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**RECOGNITION AND SOCIAL BEHAVIOUR IN *FORMICA*
ANTS**

ANTON CHERNENKO

ACADEMIC DISSERTATION

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DEDICATED TO MY FATHER **VLADIMIR CHERNENKO**, WHO MADE IT ALL POSSIBLE,
BUT COULD NOT WITNESS MY PROGRESS.

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Chapter I - Egg recognition and social parasitism in *Formica* ants

Chapter II - Colony kin structure and queen recruitment in the ant *Formica fusca* (Hymenoptera: Formicidae)

Chapter III - Colony take-over and brood rearing success in socially parasitic *Formica* ants

Chapter IV - Cuticular hydrocarbons and genetic similarity in sexuals of *Formica fusca* ants

List of original publications

This thesis is based on the following publications:

- I) Chernenko, A., Helanterä, H. & Sundström, L. 2011. Egg recognition and social parasitism in *Formica* ants. *Ethology* 117(12): 1081-1092.
- II) Chernenko, A., Helanterä, H. & Sundström, L. 2012. Colony kin structure and queen recruitment in the ant *Formica fusca* (Hymenoptera: Formicidae). *Myrmecological News* 16: 93-100.
- III) Chernenko, A., Vidal-Garcia, M., Helanterä, H. & Sundström, L. Colony take-over and brood rearing success in socially parasitic *Formica* ants. *Manuscript*.
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The publications are referred to in the text by their Roman numerals.

Contributions

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Abstract

Communication is probably one of the major means of life maintenance. Communication involves the use of signals, which can be visual, audial, olfactory etc. Organisms communicate in many different contexts, ranging from establishing own identity, foraging for food, finding a mate, protecting their territory, to more sophisticated ones such as engaging in social behaviour.

Recognition is the action or process of recognizing or being recognized. Recognition based on olfactory cues is perhaps best known from many insect species and is mediated by a wide range of volatile compounds, e.g. esters or terpenes, or non-volatile compounds such as cuticular hydrocarbons. In insects cuticular hydrocarbons are often the main agents involved in species recognition, predator avoidance, fertility and dominance signalling, cues that help parasites intrude their host nests but also those that help hosts to fend off the parasites; and also as cues facilitating sexual selection. In social insects in addition to above mentioned, cuticular hydrocarbons are involved in coordinating division of labour and nest mate recognition.

The aim of my thesis was to study recognition system underlining social behaviour in *Formica* ants such as recognition of con- and hetero-specifics, e.g. nestmates and social parasites, and

their brood as well as to examine cuticular hydrocarbon profiles of males and females.

The results show that queens of potential social parasites have a very low chance to invade host colonies of *Formica* ants and become fully integrated. Moreover, social parasitism pressure, beside from affecting how the parasites are discriminated against, may also have an effect on the host kin recognition system and lead to rejection errors of descendant brood. Thus selection mediated by temporary social parasitism may drive enhanced recognition abilities. Colony kin structure does not seem to have any effect on recognition system towards con-specific non-nestmate and nestmate queens as both low- and high-relatedness colonies were as stringent towards unrelated individuals, although colonies with presumably high genetic diversity also showed significantly greater chemical diversity based on chemical profiles of sexuals. Hence nest mate recognition entails more complex interactions between individual genotypes and colony recognition cue phenotype than previously assumed.

This thesis provides important insights on host-parasite interactions and highlights the complex interactions between different selection regimes affecting recognition system.

Summary

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1. Communication and recognition

Communication undoubtedly plays a great role in life maintenance. All communication involves the use of signals, which can be a sound, a look, a motion, an odour etc. Signals have evolved for communication between a sender and a receiver, whereas cues are used as information for one party, but are not necessarily an adaptation of the other party (Maynard Smith and Harper 2003). There are several contexts in which organisms need to communicate, e.g. establishing own identity, foraging for food, finding a mate, engaging in social behaviour and protecting their territory. At the cellular level communications and information use are mediated chemically, whereas whole organisms employ visual, auditory and olfactory pathways (Tomecek 2009).

Recognition based on olfactory cues is perhaps best known from many insect species that live in darkness, for example ants or termites, for which visual recognition is difficult. In contrast, their recognition is mediated by a wide range of volatile compounds, e.g. esters or terpenes, or non-volatile compounds such as cuticular hydrocarbons (Blomquist and Bagnères 2010). The principal role of cuticular hydrocarbons is to prevent desiccation and protect against environmental forces (Baker et al. 1963). However, they have secondarily evolved

as the main agents involved in species recognition, predator avoidance, fertility and dominance signalling, cues that help parasites intrude their host colonies but also those that help hosts to fend off the parasites; and also as cues facilitating sexual selection (Blomquist and Bagnères 2010). In social insects in addition to above mentioned, cuticular hydrocarbons are involved in coordinating division of labour and nest mate recognition (Howard and Blomquist 2005; van Zweden and D'Ettorre 2010).

Recognition involves the matching of a label (an odour cue), which is produced by a sender, with a template of a receiver (the neural representation of the cue stored in the memory). Depending on the match/mismatch of the label to the template, the sender is accepted or rejected and particular behaviour is demonstrated. The expression of the label can be split into a heritable component and an environmentally derived component (e.g. from food, nest material etc.), or could have both components simultaneously. Thus the label is a variable trait. As the environmental component can vary greatly both between individuals and over time, recognition system should be flexible to allow for discrimination even with constantly fluctuating recognition cues (van Zweden and D'Ettorre 2010).

Moreover, in social insects colony kin structure may vary to a large extent, both between and within colonies. As a result in colonies with low relatedness variability of cues may be rather high, thus further complicating recognition (Vander Meer and Morel 1998).

Different selection regimes may affect recognition system. For example, empirical studies suggest that parasitism and nest mate recognition may refine

recognition cues and lead to their diversification (Martin et al. 2011), whereas a need for species recognition may tend to even out recognition cues (Martin et al. 2009). In some cases selection might work in opposite directions, for example selection on sexually dimorphic profiles may counteract selection for a distinct nest profile (Martin et al. 2009).

1.1. Recognition and social parasitism

The recognition template in colonies with varying kin structure should be wider to allow for nest mate recognition and rejection of intruders. Such wider and less restrictive template may perhaps weaken recognition system and facilitate colony invasion by parasites.

Social organisms, such as social insects, represent a particularly favourable host for parasites due to high densities of genetically similar individuals and accumulation of resources (Schmid-Hempel 1998). Social insects are targeted by a wide range of micro and macroparasites and in some cases social insects themselves have secondarily evolved into parasites and exploit host colonies of other, often closely related social insects (Buschinger 1986; Hölldobler and Wilson 1990). Such social parasites may utilize their hosts in many ways, from relatively non-disturbing such as nest sharing, to brood or food theft, slave-making and nest take-over, and finally to permanent social parasitism, inquilinism (Wojcik 1989; Lenoir et al. 2001; Buschinger 2009). To invade their host social parasites can use either chemical camouflage (recognition

cues are acquired from its host) or chemical mimicry (recognition cues are synthesized by the parasite), or a combination of both (Lenoir et al. 2001)

In temporary social parasitism newly mated parasite queens enter host colonies and use available resources to establish a colony of their own (Buschinger 2009). These queens must first gain entry into a colony and blend in as colony members, and second, they must have their first brood reared by the host. During this process the host queen(s) are often killed by intruding parasite queens or by the host workers (Buschinger 2009). Parasite queens use various tricks to enter the host colonies, ranging from stealth, to playing dead and then being carried inside a colony by host workers or even awaiting for a slave raid to find and enter a new colony (Buschinger 2009; Mori and Le Moli 1998). Costs of parasitism are immense as parasitized colonies become moribund because host workers eventually disappear due to ageing and fatalities (Wilson 1971; Schmid-Hempel 1998). Therefore selection should hone precise recognition abilities of the host species (Davies et al. 1989) both with

respect to rejecting intruding queens and selectivity in the rearing of non-nest mate eggs. Indeed, host workers of slave-making ants are shown to guard their own colonies and prevent parasites from entering (Mori et al. 1995; Foitzik et al. 2001), whereas in temporary social parasites no defensive behaviour has been described and no studies have, to my knowledge, assessed how often parasite queens successfully usurp host

colonies. Relatively few studies have addressed whether host workers discriminate against parasite brood on different stages of their development, and shown that the parasite brood from both slave-making and temporary social parasites is mostly rejected (Johnson et al. 2005; Achenbach and Foitzik 2009) but it is not clear whether the remaining brood may survive until adulthood.

1.2. Recognition and kin structure

Presence of several reproductive queens (polygyny) in ants is common, although it involves costs for all parties involved, e.g. lower average per capita reproduction of queens and diluted relatedness that leads to lower indirect fitness benefits for workers (Elmes 1973; Herbers 1984). Nevertheless, presence of several reproductive individuals may also be beneficial if it boosts colony productivity and/or colony longevity or if increased genetic diversity brings better colony resistance against parasites as it may increase the host capacity to evolve in response to novel virulent parasite genotypes (De Castro and Bolker 2005). Also if an average queen life span is shorter than that of a colony, adopted queens may replace dying resident queens and bring the colony back to life (Nonacs 1988). This, however, begs the question on how multiple queening is maintained, and if workers are capable of recognising adoptees from potentially parasitic intruders and whether multiple queening imposes risks on colony everyday life due to a more permissive recognition system.

Based on inclusive fitness arguments single queen colonies should be less permissive to adopt additional queens than multiple queen colonies given that acceptance of additional queens reduces inclusive fitness returns (Pamilo 1991). However, this relates only to the acceptance of related queens as both single and multiple queen colonies are predicted to reject unrelated queens (Pamilo 1991). Nonetheless, unrelated queens are known to successfully enter colonies, especially multiple queen colonies are prone for such invasions (Keller 1995).

It is hypothesised that low relatedness may lead to increased diversity of genetically determined recognition cues (Hölldobler and Wilson 1977; Vander Meer and Morel 1998) assuming that low relatedness is associated with high genetic diversity (Giraud et al. 2001; Helanterä et al. 2011; but see Trontti et al. 2007). However previous studies did not find much support for direct relatedness-diversity link suggesting that the cues are either effectively mixed between nest mates or have a substantial environmental component. For example,

even in highly multiple queen species precise nest mate recognition was observed in wasps (*Polybia paulista*, Kudo et al. 2007) and ants (*Linepithema humile*, Thomas et al. 2006, 2007; *Formica paralugubris*, Chapuisat et al. 2005; Holzer et al. 2006). Also, in *Formica* ants with varying kin structure chemical diversity in workers does not seem to covary with genetic diversity

(Martin et al. 2009; Helanterä et al. 2011; van Zweden et al. 2011). In addition common ancestry of colonies within multi-nest networks, and the fact that there is a low incidence of non-network members as intruders may have selected for a more permissive recognition threshold to avoid mistaken rejection of nest mates (Chapuisat et al. 2005; but see Martin et al. 2009).

1.3. Sexual selection

In many insects recognition is mediated by chemical cues, mostly cuticular hydrocarbons and individuals may utilize these cues as a proxy to a partner quality in order to guarantee successful mating. Indeed, cuticular hydrocarbons are often sexually dimorphic with some compounds present in only one of the sexes, or quantitative differences in compounds shared between the sexes (Thomas and Simmons 2008). Moreover, unique sex-specific compounds are often found in non-social insects, such as fruit flies, crickets or longhorn beetles, where they are involved in sexual selection and play important role in partner recognition and mating rituals (Thomas and Simmons 2008; Ferveur and Cobb 2010). In

contrast, in social insects pre-copulatory sexual selection is considered to be limited (Boomsma et al. 2005) and thus there is no evolutionary pressure on cue diversification among sexes in this respect. Perhaps as a result those relatively few studies that concentrated on social insects did not find unique compounds but rather qualitative differences in cuticular hydrocarbon profiles between males and young queens (Layton et al. 1994; Cuvillier-Hot et al. 2001; Cremer et al. 2002; Antonialli et al. 2007; Abdalla et al. 2003) except for one study on *Formica truncorum* in which a few compounds were present in young queens, but absent in males (Johnson et al. 2005).

1.4. Nest mate recognition and why sexuals may be different

Nest mate recognition is a process in which social insects discriminate against alien intruders in favour of individuals belonging to their own nest (van Zweden and D'Ettorre 2010). As a result the intruders are rejected whereas nest mates are accepted. Unlike workers for which

acceptance of nest mates and rejection of aliens is the main objective of recognition, males and young queens are subject also to other selective forces, which may have a great impact on their recognition system. One of such forces may be, for example, mate choice to

guarantee correct species-recognition. As a result selection on this trait may counteract selection for a distinct colony profile and lead to a less distinct colony-specific chemical profile in sexuals than in workers. So far relatively few studies addressed this issue and all of them found colony-specific cues in sexuals (Layton et al. 1994; Butts et al. 1995; Hannonen et al. 2002; Johnson and Sundström 2012).

1.5. *Formica* ants

The genus *Formica* is particularly favourable to study recognition and social behaviour. First, newly mated queens of *Formica* s.str., including *F. truncorum*, *F. aquilonia* and *F. lugubris*, usurp colonies of the subgenus *Serviformica* (which includes *F. fusca*, *F. lemani*, *F. picea*, and *F. cinerea*), where they are believed to kill the resident queen(s) (Collingwood 1979; Buschinger 1986; Czechowski et al. 2002). In addition workers of potential host species readily lay unfertilized, male-destined eggs, sometimes in presence of a resident queen, but especially after orphaning (Helanterä and Sundström 2007) and thus can potentially alleviate parasitism costs by producing males in a final reproductive event. However, to my knowledge, to date there are no systematic studies addressing temporary social parasitism in this genus. Second, there is a large body of research with respect to population and colony kin structures in Palearctic *Formica* species (Rosengren et al. 1993), including *F. fusca*, which is one of the species used in this study. In this species variation in queen number creates variation in kin

structure, with an average effective number of queens of three (Hannonen et al. 2004). In single queen colonies a resident queen is retained across several seasons, whereas in multiple queen colonies one third of resident queens is replaced each breeding season. Also, in multiple queen colonies resident queens are often related, though relatedness may be rather low (Bargum and Sundstrom 2007). Moreover, the colony type (single or multiple queens per colony) tends to remain unchanged across seasons, though multiple queening in general is thought to arise from single queen colonies via the adoption of extra queens (Seifert 2007). Such system is ideal to study dynamics of queen replacement across breeding seasons and acceptance of related and unrelated queens into colonies with different kin structure.

2. Aims of the thesis

This work had three main aims. The first aim was to study whether workers of potential host species can mitigate costs associated with entrance of parasite queens by prohibiting the queens from entering the nest (II) and whether workers in already parasitized colonies may resist and do not accept and care for the parasite brood (I, III). This provides information on traits that help to prevent parasitism to occur in a first place (successful usurpation by a parasite queen) and persist (her successful reproduction). The second aim was to study acceptance of additional queens into *F. fusca* colonies; to what extent initially accepted queens come to reproduce and whether the outcome

depends on colony kin structure (II). This study provides insights on mechanisms of queen turnover and dynamics of colonies in species with variable kin structure. The third aim was to examine cuticular hydrocarbon profiles of male and female sexuals of *F. fusca* and test for sex-specific and colony-specific compounds; also to determine whether there is a relationship between genetic relatedness and chemical diversity within colonies (IV).

3. Material and methods

3.1. Genetic analyses

Microsatellite markers developed for *Formica* ants (Chapuisat 1996; Gyllenstrand et al. 2002; Hasegawa and Imai 2004) were used for the analysis of kin structure (I, II, III, IV), parentage (I, III) and species (I, III). Microsatellite markers are widely used in genetic studies and proved powerful tools for studying such questions (Queller et al. 1993; Goldstein and Schlötterer 1999). Genetic work was carried out in MES laboratory, University of Helsinki.

3.2. Chemical analyses

Analyses of cuticular hydrocarbons were carried out to examine chemical profiles of *F. fusca* sexuals (IV). The surface chemicals from males and young queens were extracted in pentane. The extracts were then processed using GC-mass spectrometry, which is a standard reliable method (Martin and Drijfhout

2009). The extraction and analysis of the samples was carried out in the Institute of Biology, University of Copenhagen, Centre for Social Evolution.

3.4. Experimental work and bioassays

These studies consist of four experiments that were carried out by me and/or my collaborators at Tvärminne zoological station, University of Helsinki, during summers of 2007 to 2010. Entire field colonies were collected in the vicinity of Tvärminne or Hyytiälä forestry field station and established in the laboratory. The colonies were then kept under controlled temperature and food (Bhatkar and Whitcomb 1970) and water was provided upon need. Behavioural assays were conducted to assess the extent to which the host species *Formica fusca* and *F. lemni* accept eggs of the social parasite *F. truncorum* and whether the host species differ in their discrimination abilities (I) or to examine acceptance and survival of queens of temporary social parasites *F. lugubris* and *F. aquilonia* in three potential host species, *Formica cinerea*, *F. picea* and *F. fusca* (III). Also brood care experiments were carried out to test parasite brood survival during the first 10 days of development for eggs of *F. truncorum* in *F. fusca* and *F. lemni* colonies (I) or until pupation of *F. aquilonia* brood in colonies of *F. fusca* (III). In addition laboratory-reared and mated queens of *F. fusca* were used and introduced to experimental colonies to test whether related daughter queens were more amenable for adoption than unrelated non-nest mate queens, whether

acceptance is contingent on within-colony relatedness, and whether resident queens are favoured over new ones (II). I also used laboratory colonies to rear female and male sexuals of *F. fusca* to extract their chemical profiles (IV). The use of laboratory colonies in these experiments enabled me to observe rearing of parasite and nest mate brood as well as queen survival and acceptance. Such observations are not possible in the field as colonies of *F. fusca*, *F. lemani*, *F. picea* and *F. cinerea* often inhabit places difficult to access and also easily move if disturbed.

4. Main results and their interpretation

4.1. Social parasitism

It was found that queens of temporary social parasites stand a very low chance to successfully enter the colonies of their prospective host species (III) and to have their brood reared (I, III). On average survival rate of parasite queens during 10 days was 22%, whereas all resident queens remained alive throughout the experiment and no parasite brood survived to adulthood (III).

It is commonly believed that parasite queens often kill resident queens upon entering the nest (Buschinger 2009), which is opposite to what is found in this thesis (III). Such difference may be due to the fact that parasite queens may have not had a chance to eliminate the host counterpart as the parasite queens were left on top of the respective host nest and

not inside it. However, a third of living parasitic queens were found inside the host nest and therefore had an opportunity to kill the host queen. Thus at least within 10 days neither parasite queens nor host workers kill the host queens.

Parasite queen survival differed among the host species, and none of the parasite queens survived in *Formica cinerea*, in contrast to *F. fusca* and *F. picea*, in which on average 50% and 20% of parasite queens survived respectively (III). There are several factors that may explain such disparity. For example, chemical cues may be more or less similar between hosts and parasites of some species, making recognition more or less difficult. However, *F. fusca* and *F. cinerea* demonstrate similar to each other cuticular hydrocarbon profiles, whereas *F. picea* is different (Martin et al. 2008), thus *F. fusca* should have demonstrated similar rejection of parasite queens as *F. cinerea*. Also, as colony size increases aggression towards intruders also increases (Crosland 1990; Stuart 1991; Tschinkel, Adams et al. 1995), and indeed, *F. cinerea* has the largest average colony size (Collingwood 1979; Czechowski et al. 2002) and is highly defensive against red wood ants (Dlussky 1967; Czechowski 1999; Czechowski and Markó 2005), in contrast to *F. fusca* and *F. picea*. In addition host colonies in populations with high incidence of parasitism tend to be more restrictive and defensive against intruders (Foitzik et al. 2001) and thus most parasitized host species should demonstrate the strongest defense. Nonetheless, *F. fusca*, commonly considered as a frequent host for temporary social parasites (Collingwood

1979; Czechowski et al. 2002) was the least aggressive in these experiments (I, III). Moreover, *F. fusca* accepted a high proportion of eggs of the potential temporary social parasite (I), so this argument is probably not valid.

Paper I and III show that parasite eggs introduced to the host nest are discriminated against; however, there is a discrimination delay as fewer parasite eggs remain in host colonies as the time progresses. Such delay in discrimination of parasite brood is also known from other ant species, including *Formica* (Johnson et al. 2005; Achenbach and Foitzik 2009) and suggests that brood discrimination at early developmental stages is a difficult task and attempts to remove parasite brood may lead to the accidental removal of own brood (Reeve 1989; Sherman et al. 1997; Lenoir et al. 2001). Indeed fresh brood or callow workers often have fewer chemicals on their cuticle, known as "chemical insignificance" (Lenoir et al. 2001). Perhaps multiple encounters of individual brood items and time are needed to reliably discriminate the brood to minimize costs of accidentally removing preferable, i.e. own brood. Indeed such interpretation is supported by the fact that there is a decline in the proportion of parasite versus descendant brood with increasing brood development (III).

Comparing social parasite hosts with avian parasite hosts, it seems that their defence mechanisms are fundamentally different in respect to how both host types bear costs of parasitism. Avian hosts often opt for paying the costs rather than rejecting their own brood (Davies 2000; Davies and Welbergen 2008; Welbergen and Davies 2009). In contrast,

social insect hosts invest in both prevention of parasites from entering and post-infection defence. Perhaps, the costs of brood parasitism in birds may have larger fitness effects, and paying the costs of parasitism is related to overall life-time reproductive ability, given that rather few eggs are laid in bird nest, comparing to numerous eggs in ant colonies. Moreover, there is an indication that workers in parasitized ant colonies are perhaps not as moribund as expected. It is shown that workers of many *Serviformica* ant species initiate egg-laying sometimes in presence of their resident queen(s), but more often when orphaned (Helanterä and Sundström 2007). Such unfertilized eggs develop into males. My experiments showed that workers start laying eggs already on 6th day after orphaning (I) and eventually all parasite brood is replaced by worker-laid brood (III). As a result workers may gain direct fitness returns even when the colony is parasitized and became orphaned.

4.2. Multiple queening, colony dynamics and recognition

Several factors may be responsible for whether introduced queens are accepted or rejected, e.g. adoptee relatedness to colony workers and fecundity. A related adoptee may be more preferable if the fecundity of the resident queen is lower (Forsyth 1980; Hannonen et al. 2004), whereas unrelated queens should be rejected under all circumstances. My thesis work show that workers of *Formica fusca* discriminated against non-nest mate and nest mate young mated queens introduced to their

nests in favour of the old resident queens (II, Fig. 1). Thus, workers preferred a closely related or familiar queen to a queen with a potentially greater residual reproductive value, which conforms to theoretical predictions (Bourke and Franks 1995). Fecundity is communicated by chemical cues (Holman et al. 2010; Liebig 2010) and perhaps workers of *F. fusca* are capable

of its assessment long before the onset of reproduction in spring and execute unfavourable queens already during hibernation (II). Nevertheless, if this is the only mechanism, no young queens would ever be adopted and multiple queening would not be maintained as fecundity in independently founding species tend to slowly increase with time (Keller and Passera 1990).

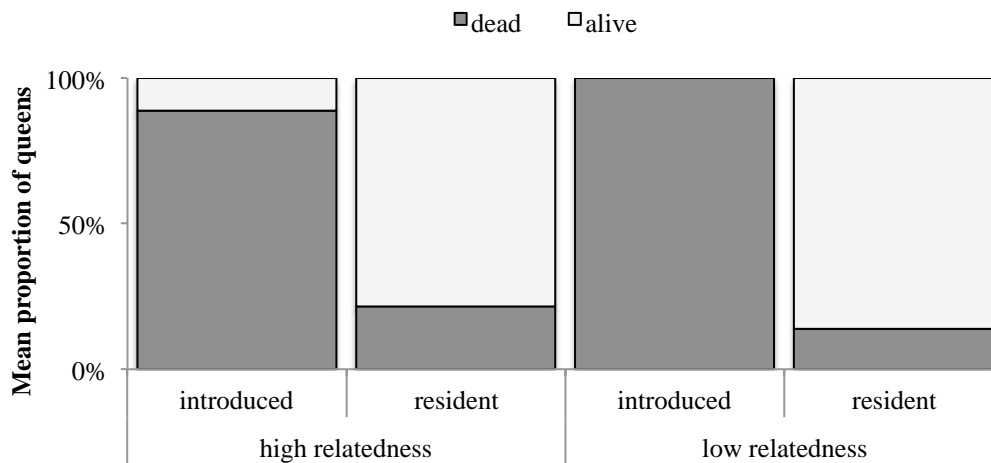


Fig. 1 Survival among introduced and resident queens in colonies with high and low relatedness (II).

This thesis shows that in *Formica fusca* low-relatedness colonies are not more permissive towards intruding conspecific queens compared to colonies with high relatedness (multiple and single queen colonies respectively) (II). Thus, high genetic diversity does not necessarily lead to compromised recognition systems as previously suggested (Hölldobler and Wilson 1977). Accordingly, no increase in the diversity of odour cues with increased genetic diversity in workers was found in *F. fusca* (Helanterä et al. 2011; but see (IV). This begs the question why previous

studies on queen adoption found that adoption of queens was more permissive in multiple queen colonies (Fortelius et al. 1993; Holzer et al. 2008b; Holzer et al. 2008a). It is likely that in these unicolonial or highly multiple queen species or populations a more permissive recognition threshold may have evolved to avoid rejection errors (Chapuisat et al. 2005; Martin et al. 2009) owing to shared ancestry due to budding (Helanterä et al. 2009) or rare encounters of individuals from other networks or super-colonies.

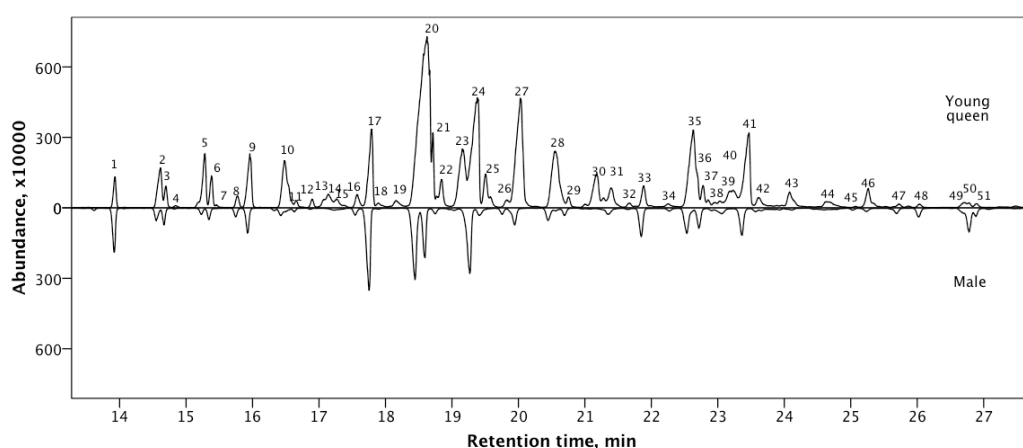
Nevertheless, a negative correlation between within-colony relatedness and

within-colony chemical diversity in *F. fusca* sexuals was found (IV), meaning that in low-relatedness chemical diversity was higher than in colonies with high relatedness. Why sexuals may be different from workers in this respect? Trophallaxis and exchange of glandular products, which are the main source of recognition cues and colony odour, occurs mostly among workers (Hölldobler and Wilson 1990) and female and male sexuals may not receive updated colony odour. To disentangle whether this is indeed the case future experiments need to assess chemical profile of sexuals and workers from the same colonies.

4.3. Cuticular hydrocarbons and genetic similarity in male and female sexuals

Males and young queens of *F. fusca* demonstrated significant differences in cuticular hydrocarbon profiles, however the differences were quantitative and no unique compounds were found (IV, Fig. 2). Such result is in agreement with most earlier studies on social insects (Layton et al. 1994; Cuvillier-Hot et al. 2001; Cremer et al. 2002; Antonialli et al. 2007; Abdalla et al. 2003) and in contrast to studies on solitary insects, in which unique sex-specific compounds are often found (Thomas and Simmons 2008).

Such differences between solitary and social insects are probably because in social insects sexual selection prior mating is questionable (Boomsma et al. 2005) whereas in solitary unique compounds are often involved in mate choice (Ferveur and Cobb 2010; Ginzl 2010). In addition, unlike solitary insects, colonies of social insects contain numerous members and maintenance of colony closure against intruders is essential. As a result a sex-specific compound would be selected against if it would lead to rejection from the nest, because the template of workers is based on individuals not carrying the sex-specific cues, i.e. workers (van Zweden and D'Ettorre 2010). Selection for a distinct sex-specific profile to guarantee successful mating is then balanced by selection for a distinct nest profile and in general selection should favour the profile that allows both species-, and sex-discrimination, as well as precise nest mate recognition. Results of this thesis suggest that this is indeed the case, as both sex-specific differences and colony-specific profiles for both males and females were found. Nevertheless, as sex ratio in *Formica fusca* is often split, it was not possible to sample males and young queens from the same colonies and thus impossible to unravel sex- and colony-specific components in cuticular hydrocarbon profile of sexuals.



Peak	Chemical	Peak	Chemical	Peak	Chemical
1	C23	18	4,12-, 4,10- & 4,18-diMeC24	35	5,13-diMeC26
2	11- & 9-MeC23	19	C25	36	4,12 and 4,10-diMeC26
3	7-MeC23	20	2,12-, 2,10-, 2,8-diMeC24	37	C27
4	5-MeC23	21	13-, 11- & 9-MeC25	38	13-, 11- and 9-MeC27
5	9,13-diMeC23	22	7-MeC25	39	7-MeC27
6	3-MeC23	23	5-MeC25	40	5-MeC27
7	7,15-diMeC23	24	11,15- and 9,13-diMeC25	41	11,15- and 9,15-diMeC27
8	5,9 & 5,13-diMeC23	25	3-MeC25	42	7,11-diMeC27
9	C24	26	7,15-diMeC25	43	3-MeC27
10	3,11-, 3,9- & 3,7-diMeC23	27	5,17-, 5,15- and 5,13-diMeC25	44	5,15-, 5,13- and 5,11-diMeC27
11	12-, 10- & 8-MeC24	28	C26	45	C28
12	6-MeC24	29	3,13-, 3,11- and 3,9-diMeC25	46	3,15-, 3,13-, 3,11- and 3,9-diMeC27
13	5-MeC24	30	13-, 12- and 8-MeC26	47	13-, 12-, 10- and 8-MeC28
14	4-MeC24	31	6-MeC26	48	8,12-diMeC28
15	10,14- and 8,12-diMeC24	32	4-MeC26	49	C29
16	6,10-diMeC24 and 3-MeC24	33	10,14- and 8,12-diMeC26	50	15-, 13-, 11-, 9-MeC29
17	5,11-diMeC24	34	6,10-diMeC26 and 3-MeC26	51	7-MeC29

Fig. 2 Cuticular hydrocarbon profile of *Formica fusca* male and female sexuals (IV).

5. Conclusions

In my thesis work I show that parasite queens have a very low chance to invade host colonies and become fully integrated. Even if host colonies become parasitized, host workers initiate egg-laying and replace parasite brood with its

own (I, III). Nevertheless, social parasitism pressure, beside from affecting how the parasites are discriminated against, may also have an effect on the host kin recognition system and lead to rejection errors of descendant brood. Thus selection mediated by temporary social parasitism may drive enhanced recognition abilities. Nevertheless, as temporary social

parasitism still exists and many parasite species cannot found their own colonies independently from the host, parasites must be able to breach the host recognition system. Perhaps enough attempts of parasitic queens to invade the hosts succeed to maintain the parasitism or else parasites might rely on acceptance errors.

Based on my results colony kin structure does not seem to have any effect on recognition system as both low- and high-relatedness colonies were as stringent towards unrelated individuals (III), although colonies with presumably high genetic diversity also showed significantly greater chemical diversity based on chemical profiles of sexuals (IV). Hence nest mate recognition entails more complex interactions between individual genotypes and colony recognition cue phenotype (Martin et al. 2009; Helanterä et al. 2011; II) than previously assumed, and also multiple queen colonies maintain a degree of genetic integrity, apparently mediated by worker behaviour.

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