiments. However, high sensitivity of this species to conditions for mating and hibernation thus far precluded long-term monitoring of laboratory colonies.
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Overcoming this challenge would allow for more rigorous experimental testing of the causal effects of queen age, relatedness and/or prior worker exposure on reproductive partitioning in this species.

5. CONCLUSIONS

In conclusion, I found that complex interactions between individuals govern reproductive partitioning in *Formica fusca*, with both queens and workers having the means and power to enhance their inclusive fitness in a polygynous colony. I show that breeding phenology of the species can profoundly shape the extent of reproductive conflicts between individuals, but may simultaneously create opportunities for enhancing fitness. Moreover, I show that at individual level life-history trade-offs provide an important insight behind the reproductive decisions of individuals and should be more frequently incorporated in studies on social species. Demonstrated kin recognition abilities and facultative responses of both queens and workers in this species, highlight that genetically based cues are present and utilized in social insect species, which goes against the general notion that these cues erode over time due to selection for colony-level recognition (Keller 1997; but see Wenseleers 2007). In addition, my thesis demonstrates that regardless of whether kin recognition occurs, passive mechanisms may lead to alignment of reproductive interests among colony individuals to ensure reproductive harmony and colony cooperation. In line with mounting evidence, within-colony chemical communication serves as a proximate basis for both active and passive mechanisms of conflict resolution in social insects.

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Lúbim Vás oboch najviac na svete. Babuľka, tie moje mravčie kráľovníčky by nikdo nevystihol lepšie.

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