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Conflict resolution and evolution of social structures in insect societies

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DEPARTEMENT D'ÉCOLOGIE ET D'ÉVOLUTION

CONFLICT RESOLUTION AND EVOLUTION OF SOCIAL STRUCTURES IN INSECT SOCIETIES

Thèse de doctorat ès sciences de la vie (PhD)

Présentée à la Faculté de Biologie et de Médecine de l'Université de Lausanne

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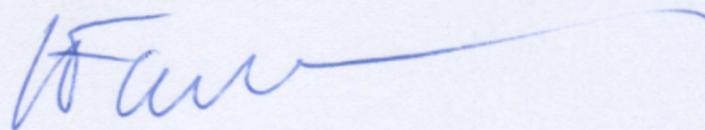
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**CONFLICT RESOLUTION AND EVOLUTION
OF SOCIAL STRUCTURES IN INSECT SOCIETIES**

Lausanne, le 26 juin 2009

pour Le Doyen
de la Faculté de Biologie et de Médecine



Prof. Christian Fankhauser

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Résolution des conflits et évolution des structures sociales dans les sociétés d'insectes

Dans les colonies de fourmis et de nombreuses espèces de guêpes et d'abeilles (les Hyménoptères sociaux), seules les reines se reproduisent. De la part des ouvrières, l'évolution de ce surprenant altruisme de reproduction peut être expliquée par la « sélection de parentèle » : les ouvrières transmettent indirectement des copies de leurs gènes en favorisant la reproduction des individus apparentés, c.à.d. les reines. L'apparentement entre reines et ouvrières peut cependant diminuer en fonction des structures sociales, par exemple lorsque le nombre de reine dans la colonie ou leur nombre d'accouplement augmente. Dans ces conditions, la transmission des gènes des ouvrières est réduite et peut donc engendrer de potentiels conflits entre reines et ouvrières. Dans cette thèse, nous avons étudié les liens entre ces conflits et les variations de structure sociale, et exploré les mécanismes induisant des variations du nombre de reines dans les colonies de fourmis.

La sélection de parentèle prédit que les ouvrières devraient favoriser l'élevage du couvain auquel elles sont le plus apparentées. Chez les Hyménoptères sociaux, les mâles sont haploïdes et les femelles (ouvrières et reines) diploïdes. En conséquence dans certaines colonies, les ouvrières peuvent être jusqu'à trois fois plus apparentées envers les femelles que les mâles, et de ce fait devraient favoriser la production des femelles. A l'inverse, dans toutes les colonies les reines sont autant apparentées à leurs filles qu'à leurs fils et devraient donc favoriser un sexe ratio équilibré. A l'aide d'une méta-analyse, nous avons démontré que généralement les ouvrières manipulent le sexe ratio de la colonie. En conséquence, l'évolution de structures sociales dans lesquelles l'apparentement des reines et des ouvrières envers les deux sexes est comparable serait un moyen de diminuer le conflit entre les deux classes.

Un autre conflit entre reines et ouvrières peut intervenir à propos de la production de mâles. Chez de nombreuses espèces, les ouvrières sont en effet capables de produire des œufs haploïdes. Dans certaines structures sociales, les ouvrières sont en moyenne plus apparentées aux mâles produits par les reines qu'aux mâles produits par les autres ouvrières. En conséquence, elles devraient éliminer les œufs d'ouvrières pour favoriser l'élevage des œufs des reines auxquels elles sont le plus apparentées. Nous avons montré que chez la fourmi *Formica selysi*, les ouvrières éliminent un plus grand nombre d'œufs produits par les autres ouvrières que d'œufs produits par les reines et ce, quelle que soit la structure sociale de la colonie. Nos résultats suggèrent donc que le comportement de « police » des ouvrières peut évoluer indépendamment des variations génétiques, potentiellement pour limiter coûts de la reproduction des ouvrières sur l'efficacité de la colonie.

Le nombre de reine dans une colonie est un paramètre clé qui influence entre autre l'apparentement des individus. La taille des nouvelles reines est généralement liée au succès de fondation de nouvelles colonies par les reines seules. Chez la fourmi *Formica selysi*, les colonies avec une reine (monogynes) produisent des reines plus grandes que les colonies avec plusieurs reines (polygynes). Nous avons montré que, chez cette espèce, la taille des reines est déterminée par des effets génétiques ou maternels transmis dans les œufs. Par contre, nous avons aussi montré que les reines produites dans les deux types de structure sociale avaient dans l'ensemble la même capacité de fonder seules de nouvelles colonies. La taille des reines peut aussi influencer leurs capacités de dispersion et contraindre les petites reines à revenir dans leur colonie d'origine après d'être accouplé à proximité. Nous avons testé l'acceptation de nouvelles reines chez une autre espèce, la fourmi *Formica paralugubris*. Dans cette espèce, toutes les colonies contiennent déjà un grand nombre de reines. Nos résultats montrent que chez cette espèce les ouvrières ne discriminent pas entre les reines du même nid et celles des nids étrangers, et de manière générale n'acceptent que peu de nouvelles reines.

En conclusion, cette thèse démontre que les mécanismes influençant le nombre de reines dans une colonie et l'influence de ces changements sur la résolution des conflits sont complexes. Les données présentées représentent donc une base solide pour explorer plus avant l'évolution et la maintenance des structures sociales chez les insectes sociaux.

Conflict resolution and evolution of social structures in insect societies

In colonies of social Hymenoptera (which include all ants, as well as some wasp and bee species), only queens reproduce whereas workers generally perform other tasks. The evolution of worker's reproductive altruism can be explained by kin selection, which states that workers can indirectly transmit copies of their genes by helping the reproduction of relatives. The relatedness between queens and workers may however be low, particularly when there are multiple queens per colony, which limits the transmission of copies of workers genes and increases potential conflicts between colony members. In this thesis, we investigated the link between social structure variations and conflicts, and explored the mechanisms involved in variation of colony queen number in ants.

According to kin selection, workers should rear the brood they are most related to. In social Hymenoptera, males are haploid whereas females (workers and queens) are diploid. As a result, workers can be up to three times more related to females than males in some colonies, where they should consequently favour the production of females. In contrast, queens are equally related to daughters and sons in all types of colonies and therefore should favour a balanced sex ratio. In a meta-analysis across all studies of social Hymenoptera, we showed that colony sex ratio is generally largely influenced by workers. Hence, the evolution of social structures where queens and workers are equally related to males and females may contribute to decrease the conflict between the two castes over colony sex ratio.

Another conflict between queens and workers can occur over male production. Many species contain workers that still have the ability to lay haploid eggs. In some social structures, workers are on average more related to sons of queens than to sons of other workers. As a result, workers should eliminate worker-laid eggs to favour queen-laid eggs. We showed that in the ant *Formica selysi*, workers eliminate more worker-laid than queen-laid eggs, independently of colony social structure. These results therefore suggest that worker policing can evolve independently from relatedness, potentially because of costs of worker reproduction at the colony-level.

Colony queen number is a key parameter that influences relatedness between group members. Queen body size is generally linked to the success of independent colony foundation by single queens and may influence the number of queens in the new colony. In the ant *F. selysi*, single-queen colonies produce larger queens than multiple-queen colonies. We showed that this association results from genes or maternal effects transmitted to the eggs. However, we also found that queens produced in colonies of the two social forms did not differ in their general ability to found new colonies independently. Queen body size may also influence queen dispersal ability and constrain small queens to be re-adopted in their original nest after mating at proximity. We tested the acceptance of new queens in another ant species, *Formica paralugubris*, which has numerous queens per colony. Our results show that workers do not discriminate between nestmate and foreign queens, and more generally accept new queens at a limited rate.

To conclude, this thesis shows that mechanisms influencing variation in colony queen number and the influence of these changes on conflict resolution are complex. Data gathered in this thesis therefore constitute a solid background for further research on the evolution and the maintenance of complex organisations in insect societies.

GENERAL INTRODUCTION

THE EVOLUTION OF SOCIALITY

Sociality is widespread among diverse animal species and is considered one of the major evolutionary transitions of life on earth (Szathmary & Maynard Smith, 1995). Social life has reached different degrees of complexity, from mutual attraction and simple parental care to highly integrated societies where individuals are specialized in specific tasks (Aron & Passera, 2000). Eusociality is the highest level of social integration and is characterized by overlapping generations, cooperative brood care and reproductive division of labour (Wilson, 1971). This last characteristic means that some of the individuals specialize in reproduction, whereas others exhibit reproductive altruism and perform other tasks such as nest maintenance, brood care, food gathering or colony defence. Eusociality is mostly present in insects, but has also evolved in taxa as diverse as crustaceans (Duffy, 1996) or mammals (Jarvis, 1981; Jarvis *et al.*, 1994). In insects, eusociality can be found in *Thrips* (Crespi, 1992), termites and social Hymenoptera that includes all ants as well as some bee and wasp species (Wilson, 1971).

Darwin considered eusociality, and more specifically reproductive altruism, as one of the most important challenges to his theory of natural selection (Darwin, 1859). Since natural selection favours individuals best adapted to their environment and that consequently have the highest reproductive success, how can it explain the maintenance of a behaviour favouring the reproductive success of others while being costly for the actor? To solve this apparent paradox, Darwin proposed that natural selection may also apply at the family level, and suggested that reproductive altruism could be selected because it increases group efficiency.

In 1964, Hamilton extended natural selection to relatives, using a genetic framework (Hamilton, 1964b; Hamilton, 1964c). This theory, later named kin selection theory (Maynard Smith, 1964), rapidly became a keystone for our modern understanding of social evolution (Bourke & Franks, 1995). Kin selection states that individuals may transmit copies of their genes not only by reproducing themselves (direct fitness), but also by helping relatives that share genes inherited from recent common ancestors (indirect fitness). Interestingly, the evolution of altruistic behaviour by kin selection can be summarized in a simple and elegant equation: $br > c$. According to this formula, altruism is selected when the benefit for the recipient of

the altruistic behaviour b multiplied by the genetic relatedness between the actor and the recipient r is higher than the cost to the actor c . When applied to social Hymenoptera, kin selection indicates that non-reproductive workers can increase their inclusive fitness (i.e. indirectly transmit copies of their genes) by helping their mother queen to raise sisters and brothers.

THE PARADOX OF POLYGYNY

Extensive variations in colony social structures can be found between species, between populations or within populations of social Hymenoptera (Bourke & Franks, 1995; Crozier & Pamilo, 1996; Queller & Strassmann, 1998). The simplest social organisation consists of colonies headed by one singly-mated queen. This basic system has secondarily evolved in more complex social structures, for example by increasing the number of mate per queen (polyandry) (Hölldobler & Wilson, 1990; Bourke & Franks, 1995; Crozier & Fjerdingstad, 2001; Hughes *et al.*, 2008a). Polyandry is common in social insects and greatly varies between species (Crozier & Pamilo, 1996; Crozier & Fjerdingstad, 2001). In *Formica* ants, queens usually mate with one or two males (e.g. Sundström & Ratnieks, 1998; Chapuisat *et al.*, 2004), whereas queen mating number may exceed 20 in honeybees and army ants (Palmer & Oldroyd, 2000; Kronauer *et al.*, 2006). A further important source of variation in social structure is the number of reproductive queens per colony. Colonies contain either one queen (monogyne colonies) or multiple queens (polygyne colonies) (Passera & Aron, 2005). Interestingly, while most species of social Hymenoptera are generally associated with colonies of a single social form, some ant species have both monogyne and polygyne colonies (e.g. Vander Meer *et al.*, 1990; Sundström, 1993; Chapuisat *et al.*, 2004; Gyllenstrand *et al.*, 2005; Bargum *et al.*, 2007).

To date, the causes of variation in social structure remain poorly understood. In particular, the evolution of polygyne is a puzzle for evolutionary biologists and a challenge to kin selection theory (Bourke & Franks, 1995). When many queens head a colony, the low genetic relatedness between queens and workers fail to compensate the costs of reproductive altruism (Hamilton, 1964a; Crozier & Pamilo, 1996). Polygyne is nevertheless very common in ants and seems to have evolved several

times independently (Hölldobler & Wilson, 1977; Rissing & Pollock, 1988; Hölldobler & Wilson, 1990; Keller & Vargo, 1993).

Several hypotheses have been proposed to explain the evolution and maintenance of multiple-queen colonies. The evolution of polygyny might be influenced by ecological factors that make solitary founding by queens costly, such as nest site limitation, predation or competition from older colonies (Nonacs, 1988; Pamilo, 1991; Herbers, 1993; Bourke & Franks, 1995; Keller, 1995). In addition, polygyny might also have evolved due to the benefits entailed by an increase in genetic diversity such as more efficient workers or better resistance to parasites (Crozier & Page, 1985; Keller & Reeve, 1994a; Schmid-Hempel & Crozier, 1999; Crozier & Fjerdingstad, 2001; Reber *et al.*, 2008).

THE SOCIALLY POLYMORPHIC ANT *FORMICA SELYSI*

Socially polymorphic species provide a good opportunity to investigate factors involved in the evolution of social structure. In particular, the ant *Formica selysi* contains colonies headed by either one or multiple queens that live in close proximity within a population located in central Valais, Switzerland (Figure 1). In this species, variation in colony queen number therefore occurs within the same habitat and under similar environmental pressures.

Our study population contains many nests for which genetic and colony traits have been studied over the past 12 years (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005; Rosset & Chapuisat, 2006; Rosset *et al.*, 2006; Rosset & Chapuisat, 2007). In monogyne colonies, queens are generally singly mated and the relatedness between workers is on average equal to $r = 0.732 \pm 0.012$, as expected in monogyne and monoandrous colonies. In polygyne colonies, the number of queens can exceed 60 (personal observation) and the relatedness between workers is on average equal to $r = 0.176 \pm 0.027$ (Chapuisat *et al.*, 2004). Importantly, there is no genetic differentiation at neutral markers between the monogyne and polygyne social forms (Chapuisat *et al.*, 2004), suggesting frequent shifts in colony social structure or gene flow between social forms. This gene flow could be mediated by males, by polygyne queens founding monogyne colonies (or inversely) and/or by queen adoption in colonies of the two social forms.

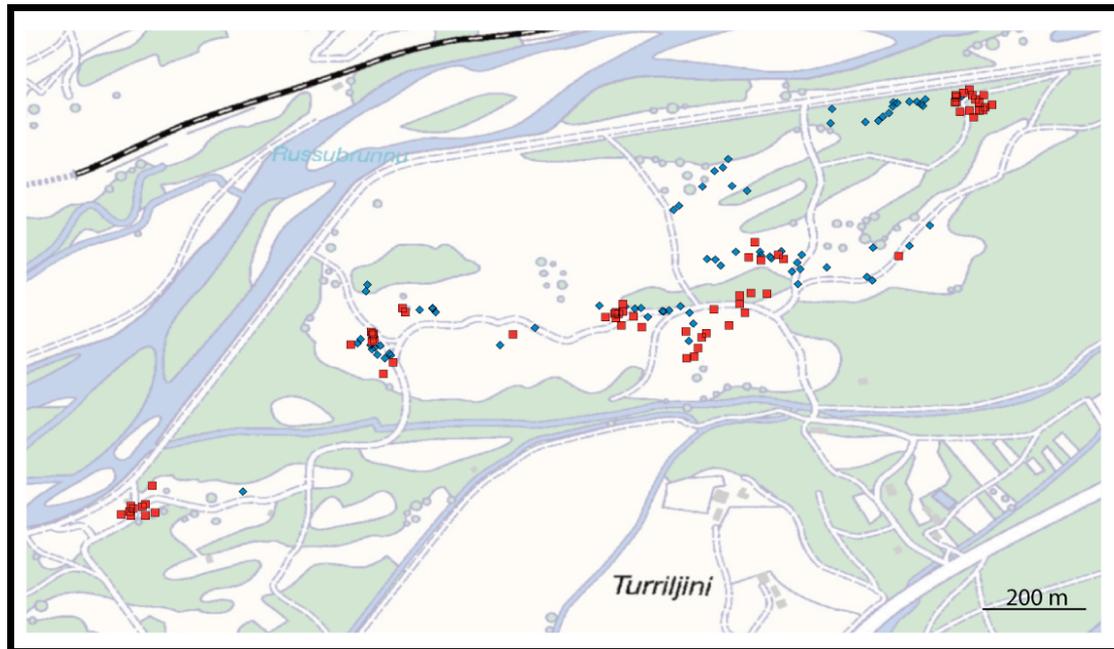


Figure 1 – Map of the study population of the ant *Formica selysi*. Monogyne (blue diamonds) and polygyne (red squares) colonies live in close proximity.

Despite the lack of genetic differentiation between social forms in this species, variation in colony social structure is tightly linked to changes in several fundamental life-history traits. Polygyne colonies are much more populous than monogyne ones. They also occur in areas of higher density, have longer colony lifespan, produce smaller queens, smaller workers and invest less in reproductive individuals relative to workers (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005; Rosset & Chapuisat, 2007). Moreover, workers show an ability to recognize conspecific workers originating from monogyne and polygyne colonies. Specifically, workers are more aggressive towards foreign workers originating from colonies with the alternative than the same social structure (Rosset *et al.*, 2006).

AIMS OF THIS THESIS

This thesis deals with two important processes involved in social evolution: conflicts resolution and variation in colony queen number. In the first part, we investigated how variation in the relatedness among colony members can influence the strength of social conflicts between queens and workers. Specifically, we explored the queen-

worker conflict over colony sex allocation across many species of social Hymenoptera, and the conflict over male parentage in the ant *Formica selysi*.

The second part focuses on the evolution of colony social structures in ants. Using the ant *Formica selysi*, we first investigated the determinants of queen size, which could be involved in the transmission of social forms over generations. Second, we tested the ability of young monogyne and polygyne queens to found new colonies without the help of workers, a process generally leading to monogyne colonies. Finally, we investigated colony traits that could influence queen adoption in the ant *Formica paralugubris*, a species contrasting with *F. selysi* in that colonies are highly polygyne and display almost no aggression between nests (Cherix, 1980; Cherix, 1983).

SOCIAL CONFLICTS

In social Hymenoptera, the relatedness between group members provides strong incentive for altruism (Hamilton, 1964c; Hamilton, 1964b), but can also induce internal conflicts over reproduction (reviewed in Ratnieks *et al.*, 2006). These conflicts generally arise because family relatedness is intermediate between 0 (unrelated individuals) and 1 (clones), so that kinship is insufficient to eliminate all incentive for individual selfishness. The conflicts among group members generally involve both direct reproduction by individuals and manipulation of the reproduction of other colony members (Trivers, 1974; Trivers & Hare, 1976; Ratnieks, 1988).

A potential conflict between queens and workers occurs over colony sex allocation (Trivers & Hare, 1976; Bourke & Franks, 1995; Crozier & Pamilo, 1996; Chapuisat & Keller, 1999b). Social Hymenoptera are haplodiploid with diploid females produced from fertilized eggs and haploid males from unfertilized ones. This sex-determination system results in relatedness asymmetries between workers (that all are females) and sexual individuals (queens and males). When colonies are headed by one singly-mated queen, workers are on average three times more related to sisters than to brothers (relatedness asymmetry), whereas queens are equally related to daughters and sons (Figure 2). In this situation, kin selection predicts a potential conflict between queens and workers, with queens favouring a balanced sex

allocation and full-sibling workers a three times larger investment in females than in males (Trivers & Hare, 1976).

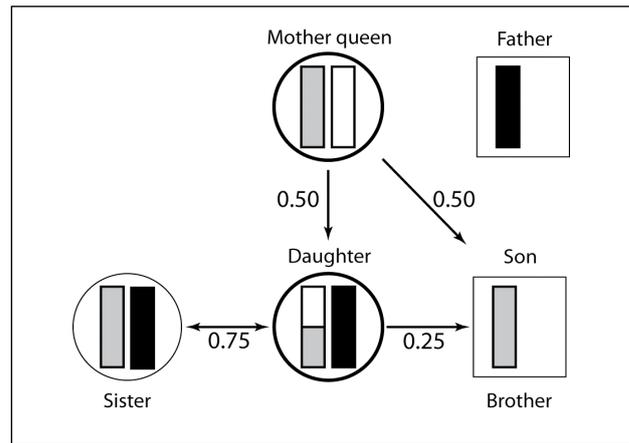


Figure 2 – Haplodiploidy and relatedness asymmetry in social Hymenoptera. Arrows indicate the relatedness between group members in a colony headed by one singly-mated queen.

The quantitative predictions of this conflict vary with changes in social structure and relatedness asymmetry. Specifically, relatedness asymmetry is expected to decrease when (i) the queens mates with more than one male; (ii) the queen is replaced by one of her daughters; (iii) multiple related queens reproduce in the same colony and (iv) workers produce males (Hamilton, 1972; Trivers & Hare, 1976; Boomsma & Grafen, 1990; Boomsma, 1991; Boomsma & Grafen, 1991; Boomsma, 1993; Foster & Ratnieks, 2001). Under worker control, these changes in relatedness asymmetry should result in less female-biased sex allocation relative to the case with one single-mated queen, and on average the degree of queen-worker conflict should decrease. The most powerful method for testing the queen–worker conflict over sex allocation is to examine if sex ratio is split according to relatedness asymmetry variation among colonies within populations. Based on this method, several studies suggested a widespread worker control over colony sex-allocation (Queller & Strassmann, 1998; Chapuisat & Keller, 1999b; Bourke, 2005). However, the magnitude of adaptive sex allocation biasing by workers had not been quantified so far. **In chapter 1**, we performed a meta-analysis to quantitatively examine if colony sex ratio was split according to relatedness asymmetry variation between colonies, using all the published studies on social Hymenoptera.

Another potential conflict between queens and workers is about the parentage of males produced in the colony (Ratnieks, 1988; Ratnieks & Visscher, 1989). In many species of social Hymenoptera, workers have functional ovaries and can lay unfertilized, male-destined eggs (Hölldobler & Wilson, 1990; Crozier & Pamilo, 1996; Helanterä & Sundström, 2007b). According to kin selection theory, workers should preferentially rear the most related male-destined eggs (Hamilton, 1964a), which are the sons of queens in colonies headed by one or more multiply-mated queens (Ratnieks, 1988), or in colonies headed by several related queens (Crozier & Pamilo, 1996). In these colonies workers policing, i.e. the selective aggression of reproductive workers or elimination of worker-laid eggs, can evolve to collectively ensure that queen-laid eggs are raised (Ratnieks & Visscher, 1989; Crozier & Pamilo, 1996; Hammond & Keller, 2004; Wenseleers & Ratnieks, 2006; Helanterä & Sundström, 2007a; van Zweden *et al.*, 2007; Bonckaert *et al.*, 2008). But the selective elimination of worker-laid eggs may also evolve independently of relatedness asymmetries in order to prevent colony-costs of worker reproduction (Ratnieks, 1988; Hammond & Keller, 2004). These costs can be multiple, such as an over-production of eggs relative to the rearing capacity of the colony, or a low colony productivity induced by the fact that reproductive workers generally work less or not at all (reviewed in Wenseleers *et al.*, 2004). Although worker policing has been observed in numerous species of social Hymenoptera, the respective influences of relatedness and colony-costs remain controversial (Hammond & Keller, 2004; Wenseleers & Ratnieks, 2006). **In the first part of Chapter 2**, we investigated the influence of variation in relatedness asymmetries on worker policing in colonies of the ant *Formica selysi*.

Mechanistically, workers generally discriminate queen-laid from worker-laid eggs through cuticular hydrocarbons exclusively carried by queen-laid eggs (Ratnieks & Visscher, 1989; Vander Meer & Morel, 1998; Martin *et al.*, 2002; D'Ettorre *et al.*, 2004; Endler *et al.*, 2004a). A conventional wisdom was that the chemical signature of queen-laid eggs was conserved across colony boundaries (Ratnieks & Visscher, 1989; Martin *et al.*, 2002; Pirk *et al.*, 2003; Endler *et al.*, 2004a), but two studies recently showed that honeybee and *Formica truncorum* workers were able to discriminate between nestmate and foreign queen-laid eggs (Helanterä & Sundström, 2007a; Pirk *et al.*, 2007). **In the second part of Chapter 2**, we tested the potential

discrimination between nestmate and foreign queen-laid eggs in monogyne and polygyne colonies of the ant *Formica selysi*.

COLONY FOUNDATION, QUEEN ADOPTION & QUEEN SIZE

In addition to their role in shaping potential conflicts, variations in colony social structure are linked to multiple changes in life-history traits. In ants, the “polygyny syndrome” defines a group of colony traits generally associated with the presence of multiple-queens (Keller, 1993b). As regarding to monogyne colonies, polygyne colonies have a larger number of workers, a relatively longer lifespan, produce smaller queens and their workers are generally less aggressive toward foreigners (Hölldobler & Wilson, 1977; Keller, 1993b; Bourke & Franks, 1995; Herbers & Banschbach, 1999).

Among the traits described in the “polygyny syndrome”, queen body size is a key parameter. This trait generally influences the mode of colony foundation, which in turn can affect social structure of the newly founded colonies (Hölldobler & Wilson, 1977; Keller, 1993a; Ruppell & Heinze, 1999). Large queens have larger energy stores that can be used to disperse and initiate new colonies alone (Hölldobler & Wilson, 1977; Keller & Passera, 1989; DeHeer *et al.*, 1999), a process called haplometrosis which leads to monogyne colonies (reviewed in Passera & Aron, 2005). In contrast, small queens may try to compensate low energy stores by associating to found a new colony and cooperate to raise the first workers, a process called pleometrosis (Cahan, 2001; Johnson, 2004). Pleometrosis is often unstable and after the emergence of workers, queens generally fight to finally produce monogyne colonies (Sommer & Hölldobler, 1995; Bernasconi & Strassmann, 1999). Interestingly, a few examples are reported in which the association can be stable during all the colony lifespan and thus results in polygyne colonies (*Pachycondyla cf inversa*, Trunzer *et al.*, 1998; *Pogonomyrmex californicus* Aanen *et al.*, 2002; Kolmer *et al.*, 2002).

In social insects, the relationship between social structure, queen size and dispersal has been studied across and within species, but the proximate factors influencing queen size remain poorly understood. Specifically, queen size can be

affected by the mother queen, through genes or maternal effects transmitted to the eggs (Bernardo, 1996), or by the social environment, i.e. the workers that can influence offspring number and resource allocation (Bargum *et al.*, 2004; Fjerdingsstad, 2005; Schwander *et al.*, 2005). In the ant *F. selysi*, monogyne colonies produce larger queens than polygyne ones (Rosset & Chapuisat, 2007). **In Chapter 3**, we tested if in this species, queen size variation was caused by a genetic polymorphism (or a maternal effect) present in the eggs, or by the social structure of the workers raising the brood. **In Chapter 4**, we experimentally investigated whether the queens produced in the two types of social structure had different ability to found new colonies independently, by haplometrosis or pleometrosis.

For young queens, an alternative option to independent colony foundation is to be accepted in an existing nest. Queen acceptance may provide benefits for the recipient colony, such as an increase in genetic diversity (Crozier & Page, 1985; Brown & Schmid-Hempel, 2003; Oldroyd & Fewell, 2007; Reber *et al.*, 2008), but may also decrease the relatedness among colony members and consequently the genetic benefits from helping close relatives (Pamilo, 1991). The trade-off between costs and benefits of queen acceptance is complex and can be shaped by characteristics of the recipient colony such as their social structure or the type of sexual produced (Pamilo, 1991; Sundström, 1997; Brown & Keller, 2000). A further important source of variation in the acceptance success comes from characteristics of the introduced queens, such as their nest of origin, their social origin or their mating status (Rosengren *et al.*, 1986; Stuart *et al.*, 1993; Evans, 1996; Sundström, 1997; Tschinkel, 2006; Kikuchi *et al.*, 2007; Vasquez & Silverman, 2008). **In chapter 5**, we tested, in the highly polygynous ant *Formica paralugubris*, whether the type of sexual (queens or males) produced in a colony, the origin of the introduced queens (nestmate or foreign) and their mating status (mated or virgin) influenced the potential acceptance of additional queens by resident workers.

CHAPTER 1

CHAPTER 1

Split sex ratios in the social Hymenoptera: a meta-analysis

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ABSTRACT

The study of sex allocation in social Hymenoptera (ants, bees and wasps) provides an excellent opportunity for testing kin selection theory and studying conflict resolution. A queen-worker conflict over sex allocation is expected because workers are more related to sisters than to brothers, whereas queens are equally related to daughters and sons. If workers fully control sex allocation, split sex ratio theory predicts that colonies with relatively high or low relatedness asymmetry (the relatedness of workers to females divided by the relatedness of workers to males) should specialize in females or males, respectively. We performed a meta-analysis to assess the magnitude of adaptive sex allocation biasing by workers and degree of support for split sex ratio theory in the social Hymenoptera. Overall, variation in relatedness asymmetry (due to mate number or queen replacement) and variation in queen number (which also affects relatedness asymmetry in some conditions) explained 20.9% and 5% of the variance in sex allocation among colonies, respectively. These results show that workers often bias colony sex allocation in their favour as predicted by split sex-ratio theory, even if their control is incomplete and a large part of the variation among colonies has other causes. The explanatory power of split sex ratio theory was close to that of local mate competition and local resource competition in the few species of social Hymenoptera where these factors apply. Hence, three of the most successful theories explaining quantitative variation in sex allocation are based on kin selection.

Keywords: sex allocation, split sex ratio, relatedness asymmetry, queen-worker conflict, social insects, meta-analysis

INTRODUCTION

Kin selection extends natural selection to include the indirect transmission of copies of genes through relatives (Hamilton, 1964a). This theory is fundamental to understanding a wide variety of evolutionary phenomena such as the evolution of altruism and spite, the emergence of eusociality and the presence of kin conflicts (Hamilton, 1964a; Hamilton, 1970; Hamilton, 1972; Bourke & Franks, 1995; Gardner & West, 2004; Ratnieks *et al.*, 2006; West *et al.*, 2007). Some of the clearest opportunities for testing kin selection theory are provided by conflicts over sex allocation in the social Hymenoptera (Trivers & Hare, 1976; Bourke & Franks, 1995; Crozier & Pamilo, 1996; Chapuisat & Keller, 1999b). Social Hymenoptera are haplodiploid with diploid females produced from fertilized eggs and haploid males from unfertilized ones. This sex-determination system results in relatedness asymmetries between workers (females who raise the brood) and sexual individuals (queens and males). When colonies are headed by one single-mated queen, workers are three times more related to sisters than to brothers, whereas queens are equally related to daughters and sons (Trivers & Hare, 1976). Hence, kin selection predicts a potential conflict between queens and workers, with queens favouring a balanced sex allocation and full-sibling workers a three times larger investment in females than in males.

The quantitative predictions vary with changes in social structure, which affect relatedness asymmetry. Specifically, relatedness asymmetry is expected to decrease when (i) the queens mates with more than one male; (ii) the queen is replaced by one of her daughters; (iii) multiple related queens reproduce in the same colony and (iv) workers produce males (Hamilton, 1972; Trivers & Hare, 1976; Boomsma & Grafen, 1990; Boomsma, 1991; Boomsma & Grafen, 1991; Boomsma, 1993; Foster & Ratnieks, 2001). Under worker control, these changes in relatedness asymmetry should result in less female-biased sex allocation relative to the case with one single-mated queen, and on average the degree of queen-worker conflict should decrease.

Variation in relatedness asymmetry can occur among species, among populations and among colonies within populations. The comparison of sex

allocation and relatedness asymmetry across ant species and populations provides evidence for partial worker control, with female-biased sex allocation in species that have a single queen per colony (= monogyne colonies), and slightly male-biased sex allocation in species with multiple queens per colony (= polygyne colonies, Trivers & Hare, 1976; Pamilo & Rosengren, 1983; Nonacs, 1986a; Pamilo, 1990; Bourke, 2005). However, this pattern is open to multiple explanations due to correlated factors. In particular, queens from polygyne colonies often stay in their natal nest while males disperse, and this local resource competition among queens also promotes male-biased sex allocation independently of the decrease in relatedness asymmetry (Crozier & Pamilo, 1996; Chapuisat & Keller, 1999b).

The most powerful method for testing the queen-worker conflict over sex allocation is to examine if sex ratio is split according to relatedness asymmetry variation among colonies within populations. The theory predicts that under worker control colonies with relatively high or low relatedness asymmetry should specialize in producing females or males, respectively (Boomsma & Grafen, 1990; Boomsma, 1991; Boomsma & Grafen, 1991). In as many as 19 out of 25 species or populations studied so far, colony sex allocation is indeed split according to measured or putative variation in relatedness asymmetry (Queller & Strassmann, 1998; Chapuisat & Keller, 1999b; Mehdiabadi *et al.*, 2003; Bourke, 2005). This general pattern is consistent with widespread worker control and provides strong qualitative support to kin selection theory (Queller & Strassmann, 1998; Chapuisat & Keller, 1999b; Bourke, 2005). However, the magnitude of adaptive sex allocation biasing by workers has not been quantified so far.

In this study, we performed a meta-analysis on empirical tests of split sex ratio theory. Our first aim was to use all published studies to quantify the impact of worker control over colony sex allocation in the social Hymenoptera. Our second aim was to examine if the degree of sex allocation adjustment depends on the source and/or magnitude of relatedness asymmetry variation. Relatedness asymmetry can vary among colonies because of queen replacement, variation in queen mating frequency, and variation in queen number under certain conditions. All types of studies have been used to qualitatively test split sex ratio theory, but they are likely to differ with respect to information constraints and strength of selection on worker

behaviour, which depends on the magnitude of variation in relatedness asymmetry (Boomsma *et al.*, 2003; Bourke, 2005). For example, the replacement of a queen by one of her daughters is probably easy to detect by workers, and it results in the highest decrease in relatedness asymmetry (3:1 to 1:1). In contrast, workers might have more difficulty to assess the number of males that have mated with the queen, because queens mate before the birth of the workers and store the sperm for the rest of their life. Workers therefore have to infer mate number from the level of colony genetic diversity, which might be a difficult task, particularly if the cost of nepotistic behaviour selects against genetically-based odour cues (Boomsma *et al.*, 2003). Higher number of queens also decreases relatedness asymmetry when queens are related (Boomsma, 1993; Bourke & Franks, 1995). However, changes in relatedness asymmetry might be small, continuous and somewhat erratic because of the dynamics of queen replacement, and therefore they are likely to be difficult to assess for workers in polygyne colonies. Our third aim was to compare the explanatory power of split sex ratio theory to the other most successful areas of sex allocation - local mate competition (which predicts a bias towards females when related males compete over access to females, Hamilton, 1967) and local resource competition (which predicts a bias towards males when related females compete over resources, Clark, 1978).

METHODS

Collection of data

We performed a large-scale search for studies that contained relevant data, and read abstracts to select studies on social Hymenoptera. We combined several methods: (1) searching for references in reviews of the subject (Herbers, 1979; Nonacs, 1986b; Nonacs, 1986a; Bourke & Franks, 1995; Crozier & Pamilo, 1996; Queller & Strassmann, 1998; Chapuisat & Keller, 1999b; Mehdiabadi *et al.*, 2003; Bourke, 2005; Ratnieks *et al.*, 2006); (2) searching the Institute for Scientific Information web of science on 7th May 2007 for all articles containing at least one of the following expressions: “sex ratio variation”, “relatedness asymmetry”, “sex investment ratio”, “queen mating”, “monoandrous”, “monandrous”, “polyandrous”,

or “split sex ratio”; (3) searching citations in all papers found by the above method. We obtained over 700 studies out of which 27 were relevant for our aims.

We did not include studies for which appropriate effect sizes could not be calculated, such as studies without data on both colony sex allocation and variation of colony relatedness asymmetry or breeding system (Brian, 1979; Pamilo & Rosengren, 1983; Ward, 1983; Strassmann, 1984; Elmes, 1987; Herbers, 1990; Stark, 1992; Fuchs & Schade, 1994; Vargo, 1996; Helms, 1999). Because the meta-analysis requests an estimate of the correlation between sex allocation and relatedness asymmetry, we had to exclude studies in which there was no variation in relatedness asymmetry among colonies within populations (Passera *et al.*, 2001 ; Duchateau *et al.*, 2004). We also excluded studies based on experimentally selected colonies (Kikuchi *et al.*, 2002) or on worker relatedness without information on queen number, queen relatedness or queen mating frequency in a slave-making ant species (Pamilo & Seppä, 1994). In a few cases, we contacted the authors to obtain additional information on published data sets (Yanega, 1989; Queller *et al.*, 1993; Pearcy & Aron, 2006).

We separately collected studies that investigated the impact of competitive interactions among relatives on sex allocation in social Hymenoptera. We used the data set of West *et al.* (2005), complemented by searching the Institute for Scientific Information web of science on 7th May 2007 for all articles containing at least one of the following expressions “local resource competition” (LRC) or “local mate competition” (LMC) in social Hymenoptera. As a result, we added three new studies published since 2005 to the nine studies on social Hymenoptera reviewed in West *et al.* (2005).

Data analysis

We analysed our data using meta-analysis methods, where the calculated effect size of each study is used as a response variable in a global analysis (Rosenthal, 1991; Rosenberg *et al.*, 2000). Each effect size (r) is a correlation coefficient providing an estimate of how colonies adjust their sex allocation in response to relatedness asymmetry variation, queen number variation or competitive interactions (LRC plus LMC). We defined a positive effect size when colonies with higher relatedness

asymmetry (or smaller queen number, lower LRC and higher LMC) had a more female-biased sex allocation, and negative when colonies with lower relatedness asymmetry (or larger queen number, higher LRC and lower LMC) had a more female-biased sex allocation. Hence, a positive large effect size indicates that sex allocation followed the predicted pattern.

We calculated effect sizes using standard methodology (Rosenthal, 1991; Rosenberg *et al.*, 2000). The values sometimes come from the Spearman rank correlation coefficient (r_s) provided in the publication. In other cases, the effect size could be calculated from the statistics (e.g. t , χ^2 , F , Z or P values) and sample size using standard formulas (Rosenthal, 1991; Rosenberg *et al.*, 2000). If the test statistics were derived from ANOVA with more than two treatments, we applied an ordered heterogeneity test (OH test, see Rice & Gaines, 1994). Finally, when values were not available, we used raw data given in figures or tables. The proportion of variance in colony sex allocation that is explained by the factor is given by r^2 .

All analyses were performed using the software package Metawin 2.0 (Rosenberg *et al.*, 2000) with random-effect model (Møller & Jennions, 2002; West *et al.*, 2005) and the statistical software R.2.5.0 (Ihaka & Gentleman, 1996). Statistical analyses were conducted on Z -transformed r -values (Zr) to correct for asymptotic behaviour of large values of r (Sheldon & West, 2004), and the bias-corrected 95% confidence interval were obtained by bootstrapping (Rosenberg *et al.*, 2000). We tested for statistical differences between the mean effect sizes with randomized ANOVA in which effect sizes were randomly permuted 10000 times between factors (Manly, 1997). Results were back transformed to r values for presentation.

We conducted each analysis with one mean effect size per species in each factor category (relatedness asymmetry variation, queen number variation and competitive interactions among relatives; Table 1 and Appendix). When the same species was studied in several populations or over several years, we calculated an average Zr , weighted by sample size. We summed up sample sizes when different colonies were sampled, and calculated an average when the same colonies were sampled repeatedly.

We used several methods to detect a potential publication bias – a tendency to be more likely to publish studies with significant results. First, we plotted the effect sizes against sample sizes. In absence of publication bias, the plot should have a funnel shape with the values of effect sizes equilibrating to the average when sample size increases (Møller & Jennions, 2001). In contrast, a significant negative correlation between effect size and sample size suggests that studies with significant results have been preferentially published, which causes a deficit of studies with non-significant results and small sample sizes. Second, we applied the “trim and fill” method to evaluate the bias in the funnel plot and the significance of the result (Johnson *et al.*, 2000). This method estimates the number (L_0) and effect size of studies that are missing from a meta-analysis due to publication bias, and then adds them to the dataset, recalculates the mean effect size and derives its statistical significance (Møller & Jennions, 2002). Finally, we calculated the “Fail-safe number” (X), which is the number of unpublished studies with an effect size of zero that would be needed to change the result from significant to not significant (Rosenthal, 1991). Interpretation of the meaning of X depends in part on the subjective assessment of whether so many unpublished studies are likely to exist. A quantitative criterion is that a result is robust if $X > 5n + 10$, where n is the number of studies on which the meta-analysis was based, although this criterion is hard to meet with small sample sizes (Rosenberg *et al.*, 2000).

We investigated whether the degree of worker control was linked to the magnitude of relatedness asymmetry variation between colonies in the population. This magnitude of relatedness asymmetry variation was estimated as the

proportion $\frac{RA_{high}}{RA_{high} + RA_{low}}$ in which RA_{high} and RA_{low} were mean relatedness

asymmetries in the highest and lowest relatedness asymmetry classes, respectively (see Appendix for details). In some cases, these relatedness asymmetries were directly measured with microsatellite or allozyme markers. In other cases, they were inferred from social structure variation (mate number, mother or sister queen, queen number). The relatedness asymmetry within polygyne colonies was estimated as

$1 + \frac{2}{1 + r_q(n-1)}$, where n is the number of queens and r_q the relatedness among

queens, that we assumed to be equal to the relatedness among workers because queens are usually recruited back into their natal colony in species with polygyne colonies (Boomsma, 1993; Crozier & Pamilo, 1996). When not available, the number

of queens was estimated as $\frac{3 - r_q}{\left(r_w - \frac{r_q}{4}\right) \times 4}$, where r_w is the relatedness among workers

(Hughes *et al.*, 1993, Boomsma, 1993).

RESULTS

Relatedness asymmetry variation

We found seven studies with quantitative data on sex allocation adjustment in response to relatedness asymmetry variation among colonies due to queen replacement or mate number variation (Appendix). Data on queen replacement by daughter were available for three species of sweat bees, and data on mate number variation were available for three ant and one bumblebee species.

Overall, sex allocation was significantly correlated with relatedness asymmetry, in the direction predicted by worker control, with a mean effect size of $r = 0.457$ (Table 1). Hence, worker control according to relatedness asymmetry explains 20.9% of the variance in sex allocation. The extent of sex allocation adjustment did not depend upon the cause of relatedness asymmetry variation. Specifically, there was no significant difference between the mean effect size of studies on queen replacement by daughter ($r = 0.552$) and variation in mate number ($r = 0.368$; randomized ANOVA, $n = 7$, $P = 0.54$). However, the number of species studied was small and the trend was in the direction predicted by the information constraints, which are higher for mate number variation than queen replacement.

The effect sizes were highly variable but seemed to be uniformly distributed and showed no sign of a publication bias (Figure 1). The trim and fill analysis did not detect missing studies ($n = 7$, number of missing studies $L_0 = 0$) and there was no significant correlation between effect size and sample size (Spearman rank correlation test, $n = 7$, $r_s = -0.036$, $P = 0.94$). The fail-safe number was small ($X = 15$,

quantitative criterion = 45) but the criterion is extremely hard to meet with small sample sizes (Rosenberg *et al.*, 2000).

Table 1 - Mean effect sizes of studies investigating sex allocation adjustment in response to relatedness asymmetry variation, queen number variation and competitive interaction among relatives.

Class of study Factor	Mean effect size (<i>r</i>)	95% confidence interval	Number of species
Relatedness asymmetry variation	0.457 **	0.211 - 0.674	7
Queen replacement	0.552 **	0.300 - 0.786	3
Mate number	0.368 *	0.003 - 0.648	4
Queen number variation	0.223 **	0.107 - 0.323	15
Monogyne versus polygyne colonies	0.090	-0.216 - 0.320	9
Count of queens in polygyne colonies	0.240 **	0.071 - 0.426	4
From relatedness variation	0.354 **	0.292 - 0.484	6
Competitive interactions among relatives	0.501 **	0.375 - 0.619	10
Local Resource Competition (LRC)	0.496 **	0.285 - 0.660	7
Local Mate Competition (LMC)	0.473 **	0.222 - 0.601	4

Asterisks indicate effect sizes that are significantly greater than zero (* $P < 0.05$, ** $P < 0.01$).

Queen number variation

We found 16 studies examining sex allocation adjustment and queen number variation among colonies (Appendix). There were data on sex allocation in monogyne and polygyne colonies for eight ant and one wasp species, data on the correlation between sex allocation and counts of queens in polygyne colonies for four ant species, and data on sex allocation with respect to queen number inferred from relatedness variation for one ant and five wasp species.

Overall, sex allocation was significantly correlated with queen number variation, in the predicted direction that colonies with higher queen numbers produced more males (Table 1, $r = 0.22$). Hence, changes in queen number explained on average 5% of the variance in sex allocation. This value is conservative, because six out of the 15 effect sizes had to be calculated from *p-value* thresholds or without applying OH test, resulting in slightly underestimated effect sizes (see Appendix).

The impact of queen number variation on sex allocation did not depend on the group of study. The mean effect size of the comparison between monogyne and polygyne colonies, queen number variation in polygyne colonies and queen number variation inferred from relatedness were not significantly different (randomized ANOVA, $n = 19$, $P = 0.263$). It is possible that the comparison between monogyne and polygyne colonies had a lower and non significant effect size because polygyne colonies can occasionally be headed by unrelated queens, and thus have high relatedness asymmetries (e.g. Fournier *et al.*, 2003). However, interpreting the differences is delicate, as there was overall no significant difference between the mean effect sizes of relatedness asymmetry variation and queen number variation (randomized ANOVA, $n = 22$, $P = 0.20$).

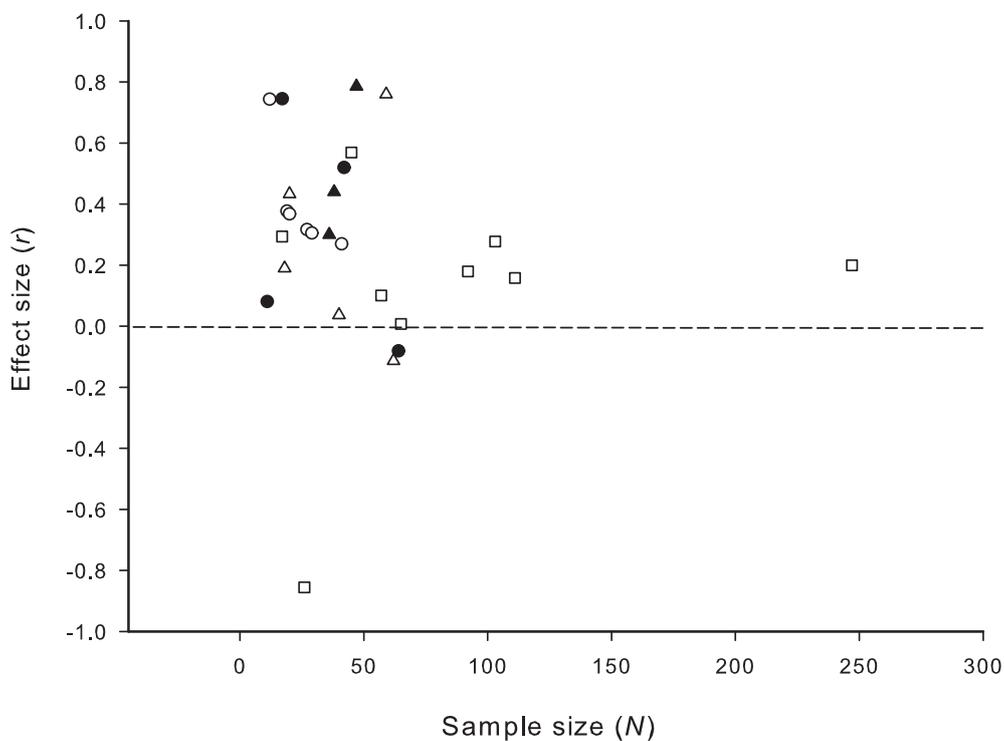


Figure 1 - Relationship between effect size and sample size for studies on queen replacement by daughter (black triangles), variation in mate number (black circles), comparison between monogyne and polygyne colonies (white squares), queen number variation inferred from the count of queens in polygyne colonies (white triangles) and queen number variation inferred from relatedness (white circles). Each point corresponds to one species.

The mean effect size became non-significantly different from zero when carrying out a trim and fill analysis ($n = 15$, number of missing studies $L_0 = 5$, adjusted mean $r = 0.132$, 95% CI: 0.08 - 0.253, $P > 0.05$). This small adjusted mean effect size might be partly due to the six studies for which the effect size was slightly underestimated (see Appendix). Otherwise, there was no significant correlation between sample size and effect size (Spearman rank correlation test, $n = 15$, $r_s = -0.357$, $P = 0.19$) and the fail-safe number was large ($X = 78$, quantitative criterion = 115).

Effect size and magnitude of relatedness asymmetry variation

The magnitude of variation in relatedness asymmetry among the most differing classes of colonies could be measured for 17 studies (Appendix). Overall, it was not significantly correlated with effect size (Figure 2, Spearman rank correlation test, $n = 17$, $r_s = 0.331$, $P = 0.20$). This non-significant pattern held when analyses were restricted to studies on queen replacement and mate number variation (Spearman rank correlation test, $n = 6$, $r_s = 0.736$, $P = 0.10$), or queen number variation (Spearman rank correlation test, $n = 11$, $r_s = 0.10$, $n = 6$, $P = 0.77$). It should however be noted that these analyses are based on small data sets and that the correlations tend to be positive, particularly in the case of queen replacement and mate number variation.

Competitive interactions among relatives

We found twelve studies on ten species examining how competitive interactions among relatives influence sex allocation in the social Hymenoptera. These involved seven studies on LRC and six studies on LMC, in ten ant species. Overall, sex allocation shows a significant correlation with the extent of competitive interactions between relatives, with a mean effect size of $r = 0.501$ (Table 1). Hence competitive interactions among relatives explain 25.1% of the variance in sex allocation. This did not depend on the group of study - the mean effect size of the local resource competition ($r = 0.496$) and the mean effect size of the local mate competition ($r = 0.473$) were not significantly different (randomized ANOVA, $n = 9$, $P = 0.92$).

The effect sizes were highly variable but seemed to be uniformly distributed and showed no sign of publication bias. The trim and fill analysis did not detect missing studies ($n = 10$, number of missing studies $L_0 = 0$), we did not detect a lack of studies with both non-significant result and small sample size (Spearman rank correlation test, $n = 10$, $r_s = -0.195$, $P = 0.59$), and the fail-safe number was large and above the quantitative criterion ($X = 155$, quantitative criterion = 60), which strongly suggests that there was no significant publication bias.

There was no significant difference between the mean effect sizes of competitive interactions, relatedness asymmetry variation and queen number variation (randomized ANOVA, $n = 32$, $P = 0.15$).

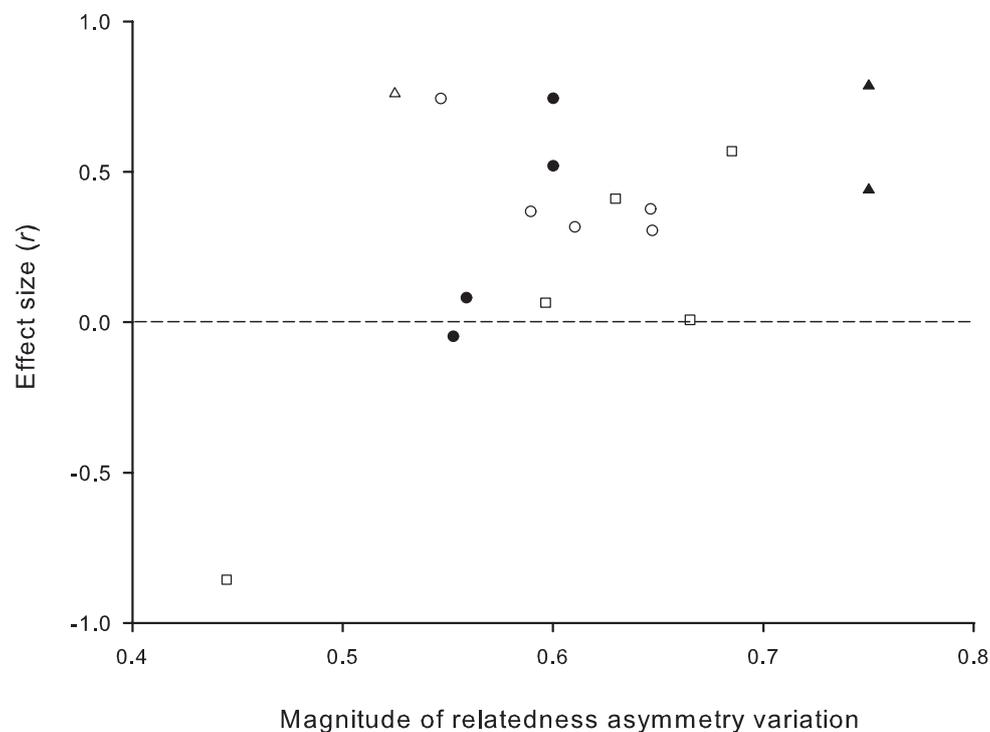


Figure 2 - Relationship between effect size and magnitude of relatedness asymmetry variation for studies on queen replacement by daughter (black triangles), variation in mate number (black circles), comparison between monogyne and polygyne colonies (white squares), queen number variation inferred from count of queen in polygyne colonies (white triangle), and queen number variation inferred from relatedness (white circles).

DISCUSSION

Overall, this meta-analysis reveals that workers of social Hymenoptera bias colony sex allocation in their favour when relatedness asymmetry varies among colonies, as predicted by split sex ratio theory. When the queen was replaced by a daughter or mated with more than one male, variation in relatedness asymmetry explained 20.9% of the variance in sex allocation among colonies. This value is considerably higher than the average (3.6%) amount of variance explained in ecological and evolutionary studies (Møller & Jennions, 2002), and hence provides a quantification of the success of split sex ratio theory.

While confirming the significant and pronounced impact of workers on colony sex allocation, the meta-analysis also reveals that worker control is far from complete, as approximately 80% of the variance in sex allocation among colonies remains unexplained. Many uncontrolled stochastic factors can affect the sex ratio of field colonies, and a large amount of the variance in the data set might be due to such noise. However, part of the variance might also come from yet unrecognized adaptive predictors of sex allocation. One such potential source of variation among colonies that deserves further investigation is that queens might prevent worker manipulation by providing male-destined haploid eggs in some colonies, and female-destined diploid eggs in other colonies (Passera *et al.*, 2001; Roisin & Aron, 2003; de Menten *et al.*, 2005b; Rosset & Chapuisat, 2006).

A somewhat surprising result of our survey was that very few studies contained quantitative data on both sex allocation and relatedness asymmetry variation. This contrasts with recent reviews which listed numerous qualitative results (Queller & Strassmann, 1998; Bourke, 2005), and suggests that further studies documenting quantitative variation are still needed to permit a more detailed analysis of the factors influencing the degree of sex ratio adjustment by workers.

One important aspect of the meta-analysis is that despite the small sample size, there was little sign of publication bias. In particular, there was no lack of studies on relatedness asymmetry variation that had small sample sizes and small effect sizes. Hence, the conclusion that workers bias colony sex allocation in their favour is unlikely to be due to the non-publication of studies with negative results.

Queen number variation explained 5% of the variance, a value that is also significantly greater than zero. This result is consistent with worker control, as relatedness asymmetry generally decreases when queen number increases. It is however difficult to evaluate the precise degree of adaptive sex allocation manipulation by workers, because queen number variation is a variable of a different order that generally correlates with variation not only in relatedness asymmetry, but also in local resource competition, life-histories, and ecological factors (Ross & Keller, 1995; Chapuisat & Keller, 1999b; Rosset & Chapuisat, 2007).

The degree of worker control did not differ significantly among studies with different sources of variation in relatedness asymmetry (queen replacement, mate number, queen number). Hence, we found no support for the hypothesis that workers have more control when relatedness asymmetry is easier to assess (e.g. queen replacement versus mate number). Similarly, the degree of sex ratio adjustment by workers did not correlate significantly with the magnitude of the variation in relatedness asymmetry among colonies, suggesting that workers were not more likely to bias the sex ratio in their favor when differences in relatedness asymmetry among colonies were large. This contrasts with several previous analyses which have suggested that the strength of selection and information constraints may limit the extent of sex allocation adjustment in both vertebrates and invertebrates (Herre, 1987; West & Sheldon, 2002; Boomsma *et al.*, 2003; Schino, 2004; Sheldon & West, 2004; Griffin *et al.*, 2005). However, both analyses in this study suffer from a lack of power due to the very small and heterogeneous data set, and there were trends in the predicted direction, which stresses the importance of obtaining data from a greater range of species.

Overall, split sex ratio theory explained approximately 20% of the variance in sex allocation among social Hymenoptera colonies exhibiting variation in relatedness asymmetry, which is close to the 25% explained by local mate competition and local resource competition in the few species of social Hymenoptera where relatives compete. These values are very high compared to many ecological and evolutionary studies, and confirm the remarkable predictive power of sex allocation theory (West *et al.*, 2005). Moreover, worker control of sex ratio, local mate competition and local resource competition are three processes based on kin selection. Hence, kin selection

proves central and very successful at explaining sex allocation variation in social animals.

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APPENDIX - Studies used in the meta-analysis. The “factor” indicates the source of variation being tested in each class of study, the “effect size” is the correlation between colony sex allocation and the relevant factor, and the “magnitude of RA variation” is a measure of the proportion of variation in relatedness asymmetry among colonies in the population, estimated as $\frac{RA_{high}}{RA_{high} + RA_{low}}$ in which RA_{high} and RA_{low} are mean relatedness asymmetries in the highest and lowest relatedness asymmetry classes, respectively.

Species	Factor	Effect size (r)	Magnitude of RA variation	Sample size	Reference
(a) Studies on relatedness asymmetry variation					
Ants					
<i>Formica exsecta</i>	Mate number	0.520 ¹	0.60	42	Sundström <i>et al.</i> 1996
<i>Formica truncorum</i>	Mate number	0.745 ²	0.60	17	Sundström and Ratnieks 1998
<i>Lasius niger</i>	Mate number	-0.047 ³	0.55	64	Fjerdingstad <i>et al.</i> 2002
Bees					
<i>Augochlorella striata</i>	Queen replacement	0.440 ⁴	0.75	38	Mueller 1991
<i>Bombus hypnorum</i>	Mate number	0.081 ⁵	0.56	11	Paxton <i>et al.</i> 2001
<i>Halictus rubicundus</i>	Queen replacement	0.786 ⁶	0.75	47	Yanega 1989, Boomsma 1991
<i>Lasioglossum laevissimum</i>	Queen replacement	0.300 ⁷	na	36	Packer and Owen 1994
(b) Studies on queen number variation					
Ants					
<i>Formica exsecta</i>	Queen number inferred from relatedness	0.270 ⁸	na	41	R Kümmerli, Pers. comm.
<i>Formica exsecta</i>	Count of queens in polygyne colonies	0.476 ⁹	0.52	59	Brown and Keller 2000
<i>Formica exsecta</i> (mean of the two above studies)		0.386		50	
<i>Formica podzolica</i>	Monogyne versus polygyne colonies	0.180 ¹⁰	na	84	Deslippe and Savolainen 1995
<i>Formica selysi</i>	Monogyne versus polygyne colonies	0.008 ¹¹	0.67	65	Rosset and Chapuisat 2006
<i>Lepthorax acervorum</i>	Monogyne versus polygyne colonies	0.411 ¹²	0.63	116	Hammond <i>et al.</i> 2002
<i>Lepthorax acervorum</i>	Monogyne versus polygyne colonies	0.068 ¹³	na	80	Chan <i>et al.</i> 1999
<i>Lepthorax acervorum</i>	Monogyne versus polygyne colonies	0.065 ¹⁴	0.60	51	Heinze <i>et al.</i> 2001
<i>Lepthorax acervorum</i> (mean of the three above studies)		0.200		247	
<i>Leptothorax longispinosus</i>	Monogyne versus polygyne colonies	0.278 ¹⁵	na	103	Herbers 1984
<i>Myrmica ruginodis</i>	Monogyne versus polygyne colonies	0.569 ¹⁶	0.68	45	Walín and Seppä 2001
<i>Myrmica ruginodis</i>	Count of queens in polygyne colonies	0.434 ¹⁷	na	20	Walín and Seppä 2001
<i>Myrmica ruginodis</i> (mean of the two above studies)		0.533		33	
<i>Myrmica tahoensis</i>	Monogyne versus polygyne colonies	0.159 ¹⁸	na	111	Evans 1995
<i>Pheidole pallidula</i>	Monogyne versus polygyne colonies	-0.856 ¹⁹	0.45	26	Fournier <i>et al.</i> 2003

<i>Pheidole pallidula</i>	Count of queens in polygyne colonies	0.190 ²⁰	na	18	Helms <i>et al.</i> 2004
<i>Pheidole pallidula</i> (mean of the two above studies)		-0.501		22	
<i>Stenamma debile</i>	Monogyne versus polygyne colonies	0.101 ²¹	na	57	Buschinger and Heinze 2001
<i>Stenamma debile</i>	Count of queens in polygyne colonies	0.037 ²²	na	40	Buschinger and Heinze 2001
<i>Stenamma debile</i> (mean of the two above studies)		0.075		49	

Wasps

<i>Brachygastra mellifica</i>	Queen number inferred from relatedness	0.744 ²³	0.55	12	Hastings <i>et al.</i> 1998
<i>Parachartergus colobopterus</i>	Queen number inferred from relatedness	0.377 ²⁴	0.65	19	Queller <i>et al.</i> 1993
<i>Polistes fuscatus</i>	Monogyne versus polygyne colonies	0.294 ²⁵	na	17	Noonan 1978
<i>Polybia emaciata</i>	Queen number inferred from relatedness	0.368 ²⁴	0.61	20	Queller <i>et al.</i> 1993
<i>Polybia occidentalis</i>	Queen number inferred from relatedness	0.317 ²⁴	0.65	27	Queller <i>et al.</i> 1993
<i>Protopolybia exigua</i>	Queen number inferred from relatedness	0.305 ²⁴	0.59	29	Queller <i>et al.</i> 1993

(c) Recent studies on competitive interactions among relatives that complement the data set of West *et al.* (2005)

Ants

<i>Cardiocondyla minutior</i>	Local mate competition	0.225 ²⁶		37	Heinze <i>et al.</i> 2004
<i>Cardiocondyla obscurior</i>	Local mate competition	0.593 ²⁷		14	De Menten <i>et al.</i> 2005
<i>Cataglyphis cursor</i>	Local resource competition	0.089 ²⁸		14	Pearcy and Aron 2006

Notes on the calculation of effect sizes and magnitudes of relatedness asymmetry variation in Appendix:

- ¹ Proportion of females produced by single-mated queen versus multiple-mated queen colonies, $P_{(1994)} = 0.006$, $P_{(1995)} = 0.0001$. The relatedness asymmetry was inferred to be 3:1 and 2:1 in single-mated queen and multiple-mated queen colonies, respectively.
- ² Proportion of females produced by single-mated queen versus multiple-mated queen colonies, $F_{(1,14)} = 12.7$ for 1989-1991 and, $F_{(1,16)} = 25.9$ for 1992-1995. The relatedness asymmetry was inferred to be 3:1 and 2:1 in single-mated queen and multiple-mated queen colonies, respectively.
- ³ Sex investment ratio in single-mated queen versus multiple-mated queen colonies, Lausanne $Z_{(1997)} = -0.25$ and $Z_{(1998)} = -1.16$, Uppsala $Z = -0.19$. We calculated a weighted average from the relatedness asymmetries in Table 5, which were measured with two microsatellite markers.
- ⁴ Sex-investment ratio in eusocial (queen present) versus parasocial (queen replaced by a daughter) colonies, $t = 2.08$. The relatedness asymmetry was inferred to be 3:1 and 1:1 in eusocial and parasocial colonies, respectively.
- ⁵ Sex-investment ratio in single-mated queen versus multiple-mated queen colonies, data from Table 2, excluding the 3 colonies that produced fewer than five individuals, one tailed Wilcoxon-rank sum test, $P = 0.394$. The relatedness asymmetry, measured with four microsatellite markers, was 3:1 and 2.37:1 for single-mated queen and multiple-mated queen colonies, respectively.
- ⁶ Proportion of females produced by eusocial (queen present) versus parasocial (queen replaced by a daughter) colonies, Chi-squared test, $\chi^2 = 29.02$. The relatedness asymmetry was inferred to be 3:1 and 1:1 in eusocial and parasocial colonies, respectively.
- ⁷ Correlation between sex-investment ratio and relatedness asymmetry, $r_s = 0.03$. The relatedness values between classes of nestmates indicate that queen replacement is the most likely source of relatedness asymmetry variation among colonies.
- ⁸ Correlation between sex-investment ratio (proportion or resources allocated to females) and worker brood relatedness in polygyne colonies, unpublished data, $r_s = 0.270$.
- ⁹ Genetic effective queen number in female- versus male-producing polygyne colonies, $F_{(1,39)} = 11.43$. We calculated the relatedness asymmetry in polygyne colonies as described in methods, using effective queen number of 2.7 and worker relatedness of 0.087 in female-producing colonies, and effective queen number of 6.7 and worker relatedness of 0.062 in male-producing colonies.
- ¹⁰ Proportion of males produced by polygyne versus monogyne colonies, $F_{(2,78)} = 1.16$, $P = 0.03$. The OH test on these statistics gave $r_s = 1$, $P_c = 0.70$ and final $P = 0.05$.
- ¹¹ Sex-investment ratio in monogyne versus polygyne colonies, $Z_{(2001)} = -0.11$ and $Z_{(2002)} = -0.01$. The relatedness asymmetry, measured with nine microsatellite markers, was 2.76:1 and 1.39:1 in monogyne and polygyne colonies, respectively.
- ¹² Sex-investment ratio in monogyne versus polygyne colonies, $F_{(1,110)} = 22.3$. The relatedness asymmetry, measured with 5 microsatellite markers, was 3.4:1 and 2.0:1 for monogyne and polygyne colonies, respectively.
- ¹³ Sex-investment ratio in monogyne versus polygyne colonies, excluding data from the Santon population which were presented in Hammond *et al.* (2005), Aberfoyle $F_{(1,29)} = 0.02$ and Roydon $F_{(1,23)} = 0.39$.
- ¹⁴ Sex-investment ratio in monogyne versus polygyne colonies, $P = 0.64$. The relatedness asymmetry in monogyne colonies was inferred to be 3:1. We calculated the relatedness asymmetry in polygyne colonies as described in methods, using the median queen number of three and worker relatedness of 0.493.

- ¹⁵ Proportion of males produced by polygyne versus monogyne colonies, $P = 0.07$. The OH test on these statistics gave $r_s = 1$, $P_c = 0.93$ and final $P = 0.0024$.
- ¹⁶ Sex-investment ratio in monogyne versus polygyne colonies, Leimann $P = 0.031$, Täktom $P = 0.002$. The relatedness asymmetry was measured with four allozyme loci. In the Leimann population, it was 2.71:1 and 1.55:1 for monogyne and polygyne colonies, respectively. In the Täktom population, it was 2.68:1 and 0.92:1 for monogyne and polygyne colonies, respectively.
- ¹⁷ Correlation between sex-investment ratio and queen number in polygyne colonies, from the data in Appendix, Leimann $r_s = -0.457$, Täktom $r_s = -0.395$.
- ¹⁸ Sex-investment ratio in queenless, monogyne and polygyne colonies, $\chi^2_{(2)} = 2.79$. The data set was not amenable to OH test, so the effect size for monogyne versus polygyne colonies is conservative.
- ¹⁹ Sex-investment ratio in monogyne versus polygyne colonies, $F_{(1,22)} = 60.13$. The relatedness asymmetry, measured with 4 microsatellite markers, was 2.66:1 and 3.32:1 for monogyne and polygyne colonies, respectively. These values do not differ significantly, and the magnitude of relatedness asymmetry variation was estimated with the RA of monogyne colonies in the numerator.
- ²⁰ Correlation between proportion of females and queen number in polygyne colonies, $r_s = -0.19$.
- ²¹ Proportion of females produced by monogyne versus polygyne colonies, data from Table 2 to 4, excluding queenless colonies, one-tailed Wilcoxon rank sum test, $W = 369$, $P = 0.223$.
- ²² Correlation between sex-investment ratio and queen number in polygyne colonies, data from Table 2 to 4, excluding queenless colonies, Spearman rank correlation test, $r_s = -0.037$.
- ²³ Relatedness among females in female-producing colonies versus relatedness among workers in male-producing colonies, one tailed t -test, $P < 0.005$. The effect size is conservative, because it had to be calculated from the p -value threshold. We calculated the relatedness asymmetry as described in methods, estimating queen number from worker relatedness given in Table 1.
- ²⁴ Relatedness among females in female-producing colonies versus relatedness among workers in male-producing colonies, t -test, $P < 0.05$ for each of the four species. Effect sizes are conservative, because they had to be calculated from the p -value thresholds. We calculated the relatedness asymmetry as described in methods, using relatedness among queens and relatedness among workers in female- and male-producing nests, respectively.
- ²⁵ Sex-investment ratio in colonies founded by one versus several queens, data from Table 1, Wilcoxon rank sum test, $W = 41.5$, $P = 0.113$.
- ²⁶ Proportion of haploid eggs in polygyne versus monogyne colonies, $\chi^2_{(4)} = 2.161$. The OH test on these statistics gave $P = 0.086$.
- ²⁷ Proportion of ergatoid males in polygyne versus monogyne colonies, $\chi^2 = 4.93$.
- ²⁸ Correlation between proportional investment in females and total sexual productivity, $Z = -0.334$.

CHAPTER 2

CHAPTER 2

Reproductive conflicts and egg discrimination in a socially polymorphic ant

Joël Meunier, Luma Delaplace & Michel Chapuisat

This chapter is a manuscript being prepared for publication

ABSTRACT

The maintenance of sociality despite potential conflicts among group members requires robust regulatory mechanisms. In eusocial Hymenoptera, workers generally care for the brood of queens, but they often have retained the ability to lay haploid eggs. Such eggs can develop into males if they are not destroyed by policing workers. We here tested whether workers from the socially polymorphic ant *Formica selysi* discriminated eggs laid by nestmate workers, nestmate queens, or foreign queens. We also examined if egg discrimination varied according to colony kin structure. To answer these questions, we introduced nestmate worker-laid eggs, nestmate queen-laid eggs and foreign queen-laid eggs in groups of workers originating from either single-queen or multiple-queen colonies. We then recorded the speed at which eggs were collected by workers, as well as the survival of eggs. Workers from single- and multiple-queen colonies discriminated worker-laid from queen-laid eggs, and eliminated the former. This suggests that workers collectively police the reproduction of nestmate workers in both types of colonies. Workers from single-queen colonies discriminated eggs laid by foreign queens from eggs laid by nestmate queens, whereas workers from multiple-queen colonies did not make this distinction. The absence of discrimination of matriline among queen-laid eggs in multiple-queen colonies may limit the costs of discrimination errors. Overall, workers exhibit a remarkable ability to discriminate eggs, which probably restrains worker reproduction and increases colony productivity.

INTRODUCTION

The evolution and maintenance of sociality require that inclusive fitness returns overtake the costs of helping other group members (Keller, 1999; Frank, 2003; Foster *et al.*, 2004). Altruistic help is particularly developed in colonies of social Hymenoptera (which include many bee, wasp and all ant species), where queens reproduce, whereas workers generally perform other tasks such as food gathering or brood rearing (Wilson, 1971). Reproductive altruism is generally directed towards related individuals, and thus increases the inclusive fitness of workers (Hamilton, 1964a). However in many species workers are able to lay unfertilized, male-destined eggs, to which they are highly related (Hölldobler & Wilson, 1990; Crozier & Pamilo, 1996; Helanterä & Sundström, 2007b). The maintenance of colony integrity therefore requests regulatory mechanisms that moderate potential conflicts between queens and workers over reproduction (Ratnieks, 1988; Keller, 1999; Ratnieks *et al.*, 2006).

Worker policing is a mechanism by which workers collectively prevent each others from reproducing, which limits the conflict between queens and workers over male production (Ratnieks, 1988; Ratnieks & Visscher, 1989; Frank, 1995; Frank, 2003; Wenseleers & Ratnieks, 2006). Workers can police the reproduction of other workers by aggressing the workers with developed ovaries, or by selectively destroying worker-laid eggs (Ratnieks & Visscher, 1989; Crozier & Pamilo, 1996; Monnin & Ratnieks, 2001; reviewed in Wenseleers & Ratnieks, 2006; Helanterä & Sundström, 2007a; van Zweden *et al.*, 2007; Bonckaert *et al.*, 2008). Selection for worker policing generally depends upon two variables: the colony-level costs of worker reproduction and the relatedness between workers and worker-laid eggs (Ratnieks, 1988; Hammond & Keller, 2004; Wenseleers & Ratnieks, 2006).

Colony-level costs of worker reproduction favour the evolution of worker policing because worker reproduction is likely to reduce the colony productivity and consequently the fitness of colony members (Ratnieks, 1988; Hammond & Keller, 2004). Worker reproduction can decrease their working capacity (Cole, 1986; Gobin *et al.*, 2003) or entail an overall inefficiency in brood rearing due to an over-production of eggs relative to the rearing capacities of the colony (Hardin, 1968;

Wenseleers *et al.*, 2004). Empirical studies estimating the costs of worker reproduction remain however limited and report mixed results (Cole, 1986; Gobin *et al.*, 2003; Dijkstra & Boomsma, 2007).

The selective elimination of worker-laid eggs by other workers may be also be driven by kin selection based on relatedness asymmetry (Hamilton, 1964a; Wenseleers & Ratnieks, 2006). According to this relatedness-based hypothesis, workers should preferentially rear the most related male-destined eggs in their colonies, which are the sons of queens in colonies headed by one or more multiple-mated queens (Ratnieks, 1988), or in colonies headed by several related queens (Crozier & Pamilo, 1996). A comparative analysis of 109 species of social Hymenoptera generally supports the relatedness-based hypothesis (Wenseleers & Ratnieks, 2006). However, worker policing also frequently occurs in species in which workers are more related to sons of other workers than to sons of queens (e.g Foster *et al.*, 2002; Iwanishi *et al.*, 2003; Hammond & Keller, 2004).

The influence of colony queen number on worker policing has only been explored in one species of ant. In *Pachycondyla cf inversa*, D'Ettorre and colleagues (2004) showed that worker-laid eggs introduced into laboratory colonies were more likely to be eliminated by workers from multiple-queen (polygyne) than single-queen (monogyne) colonies. However, eggs and workers originated from foreign colonies with alternative social structures, which may have played a key role in their elimination by workers (Helanterä & Sundström, 2007a). Therefore, further studies investigating the effect of queen number variation on worker policing remain needed.

Worker-laid eggs are generally discriminated from queen-laid eggs through the absence of a chemical signal that queens apply on their eggs (Ratnieks & Visscher, 1989; Vander Meer & Morel, 1998; Martin *et al.*, 2002; D'Ettorre *et al.*, 2004; Endler *et al.*, 2004a). Many studies suggested that this queen signal is conserved across colonies and even subspecies, resulting in a general acceptance of queen-laid eggs by workers from foreign colonies (Ratnieks & Visscher, 1989; Martin *et al.*, 2002; Pirk *et al.*, 2003; Endler *et al.*, 2004a). Two recent studies did however challenge this hypothesis. In the honeybee *Apis mellifera* and the ant

Formica fusca, workers eliminated more eggs laid by foreign queens than by nestmate queens (Helanterä & Sundström, 2007a; Pirk *et al.*, 2007; Helanterä & Ratnieks, 2009). This apparent variation among species in the worker response towards foreign queen-laid eggs raises the question of the factors influencing nestmate discrimination of queen-laid eggs.

Colony queen number might influence workers ability to discriminate between nestmate and foreign queen-laid eggs. In social insects, the process of nestmate discrimination by workers is generally mediated by the comparison between chemical cues carried by intruders and the learned chemical profile defining the colony-template (Vander Meer & Morel, 1998). It has been proposed that the simultaneous presence of multiple queens in a colony broadens the mix of chemical cues characterizing colony members (Hölldobler & Wilson, 1977; Breed & Bennett, 1987; Vander Meer & Morel, 1998). As a result, the rate of discrimination errors should increase with queen number, so that a general tolerance of workers towards foreign adults is more likely to evolve in polygyne than monogyne colonies (examples reviewed in Bourke & Franks, 1995). Since the mix of chemical cues displayed on nestmate queen-laid eggs should also increase with queen number, we may expect that polygyne workers would exhibit a greater tolerance towards foreign queen-laid eggs, as compared to monogyne workers.

The socially polymorphic ant *F. selysi* provides an excellent opportunity to investigate the influence of colony queen number on worker policing and nestmate discrimination of queen-laid eggs. Our study population contains monogyne and polygyne colonies living in close proximity and headed by generally singly-mated queen(s) (Chapuisat *et al.*, 2004). The influence of colony queen number on egg discrimination by workers can therefore be tested within the same population and species, which permits to avoid some of the confounding variables affecting population or species comparisons. In addition, workers have functional ovaries and readily lay eggs in queenless condition, whereas no worker-laid egg was detected in large survey of eggs laid in queenright monogyne field colonies (Rosset & Chapuisat, 2006, see material and methods for details). Hence, workers either quickly police worker-laid eggs or self-restrain from reproducing in queenright colonies.

The aims of this study were to test the influence of colony social structure on egg recognition by workers in the ant *F. selysi*. Specifically, we investigated the ability of workers to discriminate between eggs laid by nestmate workers or nestmate queens, as well as between eggs laid by foreign or nestmate queens. We used egg-acceptance bioassays during which we estimated the speed of egg collection and rate of egg elimination by workers originating from monogyne and polygyne colonies.

MATERIAL AND METHODS

Study species and egg source

Workers and eggs of the ant *Formica selysi* were collected in a population located along the river Rhône in central Valais, Switzerland (7°36'30"E, 46°18'30"N, altitude 565 m). This population contains a mix of monogyne and polygyne colonies, with no sign of genetic differentiation between social forms (Chapuisat *et al.*, 2004). The social structure of each colony used in this experiment had been previously determined by genotyping eight to 100 workers at nine microsatellite markers (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005; Rosset & Chapuisat, 2006; Reber *et al.*, 2008).

Queen-laid eggs were sampled from 23 monogyne and 17 polygyne field colonies, respectively. These eggs were collected in the last week of April 2008. They are the first produced in the season, and generally develop into queens or males. We assumed that eggs collected in field colonies were queen-produced, because a previous study had shown that worker reproduction is very rare in queenright field colonies of our study population (Rosset & Chapuisat, 2006).

Worker-laid eggs were obtained from queenless groups of 200 workers established in the laboratory. The workers were sampled in the first week of April 2008 from 25 monogyne and 20 polygyne field colonies, respectively. Out of these 45 queenless laboratory groups, 16 originating from monogyne colonies and 14 originating from polygyne colonies produced more than 20 eggs. Eggs from these 30 groups were used in the experiments.

Egg-acceptance bioassays

Egg discrimination by workers was investigated by introducing eggs into groups of 100 "recipient" workers originating from monogyne ($n = 57$) or polygyne ($n = 45$) colonies, respectively. In separate assays, we tested the acceptance of eggs laid either by (1) nestmate workers, (2) nestmate queens or (3) queens originating from a foreign colony of the same social structure as the recipient workers. In each assay, a mean of 29 ± 7 (SD) eggs were placed on a small plastic tray and introduced in a group of recipient workers. Prior to transfer, each egg was individually inspected under a stereomicroscope to ensure that it was not damaged. We endeavoured to standardize the age of introduced eggs by using them approximately 10 days after queens and workers had started to lay.

Recipient workers were sampled from 29 monogyne and 25 polygyne field colonies during the last week of April 2008. We established 102 groups of recipient workers in fluonlined plastic boxes (15 x 15 x 5 cm) covered by a transparent lid. The recipient groups were kept in the laboratory at 22 ± 2 °C. To limit the influence of orphaning on workers responses, egg-acceptance bioassays were set up on the day of workers sampling. One hour before egg introduction, the workers were provided with *ad libitum* water and standard ant food (a mix of beef meat, chicken egg, mealworms, baby vitamins, sugar, salt, gelatine and water). In order to avoid pseudo-replication, workers and eggs from each field colony were used once per type of assay (eggs from nestmate workers, from nestmate queens or from foreign queens).

Fifteen minutes after egg introduction, we estimated the egg collection rate by counting the number of eggs remaining on the trays in a subsample of 66 recipient groups of workers (see Figure 2 for detailed sample sizes). Twenty-four hours later, we estimated the egg survival rate by counting the total number of undamaged eggs in each recipient group of workers (see Figure 1 for detailed sample sizes).

We controlled for two factors that could influence the elimination of worker-laid eggs, namely laboratory conditions and egg sex. To control for the effect of the laboratory origin of worker-laid eggs, we compared worker response towards foreign queen-laid eggs produced in field and in laboratory colonies. To this end, we

collected workers from nine polygyne field colonies in the first week of April 2008. These groups of workers received 30 eggs laid by 15 foreign queens originating from nine polygyne field colonies and kept in the laboratory since March 2007.

The potential effect of egg sex on discrimination was also investigated. Worker-laid eggs are exclusively male-destined, whereas queen-laid eggs can be a mix of female- and male-destined eggs. We therefore compared worker responses towards worker-laid eggs and towards male-destined queen-laid eggs originating from a subsample of male-producing field colonies. In *Formica selysi*, some field colonies specialize in the production of males, and contain only haploid, male-destined queen-laid eggs (Rosset & Chapuisat, 2006). We identified 9 such male-specialized field colonies by sexing 30 ± 11 (mean \pm SD) pupae per colony in a subsample of 22 field colonies.

Statistical analysis

To test whether workers discriminate between worker-laid and queen-laid eggs, we used a two-way Analysis of Variance (ANOVA) where the maternal origin of eggs (i.e. workers or queens) and the social structure of workers (i.e. monogyne or polygyne) were used as fixed factors. The response variables were the proportion of eggs collected from the trays 15 minutes after introduction (egg collection rate) and the proportion of eggs alive after 24 hours (egg survival rate). To test whether workers discriminate between foreign and nestmate queen-laid eggs, we used another two-way ANOVA where the origin of eggs (i.e. nestmate or foreign) and the social structure of workers were used as fixed factors. Interactions between factors were tested in all analyses and pairwise comparisons were done using one-way ANOVAs where the significance level was Bonferroni-adjusted to $\alpha = 0.025$. Because eggs or workers originating from the same field colony were sometimes used twice in the analysis, we included the origin of eggs and workers as random factors in our statistical models (mixed models). We tested for differences in the survival rates of nestmate male-destined eggs laid by queens and workers using non-parametric

Wilcoxon Rank Sum tests. Variables were normalized by arcsine transformations (Sokal & Rohlf, 1995) and statistical analyses were performed using JMP 7.0.

RESULTS

Discrimination between eggs laid by nestmate workers or nestmate queens

Worker-laid eggs were collected at a slower rate and had a lower survival rate than queen-laid eggs (Collection rate: $F_{1,32} = 53.73$, $P < 0.0001$, Survival rate: $F_{1,51.81} = 28.02$, $P < 0.0001$). Egg discrimination occurred in groups of monogyne workers (Figures 1 & 2, Collection rate: $F_{1,15} = 55.27$, $P < 0.0001$; Survival rate: $F_{1,29.81} = 7.21$, $P = 0.0117$) and in groups of polygyne workers (Collection rate: $F_{1,17} = 16.78$, $P = 0.0008$; Survival rate: $F_{1,21.3} = 22.02$, $P = 0.0001$). Monogyne workers collected the two types of eggs at a faster rate than polygyne workers ($F_{1,32} = 16.42$, $P = 0.0003$), whereas egg survival rate did not significantly differ between workers of the two social forms ($F_{1,42.14} = 2.53$, $P = 0.12$). Finally, there was no significant interaction between the social origin of workers and the maternal origin of eggs on collection rate ($F_{1,32} = 0.68$, $P = 0.42$) and survival rate ($F_{1,51.81} = 2.73$, $P = 0.11$).

The collection and survival rates of queen-laid eggs sampled in laboratory colonies and transferred into groups of foreign workers were 0.54 ± 0.10 (mean \pm SE) and 0.82 ± 0.04 , respectively. These values did not differ significantly from the ones obtained from foreign queen-laid eggs sampled in field colonies (one-way ANOVAs, Collection rate: $F_{1,9.13} = 1.47$, $P = 0.26$; Survival rate: $F_{1,10.76} = 0.23$, $P = 0.64$).

Six monogyne and three polygyne field colonies produced only male pupae, strongly indicating that eggs sampled from these colonies were haploid (Rosset & Chapuisat, 2006). In the sub-sample of monogyne and polygyne groups of workers originating from these colonies, the survival rates of queen-laid eggs were 0.86 ± 0.06 (mean \pm SE) and 0.79 ± 0.04 , respectively. These values were significantly higher than 0.57 ± 0.07 (mean \pm SE) and 0.35 ± 0.08 , the survival rates of workers-laid eggs transferred into groups of monogyne and polygyne workers, respectively (Wilcoxon

rank sum tests, Monogyne workers: $W = 71.5$, $P = 0.0146$; Polygyne workers: $W = 37$, $P = 0.0433$).

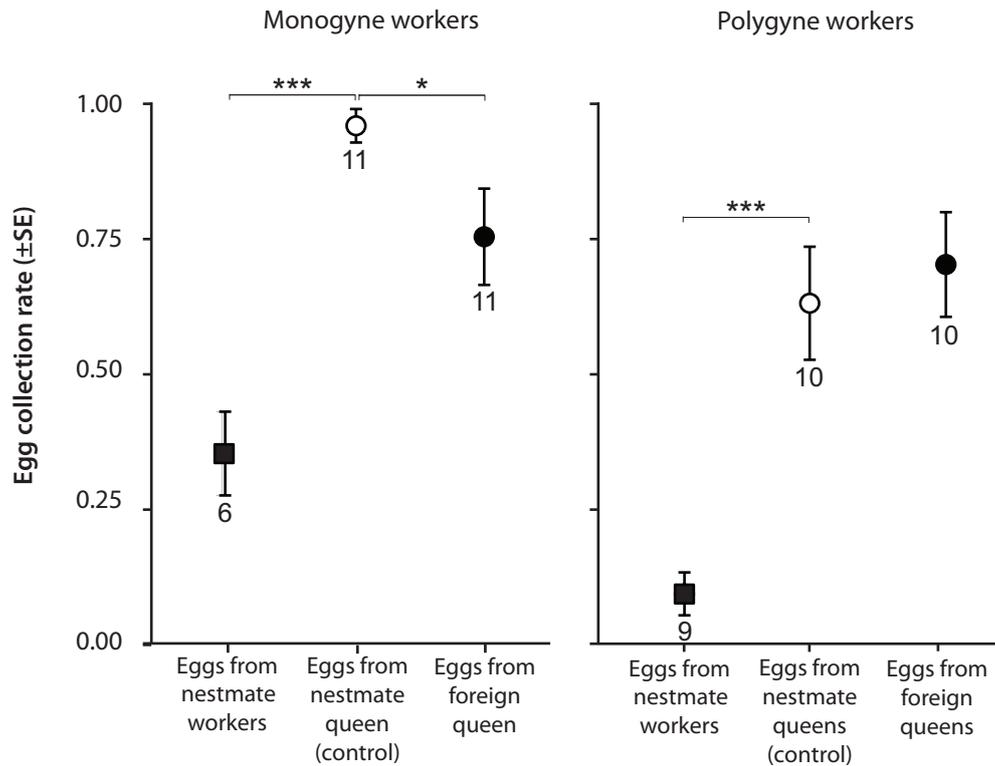


Figure 1 - Collection rates of eggs originating from nestmate workers (black squares), nestmate queens (control, white circles) or foreign queens (black circles) 15 minutes after being introduced in group of monogyne or polygyne workers. The number of recipient groups of workers is indicated below the SE bars. * $P < 0.05$, *** $P < 0.001$

Discrimination between eggs laid by foreign queens or nestmate queens

The social origin of workers significantly influenced their discrimination between foreign and nestmate queen-laid eggs (Figures 1 & 2, interactions between social structure of workers and nest of origin of eggs, Collection rate: $F_{1,38} = 8.72$, $P = 0.0054$; Survival rate: $F_{1,26.35} = 4.99$, $P = 0.0342$). In groups of monogyne workers, nestmate eggs were collected faster and survived better than foreign eggs (Pairwise comparisons, Collection rate: $F_{1,7.43} = 8.42$, $P = 0.0215$; Survival rate: $F_{1,20.42} = 6.02$, $P = 0.0232$). In contrast, nestmate and foreign eggs were collected and survived at

similar rates in groups of polygyne workers (Collection rate: $F_{1,6.06} = 1.21$, $P = 0.31$; Survival rate: $F_{1,2.81} = 0.97$, $P = 0.40$).

When considered independently of the social origin of the recipient workers, collection and survival rates of nestmate and foreign queen-laid eggs were not significantly different (Collection rate: $F_{1,38} = 2.14$, $P = 0.15$; Survival rate: $F_{1,26.35} = 2.45$, $P = 0.13$). However, queen-laid eggs were overall collected at a marginally significant faster rate and had a lower survival rate when cared for by monogyne rather than polygyne workers (Collection rate: $F_{1,38} = 4.08$, $P = 0.0501$; Survival rate: $F_{1,25.9} = 5.97$, $P = 0.0217$).

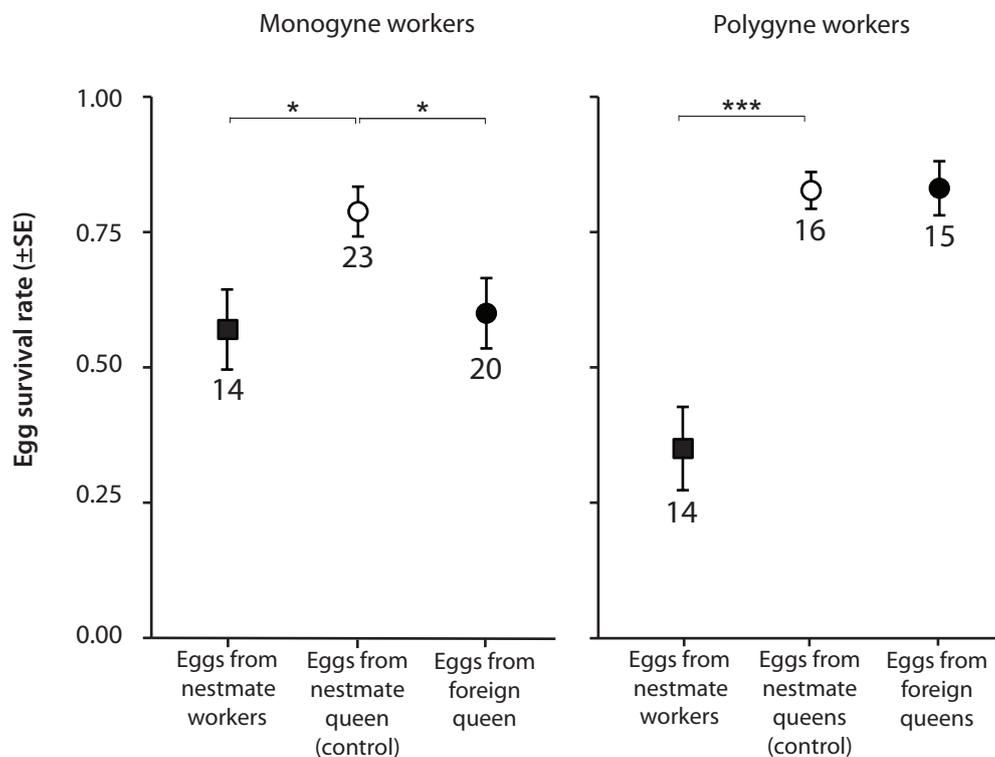


Figure 2 - Survival rates of eggs originating from nestmate workers (black squares), nestmate queens (control, white circles) or foreign queens (black circles) 24 hours after being introduced in group of monogyne or polygyne workers. The number of recipient groups of workers is indicated below the SE bars. * $P < 0.05$, *** $P < 0.001$

DISCUSSION

Workers ability to recognize and eliminate eggs according to their origin may prevent selfish individuals from exploiting the group resources (Ratnieks, 1988; Ratnieks & Visscher, 1989). Our egg-acceptance bioassays show that monogyne and polygyne workers of the ant *Formica selysi* are able to discriminate nestmate worker-laid eggs from nestmate queen-laid eggs, whereas the ability to discriminate foreign queen-laid eggs from nestmate queen-laid eggs is restricted to workers and eggs originating from monogyne colonies.

Workers originating from monogyne and polygyne colonies eliminated more worker-laid eggs than queen-laid eggs. The selective elimination of worker-laid eggs in the two social structures suggests that worker policing evolved independently from relatedness variation among group members, even if polygyne workers tend to exhibit a stronger policing than monogyne workers. Our results are therefore consistent with the hypothesis that worker policing has evolved to limit the colony-level costs of worker reproduction in *F. selysi* (Ratnieks, 1988; Hammond & Keller, 2004). Estimating the costs of worker reproduction is difficult, as colony fitness depends upon the cumulative effect of many variables such as the amount of brood produced, colony size and foraging success. Empirical studies investigating these costs are therefore limited and report mixed results. In the ants *Leptothorax allardycei* and *Pachicondyla obscuricornis*, worker reproduction induces costly competition among workers, which may reduce colony efficiency (Cole, 1986; Gobin *et al.*, 2003). In contrast, in the leafcutter ants *Acromyrmex echinator* and *A. octospinosus*, Dijkstra & Boomsma (2007) showed that worker reproduction has a very limited impact on the volume of the fungus cultivated by ants.

Our results are unlikely to be explained by the selective elimination of haploid eggs, the low intrinsic viability of worker-laid eggs or the selective elimination of eggs laid under laboratory conditions. Our bioassays indicate that within a sub-sample of haploid eggs, workers eliminated more worker-laid eggs, as compared to queen-laid eggs. This suggests that workers did not eliminate worker-laid eggs on the basis

of their ploidy, which is also consistent with the hypothesis that workers are unable to discriminate between haploid and diploid eggs (Nonacs & Carlin, 1990; Passera & Aron, 1996; Chapuisat *et al.*, 1997b; Monnin & Ratnieks, 2001).

The lower survival rate of worker-laid eggs in our experiments is also unlikely to be explained by a low intrinsic viability of these eggs, as they had stayed in the recipient colonies for only 24 hours, and worker-laid eggs that were not used in the bioassays and kept in similar conditions readily developed into adult males (Chapter 3: Meunier & Chapuisat, in press). In addition, in the honeybee *Apis mellifera* workers do not use egg-viability to recognize worker-laid eggs, as they did not discriminate between viable and CO₂-killed queen-laid eggs, whereas they discriminated between queen-laid and worker-laid eggs (Beekman *et al.*, 2001). Lastly, the elimination of worker-laid eggs is unlikely to be caused by the rejection of eggs laid in laboratory colonies, as queen-laid eggs sampled in the field and in laboratory colonies did not differ significantly in their collection and survival rates.

Our egg-acceptance bioassays indicate that nestmate discrimination of queen-laid eggs in *F. selysi* is influenced by the social origin of the recipient workers and/or the social origin of the introduced queen-laid eggs. Monogyne workers selectively eliminate queen-laid eggs from foreign monogyne colonies, whereas polygyne workers accept queen-laid eggs from foreign polygyne colonies. This result is, to our knowledge, the first evidence that colony queen number affects nestmate discrimination of queen-laid eggs in social Hymenoptera. Furthermore, the ability of monogyne workers to recognize nestmate from foreign queen-laid eggs stands in contrast to the conventional wisdom that nestmate recognition does not apply to queen-laid eggs (Ratnieks & Visscher, 1989; Ratnieks & Boomsma, 1995; Martin *et al.*, 2002; Pirk *et al.*, 2003; Endler *et al.*, 2004a; but Helanterä & Sundström, 2007a; Pirk *et al.*, 2007).

The absence of discrimination of foreign queen-laid eggs by polygyne workers is in line with the hypothesis that colony-templates that are based on more diverse recognition cues induce a greater tolerance of resident workers towards foreigners (Bourke & Franks, 1995; Vander Meer & Morel, 1998). For instance in the ant *F.*

fusca, the acceptance rate of foreign queen-laid eggs by monogyne workers is increased when workers had previous experience with queen-laid eggs from foreign colonies (Helantera *et al.*, 2007; Helantera & Ratnieks, 2009). In *F. selysi*, the lower discrimination of polygyne workers is however surprising, as the shift from monogyne to polygyne colonies did not decrease nestmate discrimination ability against adult workers (Rosset *et al.*, 2006).

Several hypotheses may contribute to explain the lack of discrimination of foreign eggs in polygyne colonies. First, nestmate discrimination of eggs and workers might be mediated through independent mechanisms, as documented in *F. fusca* for worker policing and nestmate discrimination of queen-laid eggs (Helantera & Ratnieks, 2009). For example, discrimination against foreign queen-laid eggs might have evolved as a by-product of colony closure against additional queens, which is generally more pronounced in monogyne colonies (Pamilo, 1991; reviewed in Bourke & Franks, 1995; Sundström, 1997). Workers' ability to discriminate between nestmate and foreign queen-laid eggs might also be influenced by the social origin of eggs. For instance, eggs from polygyne colonies may carry a chemical signal that is conserved across colonies, whereas eggs from monogyne colonies may carry colony-specific chemical cues. Finally, the absence of discrimination of matrilines among queen-laid eggs in multiple-queen colonies might have evolved in order to limit the costs of nepotism and discrimination errors (Keller, 1997; Holzer *et al.*, 2006b; Ratnieks *et al.*, 2006).

Overall, *F. selysi* workers exhibit a remarkable ability to discriminate eggs, which probably restrains worker reproduction and increases colony productivity. The fact that monogyne and polygyne workers are able to recognize and eliminate eggs laid by nestmate workers suggests that worker policing is induced by colony-costs of worker reproduction rather than relatedness asymmetry in this species. In addition, our results reveal that workers of monogyne origin discriminate against eggs from foreign monogyne colonies, whereas polygyne workers do not discriminate against eggs from foreign polygyne colonies. This finding is the first demonstration that colony queen number influences nestmate discrimination of queen-laid eggs in the

social Hymenoptera and runs counter to the hypothesis of a queen-specific egg acceptance signal that would be conserved across all colony types.

CHAPTER 3

CHAPTER 3

The determinant of queen size in a socially polymorphic ant

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ABSTRACT

In social animals, body size can be shaped by multiple factors, such as direct genetic effects, maternal effects, or the social environment. In ants the body size of queens correlates with the social structure of the colony: colonies headed by a single queen (monogyne) generally produce larger queens that are able to found colonies independently, whereas colonies headed by multiple queens (polygyne) tend to produce smaller queens that stay in their natal colony or disperse with workers. We performed a cross-fostering experiment to investigate the proximate causes of queen size variation in the socially polymorphic ant *Formica selysi*. As expected if genetic or maternal effects influence queen size, eggs originating from monogyne colonies developed into larger queens than eggs collected from polygyne colonies, be they raised by monogyne or polygyne workers. In contrast, eggs sampled in monogyne colonies were smaller than eggs sampled in polygyne colonies. Hence, eggs from monogyne colonies are smaller but develop into larger queens than eggs from polygyne colonies, independently of the social structure of the workers caring for the brood. These results demonstrate that a genetic polymorphism or maternal effect transmitted to the eggs influences queen size, which probably affects the social structure of new colonies.

Keywords: social structure, body size, egg size, genetic effect, maternal effect, *Formica selysi*

INTRODUCTION

Body size is a key life-history trait in plants and animals (Stearns, 1992). In a great diversity of species, body size is positively correlated with fertility or mating success (Vander Meer *et al.*, 1992; Honek, 1993; Jamieson, 2002; Kovacs *et al.*, 2008), survival (Roff, 1992; Wiernasz & Cole, 2003; Schorr *et al.*, 2009) and dispersal abilities (Sundström, 1995; Paradis *et al.*, 1998; Rüppele & Heinze, 1999; Byers, 2000; Gonzaga & Vasconcellos-Neto, 2001). In social animals, body size affects social interactions and often correlates with variation in mating system and social structure (Bourke & Franks, 1995; Lindenfors & Tullberg, 1998).

Because of their modularity and complex social organization, eusocial insects (all species of ants and termites, as well as some bee and wasp species) are particularly interesting with respect to the causes and consequences of body size variation (Oster & Wilson, 1978; Bourke & Franks, 1995; Burd, 2000; Emlen & Nijhout, 2000). Body size may be influenced by genes, maternal effects, environmental factors or social conditions, which can all affect brood development (Wheeler, 1986; Bargum *et al.*, 2004; Fjerdingstad, 2005; Schwander *et al.*, 2005). An important component of the social environment is constituted by the workers, which care for the brood and can manipulate offspring number or resource allocation (Chapuisat & Keller, 1999b; Chapter 1: Meunier *et al.*, 2008). Hence, intracolony conflicts (Chapuisat & Keller, 1999b; Chapter 1: Meunier *et al.*, 2008), social constraints (Rosenheim *et al.*, 1996) and indirect genetic effects (Linksvayer, 2006) may affect the body size of colony members.

In eusocial insects with physical castes, such as ants, the body size of young queens seems to play a key role in the evolution of social structure by mediating a feedback loop between the social structure of the natal colony, differential dispersal and mode of colony founding (Keller & Passera, 1989; Bourke & Franks, 1995). Typically, species with a single queen per colony tend to produce queens of relatively large size (Keller & Passera, 1989). These winged females have large flight muscles and elevated fat stores, so that they can fly away from their natal nest, excavate their own nest and raise their first brood independently, without the help of workers (Hölldobler & Wilson, 1990). In the monogynous ant species *Pogonomyrmex occidentalis*, the size of young queens correlated with their

probability of survival through the initial steps of independent colony founding (Wiernasz & Cole, 2003).

In contrast, species with multiple queens per colony tend to produce smaller queens that generally stay within their natal nest or disperse by budding, a process whereby queens and workers depart from a colony to jointly establish a new colony in the vicinity (Keller & Passera, 1989; Hölldobler & Wilson, 1990; Keller, 1991; Ross & Keller, 1995). Hence, variation in the body size of young queens seems to depend on queen number in the mother colony, and in turn may constrain the mode of colony founding and the number of queens in future colonies.

The link between social structure, queen size and dispersal has been mostly studied across species. A comparative survey of 24 European ant species showed that 12 out of 13 species with single-queen colonies (monogyne species) exclusively used independent colony founding, whereas 8 out of 11 multiple-queen colonies (polygyne species) used dependent colony founding (Keller, 1991). Moreover, ant species using independent colony founding produced queens with a significantly higher relative fat content than species using dependent colony founding, with no overlap in relative fat content between the two types of queens (Keller & Passera, 1989).

Several ant species exhibit intraspecific social polymorphism, and in some cases monogyne and polygyne colonies coexist within the same population (Chapuisat *et al.*, 2004; Gyllenstrand *et al.*, 2005; Bargum *et al.*, 2007). These systems are interesting to assess the link between queen number and queen size independently of phylogeographical or ecological factors that correlate with queen number variation across species or populations (Chapuisat *et al.*, 2004; Rosset & Chapuisat, 2007). So far, the relationship between colony queen number and queen size has been studied in only one species, *Leptothorax rugatulus*. Consistent with the evidence from the comparative surveys, large queens were more frequent in monogyne colonies, whereas small queens were mostly found in polygyne colonies (Rüppell *et al.*, 1998; Rüppell & Heinze, 1999). In polygynous colonies, the situation is however complicated by the fact that small queens might at least partly be intraspecific parasites exploiting unrelated colonies.

The proximate factors influencing queen size remain poorly understood. The size of young queens may be affected by their mother queen, through genes or maternal effects transmitted to the eggs (Bernardo, 1996), or by their social environment, i.e. the workers who raise the brood. Studies in four species of ants have suggested a genetic component to queen size. In the fire ant *Solenopsis invicta*, an allelic difference at the gene *Gp-9* correlated with both social structure and queen size (Ross, 1997; Ross & Keller, 1998; Keller & Ross, 1999). Reproductive queens in monogyne colonies invariably carried the *Gp-9^{BB}* genotype, whereas the ones in polygyne colonies generally carried the *Gp-9^{Bb}* genotype. At the end of the rearing period, young queens with the *Gp-9^{BB}* were larger than young queens with the *Gp-9^{Bb}* genotype. Young queens with the *Gp-9^{Bb}* genotype had lower survival, and the few that survived in polygyne colonies were the smallest queens. In *L. rugatulus*, the head widths of the mother and daughter queens were positively correlated, and there was no association with egg size (Rüppell *et al.*, 2001). In colonies of *Lasius niger* headed by one doubly-mated queen, the size of the queens produced differed significantly between the two patriline, strongly suggesting a genetic component to size (Fjerdingstad, 2005). Finally, in the ant *Formica truncorum*, queen size was heritable in one of the two study years but not in the other, suggesting that the relative importance of environmental factors may vary over years (Bargum *et al.*, 2004). A genetic polymorphism for body size and social structure may be maintained by variability in ecological and social conditions causing disruptive selection on these traits (Ross & Keller, 1995).

An effect of the social environment on queen, male and worker body size has also been documented. Workers can control the amount of food that is allocated to brood (Wheeler, 1991). They can manipulate the size of the individuals produced in their colony in response to variation in food availability, colony age, or colony composition (Oster & Wilson, 1978; Wheeler & Nijhout, 1981; Wheeler, 1991). For example, the size of worker pupae increases with colony size and decreases with larval density in experimental colonies of *S. invicta* (Porter & Tschinkel, 1985). In *Pheidole pallidula*, the production of large workers specialized in colony-defence increases when resident workers perceive the presence of foreign workers close to their nest (Passera *et al.*, 1996). Finally, workers may preferentially eliminate or underfeed one type of brood (e.g. males) to funnel resources towards their preferred

sex (Chapuisat *et al.*, 1997b; Starks & Poe, 1997; Bourke, 2005; Chapter 1: Meunier *et al.*, 2008).

We here investigate the proximate causes of queen size variation in the socially polymorphic ant *Formica selysi*. In our study population, monogyne and polygyne colonies coexist in the same habitat (Chapuisat *et al.*, 2004). In the field, colony social structure is associated with a significant difference in the body size of the new queens (Rosset & Chapuisat, 2007). Specifically, monogyne colonies produce queens that have a 59% higher dry weight and a 2.1% larger head width than queens originating from polygyne colonies, whereas the males produced in the two types of colonies have the same size (Rosset & Chapuisat, 2007). In addition to this divergence in the size of queens produced, monogyne and polygyne colonies differ in multiple life-history traits such as colony size, nest density, colony lifespan and worker size (Schwander *et al.*, 2005; Rosset & Chapuisat, 2007).

One interesting aspect of our study population is that there is no genetic differentiation at neutral markers between the monogyne and polygyne social forms and no sign of assortative mating with respect to social form (Chapuisat *et al.*, 2004); A. Reber, J. Meunier and M. Chapuisat, unpublished data). Because sex and recombination tend to break allelic associations between multiple loci, the maintenance of two social forms in sympatry suggests that the primary switch towards the development of alternative social structure is triggered by a single polymorphic gene or tightly linked group of genes, as in the fire ant *S. invicta* (Keller & Ross, 1993b; Ross & Keller, 1998). Alternatively, the social forms may result from phenotypic plasticity depending on maternal, environmental or social factors affecting the developmental trajectory of the colony (West-Eberhard, 1989; Chapuisat *et al.*, 2004).

The aim of this study was to test if the size variation in young queens of *F. selysi* was caused by a genetic polymorphism or maternal effect present in the eggs, or by the social structure of the workers raising the brood. We cross-fostered eggs among experimental colonies, so that eggs collected from either monogyne or polygyne colonies were raised by workers originating from either monogyne or polygyne colonies. If the size of new queens is influenced by genes or maternal effects transmitted to the eggs, we expect that eggs from monogyne colonies will develop into larger queens than eggs from polygyne colonies, independently of the

origin of the workers caring for the brood. In contrast, if the social environment influences the size of new queens, we expect that monogyne workers will produce larger queens than polygyne workers, independently of the origin of eggs. Understanding the causes of queen size variation will help to understand how the feedback loop linking queen number, queen size and colony founding may shape and stabilize social structure variation.

MATERIALS AND METHODS

Study species

Our study population of the ant *Formica selysi* is located between Sierre and Susten along the river Rhône in central Valais, Switzerland (7°36'30"E, 46°18'30"N, altitude 565 m). The social structure (monogyne or polygyne) of each colony used in this experiment had been previously determined by genotyping eight to 100 workers per colony at nine microsatellite markers (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005; Rosset & Chapuisat, 2006). Repeated sampling and genotyping of individuals in the same colonies over several years confirmed that the colonies have stable social structures (see Reber *et al.*, 2008).

In *F. selysi* queens lay eggs in spring and summer and no brood overwinters (Forel, 1920; Gösswald, 1989). Eggs laid early in the season (April) develop into queens and males, whereas eggs laid in late spring and summer become workers. For the cross-fostering experiment, we collected eggs and workers during the first week of April 2006. For the comparison of egg volumes, we sampled eggs on April 5th and May 15th, 2007.

Cross-fostering experiment

We cross-fostered eggs and workers in order to obtain the four following combinations: (1) workers from monogyne colonies with eggs from monogyne colonies, (2) workers from monogyne colonies with eggs from polygyne colonies, (3) workers from polygyne colonies with eggs from monogyne colonies and (4) workers from polygyne colonies with eggs from polygyne colonies (Fig 1). In total, we used eggs and workers originating from 56 independent field colonies (28 monogyne and

28 polygyne ones, respectively). The cross-fostering was organized in 14 blocks, each block corresponding to the crossing of eggs and workers among four independent field colonies (Fig 1). Hence, each block comprised four experimental colonies that had received workers from one polygyne or one monogyne field colony, and eggs from another monogyne or polygyne field colony, respectively (Fig 1).

Each experimental colony started with 290 ± 30 workers and 55 ± 6 eggs. They were established in plastic boxes ($27 \times 43 \times 16$ cm) side-lined with fluon and covered by a net to prevent ants from escaping. Each box contained ground material from the field nest of the workers and two test tubes with wet cotton wool that served as shelter for the brood and workers. Ants in experimental colonies had access to *ad libitum* water, 10% sugar water, and a protein-rich jelly provided on a daily basis (mix of beef meat, chicken eggs, mealworms, Oranol© Vitamins for baby, sugar, salt, gelatine and water). They were kept at $25 \pm 2^\circ\text{C}$ under a 14:10h light:dark cycle.

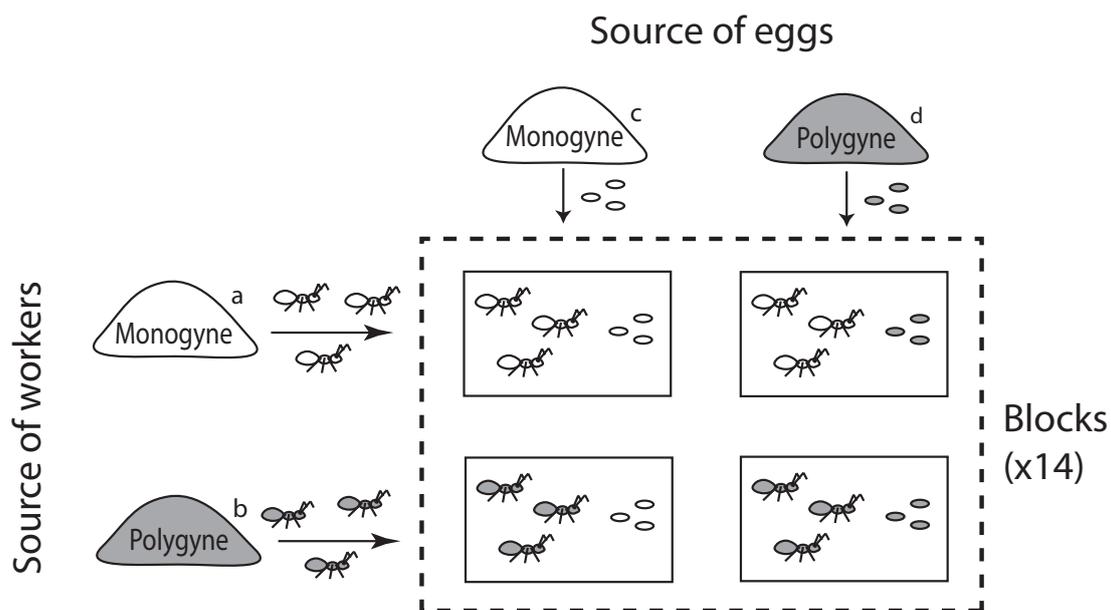


Figure 1 - Cross-fostering design. The dashed line represents one “block”, which includes four experimental colonies. The experiment had a total of 14 such blocks. Colonies *a*, *b*, *c* and *d* are independent field colonies that were used in only one block of the experiment.

Size measurement

We measured the fresh weight of queens and males produced in the experimental colonies at two points in their development, namely at the callow and mature stages. The size of the males will serve as an internal control to check that the cross-fostering experiment is not affected by confounded factors. Callow individuals are very young adults that have just finished metamorphosis and are recognizable by their pale colour and soft cuticles. Queen and male callows appeared 51 ± 4 days (mean \pm SD) and 56 ± 8 days after the start of the experiment, respectively. Mature individuals were measured when they were ready to fly away from the nests. Mature males started to fly 10 ± 7 days after they had reached the callow stage and were weighed at this time. Because queens rarely fly under laboratory conditions, we weighed queens 30 days after the callow stage, which corresponds to the time period after which queens take flight in natural conditions. Mature males and queens were then stored at -20°C until further analyses.

We measured the dry weight of queens ($n= 102$) and males ($n= 409$) to the nearest 0.001 mg, after having desiccated them for 24h at 55°C (Rosset & Chapuisat, 2007). We measured their head width (minimum width across the eyes) to the nearest 10^{-3} mm using a stereomicroscope Nikon V-12 at a magnification of 50x (see Schwander *et al.*, 2005). Finally, we extracted lipids from the thorax and abdomen of queens by placing them in petroleum ether at 52°C for 48h. After this extraction, the queens were dried again for 24h, re-weighed, and the lipid content was estimated as dry weight loss between the two measures divided by the dry weight before extraction (Keller & Passera, 1989).

We examined whether egg size differed between the two social forms, which would suggest a maternal effect based on the amount of resources packed in the eggs. We estimated the volume and shape of queen- and male-destined eggs sampled from 19 monogyne and 19 polygyne field colonies on April 5th, 2007. We also measured worker-destined eggs collected from a subsample of 9 monogyne and 15 polygyne field colonies on May 15th, 2007. We used the ImageJ© v1.40 Software (<http://rsbweb.nih.gov/ij/download.html>) to measure the width and length of 15 eggs per colony to the nearest 0.001 mm. We estimated egg volume with the ovoid

formula $\frac{4\pi}{3} \times \left(\frac{width}{2}\right)^2 \times \left(\frac{length}{2}\right)$, and egg shape with the ratio of length on width.

Statistical analysis

We analysed the fresh weight, dry weight, head width and lipid content of queens and males using two-way analyses of variance (ANOVAs) in which the social structure of origin of eggs and workers (monogyne or polygyne) were used as fixed factors. We also tested the influence of these factors on the gain of fresh weight between the callow and mature stages. In all ANOVAs, we used one mean value per experimental colony in order to avoid pseudo-replication due to the non-independence of individuals that matured in the same colony. Moreover, we used “blocks” as a random factor (mixed models), because some of the experimental colonies did not produce queens or males (Table 1) and each set of four field colonies might have had specific characteristics influencing the morphological traits of the individuals produced in a block (Fig 1). We controlled that there was no significant correlation between the number of queens or males produced in each colony and their mean fresh weight, dry weight, head width and lipid content (Spearman’s rank correlation tests, all *P-values* > 0.20).

To compare the production of queens and males in each type of experimental cross-fostering, we computed a two-way ANOVA on the odds of males and queens produced, using the social origin of eggs and workers as fixed factors. Finally, we compared egg volumes using a two-way ANOVA in which the social origin of eggs and date of sampling (April 5th or May 15th) were entered as fixed factors. Since in some cases eggs were collected in the same field colonies on the two sampling dates, we entered the colony of origin as a random factor (mixed model). To achieve residual normality, dry weights, head widths and egg volumes were log-transformed.

RESULTS

Queen and male size

Eggs originating from monogyne colonies developed into larger queens than eggs originating from polygyne colonies, independently of the social structure of the workers caring for them. Specifically, queens originating from eggs sampled in monogyne colonies reached a significantly higher fresh weight (Table 1, callow stage: $F_{1,10} = 9.19$, $P = 0.013$; mature stage: $F_{1,10} = 7.94$, $P = 0.018$), higher dry weight (Table 1, $F_{1,10} = 6.79$, $P = 0.026$) and larger head width (Table 1, $F_{1,10} = 6.53$, $P =$

0.029) than queens coming from eggs sampled in polygyne colonies. Moreover, they tended to have more lipids, even if this last difference was not statistically significant (Table 1, $F_{1,10} = 2.84$, $P = 0.12$).

In contrast, the monogyne or polygyne origin of the workers caring for the brood had little effect on queen size. Specifically, the social structure of origin of workers had no significant effect on queen fresh weight (Table 1, callow stage: $F_{1,10} = 0.001$, $P = 0.97$, mature stage: $F_{1,10} = 1.77$, $P = 0.21$), dry weight (Table 1, $F_{1,10} = 2.17$, $P = 0.17$), head width (Table 1, $F_{1,10} = 0.17$, $P = 0.69$) and lipid content (Table 1, $F_{1,10} = 1.57$, $P = 0.24$). The only significant difference was that queens raised by workers from monogyne colonies gained more fresh weight between the callow and mature stages than queens reared by workers from polygyne colonies (Table 1, $F_{1,11} = 4.84$, $P = 0.050$). This gain of fresh weight did not differ significantly between queens originating from eggs sampled in monogyne or polygyne colonies (Table 1, $F_{1,11} = 2.18$, $P = 0.17$). Finally, there was no significant interaction between the social origin of workers and the social origin of eggs with respect to queen fresh weight (callow stage: $F_{1,10} = 0.50$, $P = 0.50$; mature stage: $F_{1,10} = 0.063$, $P = 0.81$), dry weight ($F_{1,10} = 0.08$, $P = 0.78$), head width ($F_{1,10} = 0.28$, $P = 0.61$) and lipid content ($F_{1,10} = 0.080$, $P = 0.78$).

The size of males produced in experimental colonies was independent of the social structure of origin of either eggs or workers. Specifically, the fresh weight of males was not significantly affected by the origin of eggs (Table 1; callow stage, $F_{1,32} = 0.81$, $P = 0.37$; mature stage, $F_{1,32} = 0.15$, $P = 0.70$), nor by the origin of the workers caring for these eggs (Table 1; callow stage: $F_{1,32} = 0.76$, $P = 0.39$; mature stage: $F_{1,32} = 0.01$, $P = 0.92$). We also found no significant effect of the origin of eggs or workers on male head width (Table 1, eggs: $F_{1,32} = 0.02$, $P = 0.89$; workers: $F_{1,32} = 2.17$, $P = 0.15$), on male dry weight (Table 1, eggs: $F_{1,32} = 0.91$, $P = 0.35$; workers: $F_{1,32} = 0.43$, $P = 0.52$), nor on the variation of male fresh weight between the callow and mature stages (Table 1; eggs: $F_{1,33} = 0.30$, $P = 0.59$; workers: $F_{1,33} = 1.42$, $P = 0.24$). Finally, there was no significant interaction between the social origin of workers and the social origin of eggs with respect to male fresh weight (callow stage: $F_{1,32} = 0.06$, $P = 0.94$; mature stage: $F_{1,32} = 0.39$, $P = 0.54$), dry weight ($F_{1,32} = 0.96$, $P = 0.33$) and head width ($F_{1,32} < 0.001$, $P = 0.99$).

Table 1 Queens and males produced in the cross-fostering experiment. Dry weight, head width and lipid content were measured on mature individuals. Mean measures are provided with standard errors.

	Types of cross-fosterings in experimental colonies			
	Monogyne eggs		Polygyne eggs	
	Monogyne workers	Polygyne workers	Monogyne workers	Polygyne workers
Queens				
Fresh weight of callows (mg)	27.71 ± 1.16	28.37 ± 1.61	25.53 ± 0.94	25.41 ± 0.67
Fresh weight of mature individuals (mg)	32.15 ± 1.98	30.18 ± 1.99	28.27 ± 1.41	25.81 ± 1.15
Dry weight (mg)	14.71 ± 1.10	12.98 ± 1.40	12.27 ± 1.0	10.35 ± 0.59
Head width (mm)	1.24 ± 0.02	1.23 ± 0.03	1.21 ± 0.03	1.21 ± 0.01
Lipid content	0.23 ± 0.01	0.20 ± 0.01	0.20 ± 0.02	0.19 ± 0.01
Number of queen-producing colonies	7	4	10	6
Number of queens produced per colony	3.86 ± 1.20	4.75 ± 2.05	3.20 ± 0.89	4.00 ± 1.37
Survival rate of queens from emergence to day 30	0.95 ± 0.05	0.85 ± 0.12	0.85 ± 0.07	0.92 ± 0.06
Males				
Fresh weight of callows (mg)	20.33 ± 0.38	19.97 ± 0.44	20.03 ± 0.38	19.64 ± 0.33
Fresh weight of mature individuals (mg)	15.86 ± 0.22	15.98 ± 0.28	15.89 ± 0.30	15.63 ± 0.33
Dry weight (mg)	4.89 ± 0.09	4.91 ± 0.11	4.89 ± 0.09	4.70 ± 0.14
Head width (mm)	0.93 ± 0.01	0.94 ± 0.01	0.93 ± 0.01	0.93 ± 0.01
Number of male-producing colonies	13	13	13	10
Number of males produced per colony	10.38 ± 0.51	4.85 ± 0.32	11.77 ± 0.63	6.40 ± 0.37
Survival rate of males from emergence to flight	0.86 ± 0.06	0.91 ± 0.04	0.91 ± 0.11	0.89 ± 0.05

The number of queens and males produced in experimental colonies varied from 0 to 10 and 0 to 22, respectively. The number of queens produced was not significantly affected by the origin of eggs or workers (Table 1, $\chi^2 = 1.63$, $P = 0.65$). In contrast, experimental colonies containing workers originating from monogyne colonies produced significantly more males than experimental colonies containing workers originating from polygyne colonies (Table 1, $\chi^2 = 20.92$, $P < 0.001$). Experimental colonies with monogyne workers also produced a significantly higher proportion of males than colonies with polygyne workers (ANOVA on odds ratios, $F_{2,44} = 5.64$, $P = 0.007$), whereas this proportion was not significantly influenced by the social origin of eggs ($F_{2,44} = 0.44$, $P = 0.65$) or the interaction between the social origin of eggs and the social origin of workers ($F_{2,44} = 0.55$, $P = 0.58$). Finally, the proportion of queens and males that died between the callow and mature stages did not differ significantly among the types of experimental cross-fosterings (Table 1; queens: Fisher's exact test, $P = 0.46$; males: Chi-square test, $\chi^2_3 = 1.92$, $P = 0.59$).

Egg size

Eggs sampled in monogyne field colonies were 25% smaller than eggs sampled in polygyne field colonies (Fig 2; $F_{1,37} = 14.68$, $P < 0.001$). The worker-destined eggs sampled on May 15th were 28.5% smaller than the queen- and male-destined eggs sampled on April 5th (Fig 2, $F_{1,21} = 81.12$, $P < 0.001$), but there was no significant interaction between colony social structure and date of sampling ($F_{1,21} = 1.67$, $P = 0.21$). The difference in egg volume was not associated with a difference in egg shape, as the ratio of length on width was not significantly different between eggs sampled in monogyne and polygyne colonies (April 5th, 2007: monogyne colonies: 2.05 ± 0.06 (mean \pm SD), polygyne colonies: 2.02 ± 0.08 , t test, $t = 0.90$, $df = 36$, $P = 0.38$; May 15th, 2007: monogyne colonies: 1.95 ± 0.07 , polygyne colonies: 1.94 ± 0.07 , t test, $t = 0.33$, $df = 22$, $P = 0.74$).

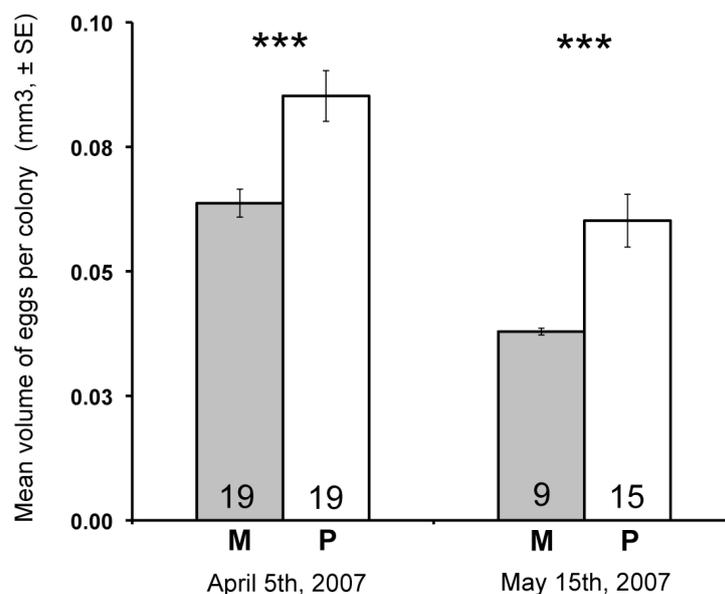


Figure 2 - Volume of the eggs sampled in monogyne (M) and polygyne colonies (P). Eggs collected on April 5th, 2007 mainly develop into queens and males, whereas eggs collected on May 15th, 2007 only develop into workers. Sample sizes are provided at the bottom of each bar (number of colonies, average egg size based on 15 eggs measured per colony). *** $P < 0.001$

DISCUSSION

The determination of queen size in ants is potentially complex and can be affected by genes, maternal effects and/or the social environment. Moreover, the size of young queens can play a central role in the transmission of social structure if it influences dispersal, colony founding and queen number in future colonies (Bourke & Franks, 1995; Wiernasz & Cole, 2003). Alternative social structures (monogyne or polygyne) indeed correlate with queen size across and within species, but the proximate factors at the basis of this relationship remain poorly understood (Keller & Passera, 1989; Bourke & Franks, 1995; Rosset & Chapuisat, 2007).

Our cross-fostering experiment in the socially polymorphic ant *Formica selysi* demonstrates that the association between the social structure of the colony and the size of the new queens produced results from genetic or maternal effects transmitted to the eggs. Specifically, queens originating from eggs sampled in monogyne colonies became larger than queens developing from eggs sampled in polygyne colonies, independently of the origin of the workers that cared for the brood.

It is generally difficult to disentangle genetic and maternal effects in cross-fostering experiments. In social Hymenoptera, several studies have indicated a positive association between the sizes of old and new queens (Rüppell *et al.*, 2001) or a genetic component to queen size (Ross & Keller, 1998; Bargum *et al.*, 2004; Fjerdingstad, 2005). One important potential source of maternal effect is egg size, which in Hymenoptera is generally correlated with the body weight of the laying female (Berrigan, 1991). Hence, large queens might simply pack more resources into larger eggs that would develop into larger queens. In *F. selysi*, we found the opposite pattern, as eggs collected in monogyne colonies were significantly smaller but developed into significantly larger queens than eggs collected in polygyne colonies. Our results therefore suggest that the variation in queen size between monogyne and polygyne colonies is not caused by a maternal effect linked to the amount of resources packed into the eggs, and is probably affected by a genetic polymorphism, even if other forms of maternal effects are also possible. Similar conclusions had been reached for the ant *L. rugatulus*, in which macrogyne queens produced larger queens, but eggs of similar size, than microgyne queens (Rüppell *et al.*, 2001). To

further distinguish between genetic and maternal effects in *F. selysi* would require us mating the queens with one male of each social structure and looking at queen size differences between patriline.

Why do queens from monogyne colonies produce smaller eggs? One simple explanation would be that there is a trade-off between egg size and egg number (Kayaalp & Schwarz, 2007). Queens in monogyne colonies have to supply all the eggs for their colony, and have therefore a much higher egg-laying rate than queens from polygyne colonies (Vander Meer *et al.*, 1992). It is also possible that queens from polygyne colonies have to lay larger eggs because they compete with each other and workers preferentially rear large eggs. In line with this hypothesis, eggs originating from polygyne colonies had a higher acceptance rate than eggs originating from monogyne colonies when they were introduced into groups of unrelated workers from each social structure (J. Meunier and M. Chapuisat, unpublished data).

One major component of the social environment, namely the social structure of the workers caring for the brood, had no significant impact on the size of young queens at the end of the rearing period. We did however detect a small difference in the developmental trajectory of queens. Specifically, queens raised by workers originating from monogyne colonies gained slightly more fresh weight between the callow and mature stages than queens reared by polygyne workers, and this independently of the origin of eggs. A similar pattern has been documented in *S. invicta* (Keller & Ross, 1993a). Workers probably influence this variation in fresh weight by controlling the amount and quality of food given to young queens. In *F. selysi*, monogyne workers are larger than polygyne ones (Schwander *et al.*, 2005), and might thus be more efficient at feeding adult queens.

In contrast to queens, we found no significant difference in the size of males produced in the four types of experimental cross-fosterings. This result is in line with the observation that monogyne and polygyne colonies produce males of similar weight in the field (Rosset & Chapuisat, 2007). It is also an important internal control demonstrating that the differences in queen size were not due to some uncontrolled factor affecting brood development and varying between the four types of experimental cross-fosterings.

Interestingly, the origin of the workers did not significantly affect the number of queens produced but had a significant impact on the number of males produced. Experimental colonies with workers originating from monogyne colonies produced twice as many males as colonies with workers originating from polygyne colonies. As in many other species of the genus *Formica* (Helanterä & Sundström, 2007b), young workers of *F. selysi* start to lay unfertilized haploid eggs shortly after being orphaned. Hence, part of the males produced in our experimental queenless colonies probably came from worker-laid eggs. Differences in male production between workers originating from monogyne or polygyne workers might thus be due to (i) monogyne workers laying more eggs than polygyne workers, (ii) polygyne workers destroying more worker-laid eggs than monogyne workers (policing', reviewed in Wenseleers & Ratnieks, 2006), and/or (iii) eggs laid by polygyne workers being less viable than eggs laid by monogyne workers. Further experiments will be needed to sort among these hypotheses.

To conclude, our cross-fostering experiment indicates that queen size in the socially polymorphic ant *F. selysi* is primarily influenced by a genetic polymorphism or possibly a maternal effect transmitted to the eggs independently of egg size. The fact that queen size is inherited may play a role in determining the social structure of future colonies, because queen size is likely to affect the success of independent colony founding by single queens (Wiernasz & Cole, 2003), and small queens may be constrained to found new colonies by budding. Further experiments will be needed to evaluate if the size and social origin of queens affect their colony founding success or acceptance rate by workers. These experiments will reveal if there is a strong and causal relationship between queen size and colony queen number, or if this association is just one component of a broader behavioural syndrome determining social structure variation.

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CHAPTER 4

CHAPTER 4

Flexible colony founding strategies in a socially polymorphic ant

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This chapter is a manuscript being prepared for publication

ABSTRACT

Social insects exhibit a great variation in the number of queens per nest. One of the proximate causes of this variation may be that queens produced by multiple-queen colonies are generally smaller, and might thus be unable to found new colonies independently. Here, we examined if the social origin of queens and males influenced the colony founding success of queens in the socially polymorphic ant *Formica selysi*. Queens originating from single-queen (= monogyne) and multiple-queen (= polygyne) colonies had similar survival rate and colony founding success, be they alone or in two-queen associations. During the first five months, queens of monogyne origin gave rise to more workers than queens of polygyne origin. Pairs of queens were also more productive than single queens. However, these differences in productivity were transient, as all types of colonies had reached a similar size after 15 months. Mating between social forms was possible and did not decrease queen survival or colony productivity, as compared to mating within social forms. Independently of the social origin of queens, mating with males of polygyne origin resulted in a higher rate of colony failure over 15 months, as compared to mating with males of monogyne origin. Overall, these results indicate that queens from each social form are able to found colonies independently, at least under laboratory conditions. Moreover, gene flow between social forms is not restricted by mating or genetic incompatibilities. This flexibility in mating and colony founding helps to explain the maintenance of alternative social structures in sympatry and the absence of genetic differentiation between social forms.

INTRODUCTION

One of the major transitions of life on earth is the monopolization of reproduction by a few members of a social group (Szathmary & Maynard Smith, 1995). This partitioning of reproduction culminates in eusocial insects, where it primarily evolved by kin selection in simple families headed by one single-mated female (Hughes *et al.*, 2008b; Hughes *et al.*, 2008c). Secondary changes in colony social structure have been frequent, particularly in ants, which show a great variation in the number of breeders per nest, both within and among species (Keller, 1993b; Bourke & Franks, 1995). The shift from a single to multiple queens per colony seems paradoxical at first, as it decreases the relatedness among nestmates and the inclusive fitness of workers (Hamilton, 1964c). However, this shift is generally associated with profound changes in dispersal and life-histories that may be beneficial in some ecological conditions (Hölldobler & Wilson, 1977; Nonacs, 1988; Keller, 1993b; Bourke & Franks, 1995; Rosset & Chapuisat, 2007).

Identifying the proximate and ultimate factors influencing the evolution and maintenance of alternative social forms has become a central question in studies of social evolution (Keller & Reeve, 1994b). In ants, variation in colony queen number is frequently associated with differences in queen dispersal and mode of colony founding (Hölldobler & Wilson, 1977; Keller, 1993a). Monogyne (= single-queen) colonies are generally founded independently by queens (= haplometrosis). Young queens disperse on the wing, mate away from their natal nest and initiate a new colony alone (Hölldobler & Wilson, 1977; Keller & Passera, 1989; DeHeer *et al.*, 1999). Haplometrosis is comparatively risky, as lone queens are exposed to predation (Nichols & Sites, 1991), nest usurpation and mortality due to excessive dryness or humidity (Johnson, 2000).

Young queens may also associate during colony founding and cooperate to raise their first workers (= pleometrosis, Helms Cahan, 2001; Johnson, 2004). Pleometrosis increases queen survival (Tschinkel, 1998; Cahan & Julian, 1999) and worker production, which are critical for colony founding success (Heinze, 1993; Bernasconi & Keller, 1996). Queen associations are often unstable: after the emergence of workers, queens generally fight until only one survives (Sommer & Hölldobler, 1995; Bernasconi & Strassmann, 1999). However, stable queen

associations resulting in permanently polygyne colonies have been reported in several ant species (Trunzer *et al.*, 1998; Johnson, 2002; Kolmer *et al.*, 2002; D'Ettorre *et al.*, 2005).

Polygyny (= the presence of multiple reproductive queens) is often associated with restricted dispersal of queens (Bourke & Franks, 1995; Chapuisat *et al.*, 1997a). As polygyne colonies readily accept additional queens, young queens have the option to stay within their natal colony or to seek adoption in a foreign colony (Heinze, 1993; Bourke & Franks, 1995). Polygyny is frequently associated with budding, a process whereby queens and workers walk away from their natal colony to establish a new colony in the vicinity (Peeters & Ito, 2001). A similar process occurs in a few monogyne species such as army ants, in which the mother and daughter queens divide up the workforce to form two independent colonies (colony fission, Peeters & Ito, 2001; Seppä *et al.*, 2008).

The divergence between monogyne and polygyne social forms in their mode of colony founding is generally associated with a difference in queen size and queen relative fat content (Keller & Passera, 1989). Queens originating from single-queen colonies need large energy reserves to disperse and found new nests independently. In contrast, queens originating from multiple-queen colonies need fewer reserves if they mate close to their natal nest and found new nests with the help of workers. As a result, queens from polygyne species are generally smaller and have lower relative fat content than queens from monogyne species (Keller & Passera, 1989; Howard, 2006). Polygyne queens also tend to have shorter lifespan, lower fecundity and earlier age of first sexual reproduction (Bourke & Franks, 1995; Keller & Genoud, 1997). If, because of their smaller size and lower energetic reserves, queens originating from polygyne colonies are unable to found new colonies independently, a feedback loop may link queen number, queen size and mode of colony founding (Hölldobler & Wilson, 1977; Keller & Passera, 1989; Seppä & Pamilo, 1995; Chapter 3: Meunier & Chapuisat, in press).

The association between colony social structure and queen dispersal has mostly been studied by comparing species or populations that are geographically separated and differ in ecological conditions. However, in some species the monogyne and polygyne social forms co-exist in sympatry (Fournier *et al.*, 2002; Fritz & Vander Meer, 2003; Chapuisat *et al.*, 2004; DeHeer & Herbers, 2004; Seppä

et al., 2004; Gyllenstrand *et al.*, 2005; Bargum *et al.*, 2007). Such populations are particularly interesting to investigate the causes and correlates of social structure variation.

Formica selysi is a socially polymorphic species in which monogyne and polygyne colonies are interspersed within the same population (Chapuisat *et al.*, 2004). Queens produced by polygyne colonies are smaller, and polygyne colonies invest less in reproductive individuals and more in workers (Rosset & Chapuisat, 2007; Chapter 3: Meunier & Chapuisat, in press), which suggests that queens originating from polygyne colonies might be constrained to found new colonies with the help of workers. A recent cross-fostering experiment has shown that queen size is influenced by genetic or maternal effects transmitted to the eggs (Chapter 3: Meunier & Chapuisat, in press), further suggesting that queens originating from monogyne or polygyne colonies might differ in their colony founding ability. However, the lack of genetic differentiation between social forms at microsatellite markers suggests that mating between social forms or flexibility in dispersal and colony founding maintain gene flow between social forms (Chapuisat *et al.*, 2004).

In order to get insight into the mechanisms contributing to the maintenance alternative social structures in sympatry, we studied the reproductive options of *F. selysi* queens. First, we compared the colony founding ability of queens originating from monogyne and polygyne colonies, alone or in association. Second, we assessed if mating between males and females belonging to alternative social forms was possible and resulted in genetically viable offspring. These experiments will permit to determine if colony founding is plastic or influenced by the socio-genetic origin of queens, and more generally if gene flow between social forms is restricted by differences in colony founding strategies or mating incompatibilities.

MATERIALS AND METHODS

Sampling of ants

The ants came from a population of *Formica selysi* located between Sierre and Susten along the river Rhône in central Valais, Switzerland. We sampled males and females from 46 monogyne and 19 polygyne colonies from which we already had detailed genetic data (Chapuisat *et al.*, 2004; Schwander *et al.*, 2005). Males and females were

collected in June 2007 under flat stones placed on each field colony. Ants were brought back to the laboratory in plastic boxes (13.5 cm long x 15 cm wide x 5 cm high) lined with fluon to prevent escape.

Experimental mating

Queens were crossed with males originating from monogyne and polygyne colonies, respectively. Experimental mating took place outside, in the morning, at a temperature comprised between 20 and 25 °C. Pairs of queens coming from the same colony of origin were placed in plastic containers (35cm long x 22cm wide x 15cm high) lined with fluon and covered with a net. Five to ten males coming from several colonies were added, using separate containers for males originating from monogyne or polygyne colonies. We isolated each queen immediately after mating.

Colony founding

In June and July 2007, mated queens were placed in glass test tubes (15.5 cm long x 6 mm diameter) either alone (one-queen founding) or in pairs originating from the same colony and mated on the same day (two-queen founding). The bottom of the test tube was filled with water retained by a small cotton wool plug, and the tube opening was closed with cotton wool (Bernasconi & Keller, 1998). Founding queens were maintained at 25°C under a 12 hours day/night cycle, without food. Test tubes were inspected daily until the first workers were five days old. At this point, the tubes were opened and placed in plastic boxes (13.5 cm long x 15 cm wide x 5 cm high) lined with fluon. These incipient colonies were then provided with ad libitum supplies of water and protein-rich jelly made of honey, chicken egg, beef meat and agar.

The growth of incipient colonies was recorded over 15 months, until the end of the second summer (September 2008). Eighty-two queens died during the first phase of colony founding, before worker emergence. These queens were dissected to check their mating status. None of them had empty spermatheca, which indicates that they had successfully mated. Fourteen queens were still alive but had not produced any brood after five months. These queens were killed and 11 of them were successfully dissected, which revealed that six had no sperm in the spermatheca. The proportion of broodless queens and the proportion of unmated queens were not

significantly affected by the social origin of queens (broodless: $F_{1,455} = 0.7$, $P = 0.4$, unmated: $F_{1,455} = 0.3$, $P = 0.6$) or the social origin of males (broodless: $F_{1,455} = 0.2$, $P = 0.7$, unmated: $F_{1,455} = 1.2$, $P = 0.3$), and there was no interaction between the two factors (broodless: $F_{1,455} = 0.6$, $P = 0.4$, unmated: $F_{1,455} = 0.01$, $P = 0.9$).

Five months after the start of the experiment, we placed the colonies in hibernation, which lasted from November 2007 to April 2008. The temperature was decreased to 15 °C for one week, before to be set at 8°C for the rest of the hibernation time. In April 2008, the temperature was progressively raised to 25°C. Colony growth (number and workers) and queen mortality were checked monthly until the end of the second summer, in September 2008. Queens were weighted at the start of the founding experiment, just before hibernation, and at the end of the second summer.

In total, we obtained data on queen mortality and colony growth for 124 incipient colonies founded by a single queen (71 and 53 of monogyne and polygyne origin, respectively) and 159 incipient colonies founded by two queens (202 and 116 of monogyne and polygyne origin, respectively).

Statistical analysis

Queen mortality was estimated as the proportion of dead queens, using one value per colony. Colony failure was characterized by the death of all queens in a colony. Queen mortality and colony failure rate were tested using likelihood ratio tests on a quasibinomial regression model with three fixed factors and their interactions: female social origin (monogyne or polygyne), male social origin (monogyne or polygyne), and mode of colony founding (one queen or two-queen founding).

Worker production (square-root+1 transformed) was tested using an ANOVA with three fixed factors and their interactions: female social origin, male social origin and the mode of colony founding. In cases of two-queen founding (pleometrosis), queen execution often occurs after worker emergence (Rissing & Pollock, 1987; Sommer & Hölldobler, 1995; Bernasconi & Strassmann, 1999). As the timing of queen mortality is likely to be important for productivity, we made a distinction between two-queen colonies in which both queens survived (2Q), two-queen colonies in which one of the queens died in the early phase of colony founding before worker

emergence (2→1Q early) and two-queen colonies in which one of the queens died after worker emergence (2→1Q late).

We tested for interactions between factors using likelihood ratio tests and sequentially removed all non-significant interactions ($P > 0.10$). Queen mortality, colony failure and worker production were compared after five months (just before hibernation) and after 15 months (at the end of the second summer). All statistical analyses were performed in R 2.8.0 (<http://www.R-project.org>).

RESULTS

Queen mortality and colony failure

Five months after the start of the colony-founding experiment, queens originating from monogyne and polygyne colonies did not differ significantly in their rates of mortality or colony failure (Table 1; mortality: $F_{1,282} = 1.1$, $P = 0.3$; colony failure: $F_{1,282} = 1.4$, $P = 0.2$). There was also no significant effect of male social origin on queen mortality ($F_{1,282} = 1.4$, $P = 0.2$) or colony failure ($F_{1,282} = 1.8$, $P = 0.2$). Finally, there was no significant interaction between queen social origin, male social origin and mode of founding (all $P > 0.1$).

In contrast, queen mortality was more than two times higher in two-queen than in one-queen associations, as 56.3% and 23.4% of the young queens died in two- and one-queen foundings, respectively ($F_{1,282} = 36.6$, $P < 0.0001$). Somewhat surprisingly, colony failure was also higher in two-queen than in one-queen foundings (42.1 % against 23.4 %, respectively; $F_{1,282}$, $P = 0.001$). In 28.3% of the two-queen foundings, one queen died while the other successfully raised workers and survived until the end of the experiment. The social origin of queens did not influence the proportion of two-queen colonies in which one queen died before ($\chi^2 = 0.04$, $df = 1$, $P = 0.8$) or after ($\chi^2 = 0.004$, $df = 1$, $P = 0.9$) worker emergence.

After 15 months, there was a marginally significant interaction between queen social origin and mode of colony founding with respect to queen mortality ($F_{1,282}$, $P = 0.06$). This was because queens originating from monogyne colonies tended to have a higher mortality in two-queen foundings than queens originating from polygyne

colonies (68.3% versus 53.4%, respectively; $F_{1,158} = 5.0$, $P = 0.03$), whereas there was no significant difference between the two types of queens in one-queen foundings ($F_{1,123} = 0.6$, $P = 0.4$). Queen social origin had no effect on colony failure after 15 months ($F_{1,282} = 1.6$, $P = 0.2$). Male social origin had no effect on queen mortality ($F_{1,282} = 3.1$, $P = 0.08$). However, an unexpected finding was that male social origin did significantly influence colony failure ($F_{1,282} = 4.4$, $P = 0.04$). Specifically, colonies in which queens had mated with males from polygyne colonies had a higher failure rate (44.9%) than colonies in which queens had mated with males from monogyne colonies (31.5%), independently of the social origin of queens. There was no significant interaction between queen social origin, male social origin and the mode of founding with respect to the rate of colony failure over 15 months (all $P > 0.08$).

Table 1 - Queen mortality and colony failure rate in the colony founding experiment. Queens originating from monogyne or polygyne colonies were experimentally mated with males originating from monogyne or polygyne colonies. Colony failure in two-queen foundings corresponds to the death of both queens. In one queen foundings, colony failure is equal to queen mortality. These rates were measured five and 15 months after the start of the colony-founding experiment, that is just before the first hibernation and at the end of the second summer, respectively. The number of colonies is indicated in brackets.

	Queen mortality				Colony failure	
	One-queen founding		Two-queen founding		Two-queen founding	
	Monogyne queens	Polygyne queens	Monogyne queens	Polygyne queens	Monogyne queens	Polygyne queens
After five months						
Monogyne males	0.17 (34)	0.28 (25)	0.55 (40)	0.45 (28)	0.38 (40)	0.32 (28)
Polygyne males	0.24 (37)	0.25 (28)	0.64 (61)	0.53 (30)	0.54 (61)	0.33 (30)
After fifteen months						
Monogyne males	0.20 (34)	0.32 (25)	0.60 (40)	0.46 (28)	0.40 (40)	0.32 (28)
Polygyne males	0.27 (37)	0.29 (28)	0.74 (61)	0.60 (30)	0.64 (61)	0.43 (30)

The lower success of two-queen foundings, as compared to one-queen foundings, persisted over time. Indeed, queen mortality and colony failure rates over 15 months were higher in two-queen foundings than in one-queen foundings (Table 1, mortality: $F_{1,282} = 44.4$, $P < 0.0001$, colony failure: $F_{1,282} = 13.4$, $P = 0.0003$).

Worker production

The social origin of the queens significantly influenced the number of workers produced by incipient colonies over the five first months of the colony-founding experiment (Fig. 1, $F_{1,171} = 5.1$, $P = 0.02$). Specifically, colonies founded by queens of monogyne origin produced more workers than colonies founded by queens of polygyne origin (Fig.1). This higher productivity might be explained by the heavier weight of monogyne queens at the start of colony founding (t-test, $t = 2.8$, $df = 169.5$, $P = 0.005$). In contrast, male social origin had no effect on worker production ($F_{1,171} = 0.007$, $P = 0.9$) and there were no interaction between queen social origin, male social origin and mode of founding (all $P > 0.4$).

The mode of founding had a strong influence on the number of workers produced after five months (Fig.1; $F_{1,171} = 17.11$, $P < 0.0001$). Two-queen colonies in which one of the queens had died after worker emergence produced significantly more workers than all other foundation types (Tukey HSD, 2→1Q late versus 2Q: $P = 0.05$, 2→1Q late versus 1Q: $P < 0.0001$, 2→1Q late versus 2→1Q early: $P < 0.0001$). Two-queen foundings in which both queens survived produced more workers than one-queen foundings (Tukey HSD, 2Q versus 1Q: $P < 0.0001$) and than two-queen foundings in which one of the queens had died before worker emergence (Tukey HSD, 2Q versus 2→1Q early: $P = 0.0001$). There was a strong positive correlation between the number of workers produced and the duration of the association between queens ($r = 0.33$, $t_{90} = 3.3$, $P = 0.001$).

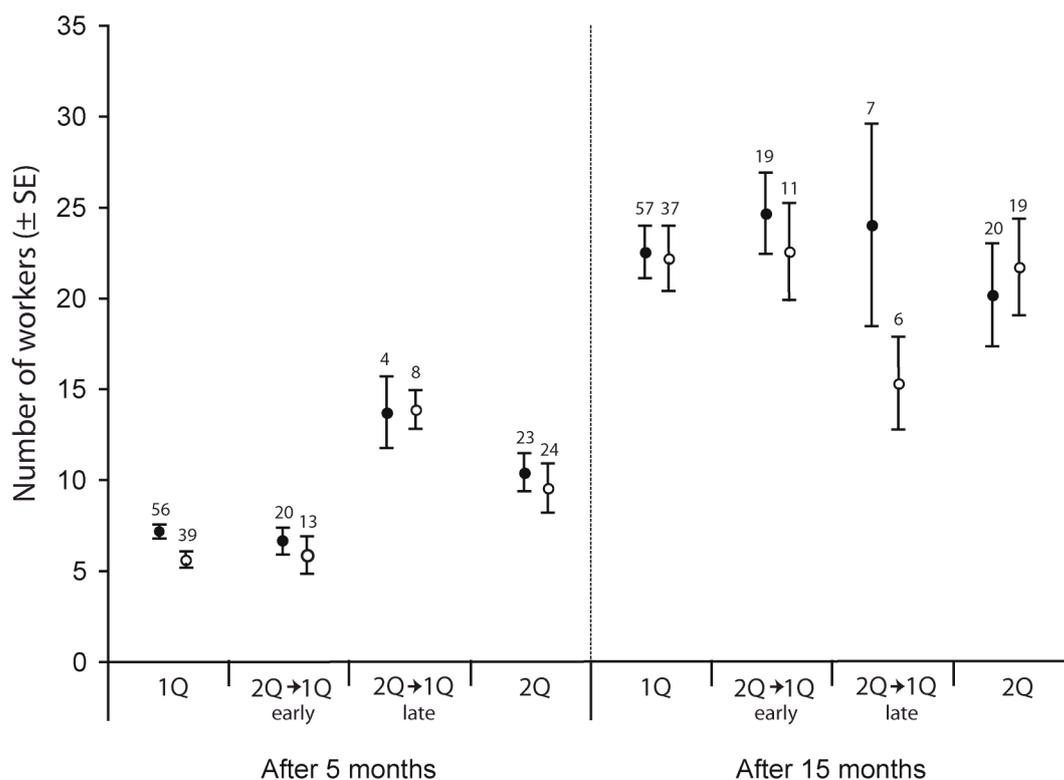


Figure 1 - Mean (\pm SE) number of workers produced by incipient colonies founded by queens originating from monogyne colonies (black dots) or from polygyne colonies (white dots). Incipient colonies were founded by a single queen (1Q), by two queens of which one died before worker emergence ($2 \rightarrow 1Q$ early), by two queens of which one died after worker emergence ($2 \rightarrow 1Q$ late), or by two queens that survived until the end of the experiment (2Q). The productivity after five and 15 months are indicated on the left and right panels, respectively. The number of colonies is indicated above the bars.

These differences in colony productivity were transient and had disappeared at the end of the second summer. After fifteen months, monogyne and polygyne queens had produced similar number of workers (Fig. 1; $F_{1,157} = 0.2$, $P = 0.7$). Moreover, the differences in queen weight had also disappeared (queen weight: $t_{126} = 0.13$, $P = 0.9$).

After fifteen months the differences in colony productivity between colony founding types (1Q, $2 \rightarrow 1Q$ early, $2 \rightarrow 1Q$ late and 2Q) had also become non significant (Fig. 1, $F_{3,157} = 1.1$, $P = 0.3$). This is because after hibernation the increase in the number of workers was significantly higher in one-queen colonies and in two-queen colonies in which one of the queens had died before worker emergence than in the other types of colony foundings (Figure 1; $F_{3,169} = 5$, $P = 0.002$; Tukey HSD 1Q versus $2 \rightarrow 1Q$ late: $P = 0.009$, $2 \rightarrow 1Q$ early versus $2 \rightarrow 1Q$ late: $P = 0.008$). The social origin of males had no effect on worker production after 15 months ($F_{1,57} = 1.1$, $P =$

0.3) and there was no significant interaction between queen social origin, male social origin and mode of founding (all $P > 0.3$).

DISCUSSION

The proximate mechanisms contributing to the maintenance of colony queen number variation in ants remain poorly understood. We found a high degree of flexibility in mating and colony founding by *F. selysi* queens originating from monogyne as well as polygyne colonies. Hence, the results of our colony-founding experiment do not support the hypothesis that a feedback loop linking queen number, queen size and colony founding contributes to stabilize social structure variation in this socially polymorphic ant population (Hölldobler & Wilson, 1977; Keller & Passera, 1989; Chapter 1: Meunier & Chapuisat, in press). Young queens produced by monogyne colonies were significantly heavier than young queens originating from polygyne colonies (this study, see also Rosset & Chapuisat, 2007; Chapter 3: Meunier & Chapuisat, in press). However, queens originating from monogyne and polygyne colonies had a similar survival rate and colony founding success, and both types of queens were able to found colonies without the help of worker.

The queens had no access to food until the first workers had matured. Hence, queens of polygyne and monogyne origin are able to rear a first cohort of workers by relying entirely on their energetic reserves. Stable and protected laboratory conditions may have increased the survival and colony founding success of queens, as compared to natural conditions. However, the fact that queens of monogyne and polygyne origin had similar mortality and colony failure rates in the laboratory suggests that both types of queens have similar capacities for independent colony founding in the field. Fire ant queens originating from polygyne colonies were also able to start new colonies in isolation under laboratory conditions, but their success was lower than the one of queens originating from monogyne colonies (DeHeer, 2002).

Despite generally similar abilities to found colonies independently, there were some subtle differences between *F. selysi* queens of monogyne and polygyne origin. There was a marginally higher rate of colony failure for monogyne queens in two-queen foundings at the end of the experiment, suggesting that monogyne queens may be less tolerant towards the presence of other queens. Monogyne queens also

produced more workers than polygyne ones during the first five months of colony founding, which is probably a consequence of their heavier initial weight. However, the difference between monogyne and polygyne queens in colony productivity was transient, as all types of colonies had reached a similar size at the end of the second summer.

Colonies founded by two queens produced more workers than colonies founded by one queen during the first five months of the experiment, independently of the social origin of queens. Reaching higher worker number during the first months of colony life may considerably increase the success of incipient colonies, as it improves hunting efficiency, brood care, task partitioning or colony competitiveness in the field (Rissing & Pollock, 1987; Vargo, 1988; Heinze, 1993; Bernasconi & Keller, 1996; Helms Cahan, 2001). Again, in our experiment these differences in colony productivity between one-queen and two-queen colonies were only observed during the first few months of colony life, at a time when the queens' energetic reserves are primordial for worker production.

The frequency of pleometrosis in the field is unknown for *F. selysi*. Our results indicate that queens in two-queen associations suffer from higher mortality and colony failure rates than single queens. However, associative colony founding by queens of monogyne or polygyne origin might still be favoured if nest sites are rare or if joint colony founding increases the nest resistance to disease, usurpation, or predation in the field (Johnson, 2002; D'Ettorre *et al.*, 2005; Feldhaar *et al.*, 2005; Reber *et al.*, 2008).

We found no sign of mating avoidance or genetic incompatibility between social forms. Males and females originating from different social forms readily mated in the same experimental conditions and in the same amount of time than individuals belonging to the same social form. Moreover, queens that had mated with a male of the same or of the other social form had similar productivity. These results suggest that there is no morphological or behavioral barrier to mating between social forms, and that the social forms are genetically compatible. In our study site, monogyne and polygyne colonies occur at close proximity (Chapuisat *et al.*, 2004) and produce queens and males in synchrony (Rosset & Chapuisat, 2007), further suggesting that mating between social forms may be frequent.

An unexpected result of our study was that colonies in which queens had mated with males of polygyne origin suffered from higher colony failure over 15 months than colonies in which queens had mated with males of monogyne origin, and this independently of the social origin of queens. However, this effect was weak and only marginally significant, and should thus be considered with caution. Speculative explanations for the observed pattern might be that males of polygyne origin transfer toxic products during mating, or produce brood of lower quality.

Flexible colony founding and mating between individuals originating from polygyne and monogyne colonies probably contribute to maintain gene flow between social forms in *F. selysi*, which is in line with the lack of genetic differentiation between social forms at nuclear microsatellite markers in our study population (Chapuisat *et al.*, 2004). Significant and apparently bidirectional gene flow between social forms has also been documented in populations of *F. fusca* and *F. podzolica* (DeHeer & Herbers, 2004; Bargum *et al.*, 2007). Interestingly, these Formica species with high gene flow between social forms and presumably plastic social structures occupy ephemeral, open or discontinuous patches of habitat that undergo frequent disturbance (Chapuisat *et al.*, 2004; DeHeer & Herbers, 2004; Sundström *et al.*, 2005; Bargum *et al.*, 2007).

F. selysi is a pioneer species that lives in open habitat along alpine rivers. At our study site, rare but severe floods deeply modify soil topology and create patches that are free of resident colonies. In contrast, colonies occur at high density in parts of the site that have not been recently flooded (Rosset & Chapuisat, 2007). Heterogeneous and disturbed environmental conditions probably contribute to the co-existence of alternative social forms in sympatry, as new areas to colonize should favor independent colony founding by single queens, while densely populated areas should favor polygyne colonies and colony budding (Ross & Keller, 1995). The observed flexibility in colony founding by *F. selysi* queens of monogyne or polygyne origin is in line with the hypothesis that variable and unpredictable environmental conditions select for plastic responses in dispersal, colony founding and queen number (Herbers, 1986; Feldhaar *et al.*, 2005).

CHAPTER 5

CHAPTER 5

Stay or drift? Queen acceptance in the ant *Formica paralugubris*

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ABSTRACT

The acceptance of new queens in ant colonies has profound effects on colony kin structure and inclusive fitness of workers. Therefore, it is important to study the recognition and discrimination behaviour of workers towards reproductive individuals entering established colonies. We examined the acceptance rate of queens in populations of the highly polygynous ant *F. paralugubris*, where the genetic differentiation among nests and discrimination ability among workers suggest that workers might reject foreign queens. We experimentally introduced young queens in their natal nest and in foreign nests. Surprisingly, the survival rate of mated queens did not differ significantly when introduced in a foreign male-producing nest, a foreign female-producing nest, or the natal nest. Moreover, the survival of virgin queens in their natal nest was twice the one of mated queens, suggesting that mating status plays an important role for acceptance. The results indicate that other factors than queen discrimination by workers are implicated in the limited long-distance gene flow between nests in this population.

Key words: queen acceptance, mating status, dispersal, social insects, ants

INTRODUCTION

In most social insects, including many ant species, individuals aggressively reject unrelated non-nestmates. This defence helps to maintain territorial colony boundaries, prevents the invasion and exploitation of the colony by unrelated conspecifics and ensures that altruistic acts are directed towards relatives (Hamilton, 1964a; Crozier & Pamilo, 1996). An increasing number of studies have investigated the discrimination ability between ant workers, but few have addressed the recognition and discrimination behaviour of workers towards reproductive individuals entering colonies. These studies are important, because accepting new queens will generally have a large impact on colony kin structure and inclusive fitness of workers (Heinze & Keller, 2000).

Ant colonies typically produce hundreds or thousands of new queens and only a tiny fraction will ever succeed in founding a new colony independently after the mating flight (Hölldobler & Wilson, 1990). In species with multiple queens per colony (polygynous species), alternative options for young queens are to stay and mate within the natal nest or to seek adoption in an established colony after the mating flight. Established colonies benefit from re-accepting queens when these new queens increase colony survival or productivity sufficiently to compensate for the decrease in inclusive fitness, or when the queens are related to the resident colony members (Nonacs, 1988). Nestmate queens are indeed generally related in many polygynous species, which confirms that queens often mate and stay in their natal nest, or return to their natal nest after the mating flight (Bourke & Franks, 1995; Keller, 1995; Chapuisat & Keller, 1999a).

The process of queen replacement in social insects has received much attention (Heinze & Keller, 2000). In the queen replenishment hypothesis, Brown and Keller (2000) proposed that the production of new queens occurs preferentially or only in nests with relatively few queens. These are the nests that benefit most from recruiting new queens to enhance colony survival and productivity. But female-producing colonies appear to produce more queens than the ones needed for optimum productivity and survivorship. Brown and Keller (2000) suggested that the overproduction of queens is a way to dilute the effect of potentially accepting foreign queens during periods of queen replenishment. If workers are unable to distinguish

between foreign and nestmate queens, they might avoid parasitism by foreign queens by killing all young queens in years when the colony does not produce queens (Rosengren & Pamilo, 1983; Brown & Keller, 2002).

Behavioural studies on queen adoption in polygynous ants report mixed results, ranging from total acceptance to complete rejection of foreign queens. In *F. exsecta* newly emerged virgin queens were more likely to be accepted in female-producing colonies, and were mostly killed in colonies that produced only males (Brown *et al.*, 2003). A study in *F. truncorum* suggests that the mating status of queens influences their acceptance. Specifically, workers in laboratory colonies did not distinguish between nestmate and non-nestmate queens, but were more likely to accept virgin than mated queens (Sundström, 1997). In *F. polycтена*, all old foreign and mated queens were accepted (Rosengren *et al.*, 1986). A recent laboratory study in the ponerine ant *Pachycondyla luteipes* showed that mated and reproductive active queens were accepted in their nest of origin, whereas 70% of the non-nestmate queens were rejected (Kikuchi *et al.*, 2007). Similarly, in the argentine ant *Linepithema humile* nestmate queens were generally accepted (90%), but most of the queens introduced into foreign colonies were killed (Vasquez & Silverman, 2008). Finally, in *Leptothorax curvispinosus* all foreign queens were rejected (Stuart *et al.*, 1993), and in *Myrmica tahoensis* the acceptance rate of old queens was about 23% (Evans, 1996).

Our study species *F. paralugubris* has an extraordinary social structure with almost no aggression between nests that can contain as many as several hundreds of reproductive queens (Cherix, 1980; Cherix, 1983). New sexuals are produced only once a year in spring and the mating flight takes place over two to three weeks in June. Young queens have alternative reproductive strategies (Cherix *et al.*, 1991). They can mate and stay within the nest (Chapuisat & Keller, 1999a). They can also fly to mating places and later join the natal or another established colony in the population, or found a new colony by parasitizing species of the subgenus *Serviformica* (Cherix *et al.*, 1991). Experimental introductions of queens in female-producing nests showed that virgin queens were accepted with a higher probability than mated queens, and suggested that the survival of introduced queens did not differ between natal and foreign nests (Fortelius *et al.*, 1993). Although interesting, the results of this pioneering study are not fully conclusive. First, they suffer from

pseudoreplication, as nests were used several times and the introduced queens originated from only three nests. Second, the duration of introduction was short (two hours) and no queen was introduced in male-producing nests, so that some discrimination might still occur over longer time period or in other types of nests.

A further investigation of queen acceptance was prompted by new genetic and behavioural data suggesting that workers might reject foreign queens. Distant nests of our study population are genetically differentiated, which results in low but significant relatedness among nestmate workers, despite the high number of reproductive queens (Chapuisat *et al.*, 1997a; Chapuisat & Keller, 1999a). This genetic differentiation and isolation by distance indicate that long-distance gene flow between established nests is limited and that most queens mate and stay within their natal nest (Chapuisat *et al.*, 1997a; Chapuisat & Keller, 1999a). Such a lack of long-distance gene flow is surprising, because many queens fly and mate away from the nests (Cherix *et al.*, 1991; Chapuisat, 1998). Another intriguing feature of this system is that although distant nests are genetically differentiated, workers do not reject conspecific workers from nests of the same population, and workers move freely between neighbouring nests (Chapuisat *et al.*, 2005; Holzer *et al.*, 2006a). However, this absence of aggression is not due to a complete lack of recognition. Workers are able to distinguish nestmates from non-nestmates, as shown by higher trophallaxis rate and longer antennation bouts between non-nestmates (Chapuisat *et al.*, 2005; Holzer *et al.*, 2006a). Together, these new data raise the question whether workers might be able to discriminate and reject foreign queens.

The main aim of this study was to examine whether resident workers reject young foreign queens that enter into their nest. We used mated queens in order to simulate the natural situation where queens seek adoption in established colonies after the mating flight. We introduced mated queens into their natal nest, a foreign female-producing nest, or a foreign male-producing nest, and measured their survival after 22 hours. This experiment also permits to test one element of the queen replenishment hypothesis, which is that queens should be equally accepted in their natal nest and in foreign female-producing nests, but rejected in male-producing nests. In addition, we also introduced young virgin and mated queens into their natal nest to examine whether the mating status of the queens influences their survival and acceptance by workers.

MATERIAL AND METHODS

The experiment was carried out within two populations of *F. paralugubris* in Bois de Peney and Chalet à Roch in the Swiss Jura Mountains. The introductions took place during the mating flight between June 8th and 18th, 2005. We collected newly emerged alate queens and males from the nest surface between 9:15 and 10:35 am. The queens from each nest were divided in two groups, one with queens destined to mate and the other with queens destined to remain virgin. Each group was placed in a transparent plastic box (23 x 39 x 15 cm) covered with a thin tissue. Males were added and were left with the queens for approximately two hours in one of the boxes, with a ratio of queens to males of approximately 1 : 1.3. Virgin and mated queens received the same treatment with the only difference that virgin queens had no contact with males.

Queens were inserted into the nests on the same day. They were placed individually into 7 to 8 cm long (diameter: 1.5 - 2 cm) bamboo tubes with 2 mm diameter holes. The holes were big enough to allow workers to freely enter and leave the tubes, while trapping the larger queen inside the tube (Fortelius *et al.*, 1993). A previous study in *Formica exsecta* has shown that this method is effective to reveal variation among nests in the degree of queen acceptance by workers. Indeed, among populations, the mortality of queens introduced into male-producing nests ranged from 73% to 98%, in sharp contrast to the mortality of queens introduced into their natal colony, which ranged from 3 to 25% (Brown *et al.*, 2003). To avoid aggressive reactions of workers towards the queens due to the odour of the bamboo tubes, we introduced the tubes into the nests for approximately three hours prior to the start of the experiment.

In the early afternoon, we introduced tubes containing one queen into the upper part of the mound, 10 to 20 cm under the nest surface. The virgin queens were introduced into their natal nest. The mated queens were introduced into their natal nest, a foreign female-producing nest, and a foreign male-producing nest (three treatments). Each queen and each nest was used only once. In total we used 33 different nests, resulting in 11 combinations of three nests, and eight queens per nest and treatment. The tubes with the queens were collected in the morning of the next

day, 22 hours after their introduction. We recorded whether the queen was alive or dead. Occasionally, we were not able to retrieve all the tubes, so the number of introduced queens per treatment was 7.48 ± 0.66 (mean \pm SD). The queens were stored in ethanol and dissected to verify their mating status, which is revealed by the presence or absence of sperm in the spermathecae.

Out of the 88 presumed virgin queens, eight turned out to be inseminated. These queens had mated within their natal nest before sampling. The mating success of queens in boxes was very high, with 249 out of 260 queens (96%) having sperm in their spermathecae. These eight mated queens and eleven virgin queens were removed prior to analysis, but we checked that transferring them into the other treatment would not have changed the results.

On June 10th and 11th 2008, we performed an additional control experiment to verify that queen mortality was due to worker behaviour and not to queen manipulation, mating or confinement *per se*. We collected newly emerged alate queens from 11 nests at Bois de Peney and let them mate in cages as described above. For each of the 11 nests, eight mated queens were placed in tubes that were introduced into a randomly selected foreign nest, whereas eight mated queens were placed in tubes that were inserted into worker-free woodpiles located on a small meadow within our study site. The tubes were left for 22 hours and mortality was recorded as described above. In addition, worker behaviour towards queens was observed shortly after retrieving the tubes from the nests, and queen injuries were recorded.

To compare the acceptance rate of mated queens in their natal nest, a foreign female-producing nest, or a male-producing nest, we computed a two-way analysis of variance (ANOVA) on the odds of survival with nest of introduction and the date of introduction as fixed factors and nest as random factor. Similarly, to compare the acceptance rate of virgin and mated queens in their natal nest, we performed a two-way ANOVA on the odds of survival with mating status and the date of introduction as fixed factors and nest as random factor. We compared the survival of mated queens in tubes introduced in nests or in woodpiles with a Wilcoxon matched-pairs signed-rank test on the proportion of surviving queens. The power analysis was performed with the software G*power (Faul *et al.*, 2007).

RESULTS

Mated queens introduced into their natal nest, a foreign female-producing nest, or a foreign male-producing nest did not differ significantly in their survival rate (transformation $\log+1$, $F_{2,20} = 1.10$, $P = 0.35$, Fig. 1). Specifically, the average survival rate \pm SD of young mated queens was 20 % \pm 22 % when introduced into their own nest, 34% \pm 32% when introduced into foreign female-producing nests, and 17% \pm 18% when introduced into foreign male-producing nests. The power analysis revealed that an increase of 57% in survival in one of the treatments would have been detected with a probability of 80%.

The survival of queens introduced into their natal nest was affected by their mating status. Specifically, virgin queens were significantly more likely to survive the introduction in their natal nest (mean \pm SD: 45% \pm 33%) than mated queens (20% \pm 22%; $F_{1,10} = 7.63$, $P < 0.05$, Figure 1).

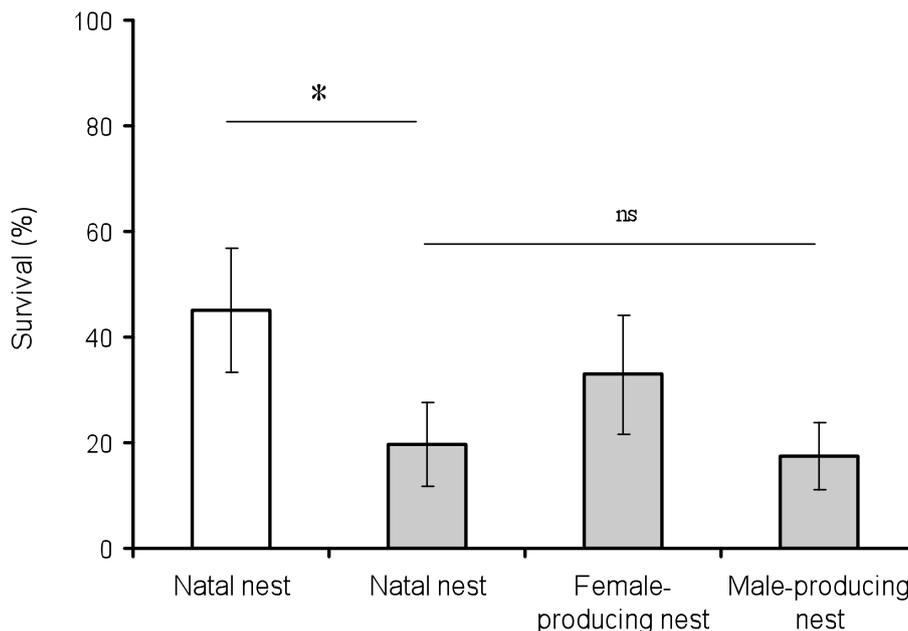


Figure 1 - Survival of virgin queens introduced into their natal nest (white bar) and of mated queens (grey bars) introduced into their natal nest, a foreign female-producing nest or a foreign male-producing nest. The mean \pm SE is shown.

The mean survival rate of virgin and mated queens introduced into their natal nest decreased significantly over time, as the flight period progressed ($F_{1,9} = 5.35$, $P < 0.05$, Figure 2). A similar tendency was found when mated queens were introduced into foreign nests, but the effect was not significant ($F_{1,9} = 2.01$, $P = 0.19$).

Mated queens introduced into foreign nests had significantly lower survival than mated queens kept in woodpiles without workers (Wilcoxon matched-pairs signed-rank test, $W = 66$, $N = 11$, $P < 0.001$). The average survival rate \pm SD of mated queens in tubes introduced into foreign nests was 23% ($\pm 29\%$), whereas this value was 98% $\pm 5\%$ when tubes were kept in woodpiles. This control experiment indicates that queen mortality is induced by the workers' behaviour towards them, and is not merely due to manipulation, mating or confinement. Moreover, we often observed workers attacking queens when opening the tubes retrieved from nests, and 37% of the dead queens showed severe physical injuries, with head, legs or antennae having been cut off.

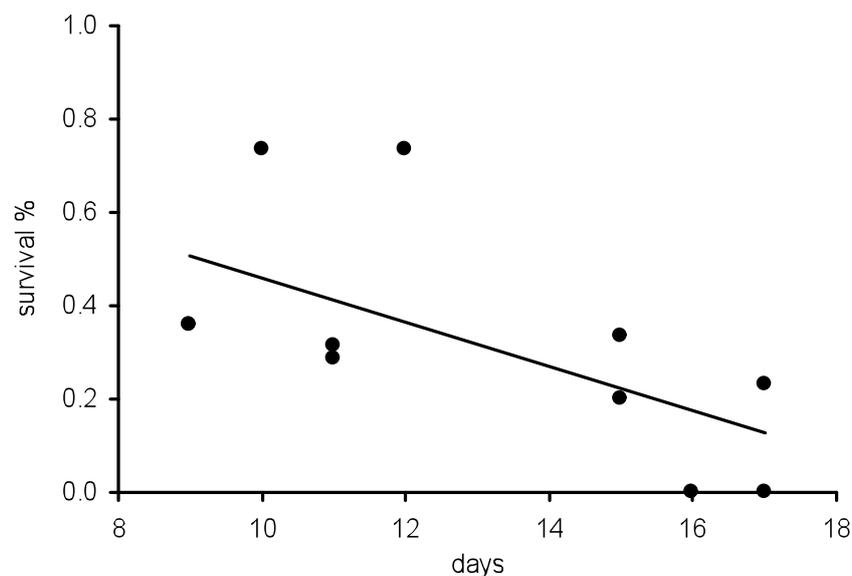


Figure 2 - Mean survival of virgin and mated queens introduced into their natal nest, in relation to the progression of the mating flight period (June 8th to 18th).

DISCUSSION

In this highly polygynous population of *F. paralugubris*, resident workers do not discriminate against foreign queens introduced into their nest. The average survival rate of mated queens in female- and male-producing foreign nests was 25% after 22 hours, which is not significantly different from the survival rate of mated queens in their natal nest (20%). This lack of discrimination is in line with the results of previous studies of highly polygynous *Formica* ants (Rosengren *et al.*, 1986; Fortelius *et al.*, 1993; Sundström, 1997).

So far only one other study has investigated queen acceptance in female- and male-producing colonies of ants. In *F. exsecta* the survival rate of virgin queens ranged from 69% to 97% for introductions into natal or foreign female-producing colonies, but was as low as 2% to 27% for introductions into foreign male-producing colonies (Brown *et al.*, 2003). In contrast, the comparatively high survival rate of mated queens in male-producing nests of *F. paralugubris* suggests that the presence of young queens within a nest is not a prerequisite for acceptance in this species, and provides no evidence for the hypothesis that workers should accept young queens only when queens are produced by the colony (Brown *et al.*, 2003).

In our experiment the survival rate of virgin queens was twice the one of mated queens in their natal nests. This higher survival of virgin queens is in agreement with the results of previous experiments in *F. paralugubris* (Fortelius *et al.*, 1993) and *F. truncorum* (Sundström, 1997). The most likely explanation for the higher mortality of mated queens is that workers react more aggressively towards mated than virgin queens. Workers might discriminate between the two types of queens on the basis of their cuticular hydrocarbon profiles, which are commonly used for recognition in ants and have been shown to differ between virgin and mated *Linepithema humile* queens (de Biseau *et al.*, 2004). The alternative explanation that mating *per se* is costly and lowers queen survival was not supported by our control experiment, which showed high survival rate of mated queens confined in tubes in absence of workers. The short-term costs of mating have been little investigated in ants, but appear to be minimal. In the leaf-cutting ant *Atta colombica*, virgin queens and queens sampled one day after mating did not differ in their immune response, but

mated queens that stored more sperm had lower immune response nine days after mating (Baer *et al.*, 2006).

The survival rate of mated and virgin queens introduced into their natal nests was highest at the beginning of the mating season and decreased as the mating flight progressed. Again, the high survival of queens in absence of workers suggests that this rise in queen mortality is due to an increase in worker aggression over the season, possibly triggered by the absence of young queens in the nests at the end of the mating flight. However, some age- or season-related factors might also increase queen mortality independently of worker behaviour.

In conclusion, the similar survival rate of queens introduced into their natal or a foreign nest indicates that the limited gene flow between distant nests observed in our study population is not due to worker discrimination of foreign queens. The fact that workers do not preferentially kill foreign queens suggests that other mortality factors during the mating flight, coupled with dilution over distance and high rates of local recruitment (Chapuisat & Keller, 1999a), are sufficient to limit effective gene flow among distant nests.

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GENERAL CONCLUSION

In social Hymenoptera, variations in colony social structures are generally associated with genetic, ecological and behavioural changes that can influence cohesion among colony members. In this thesis, we explored the link between social structure variations and conflicts among colony members. We also investigated the influence of social structure on nestmate discrimination, a mechanism preventing foreign individuals from exploiting colony resources. Finally, we tested mechanisms influencing queen number in ant colonies. As a conclusion, we would like to highlight some of the results found during this thesis and their evolutionary significance.

CONFLICTS AMONG COLONY MEMBERS

The first conflict we focused on was the conflict between queens and workers over the ratio of males and females produced by colonies. According to kin selection theory, workers should manipulate this ratio according to variation in relatedness asymmetries, whereas queens should promote an equilibrated sex ratio (Trivers & Hare, 1976). In a meta-analysis including all empirical studies published on this topic, we showed that in social Hymenoptera variation in relatedness asymmetry explains 20.9% of the variance in sex ratio among colonies (Chapter 1). This value is considerably higher than the average amount of variance explained in ecological and evolutionary studies (Møller & Jennions, 2002), indicating that colony sex-ratio is largely influenced by workers. Our results therefore suggest that the evolution of social structures with low relatedness asymmetries (e.g. colonies headed by multiple related queens or multiply-mated queen) might be at least partly selected because they limit costly conflicts over colony sex ratio.

During this thesis, we also focused on the conflict between queens and workers over the production of males. In many species of social Hymenoptera, workers retain the ability to lay male-destined eggs so that queens and workers possibly compete over reproduction. Kin selection predicts that workers should preferentially rear the most related male-destined eggs. As a result, worker policing should evolve in colonies headed by several related queens, whereas workers from colonies headed by one singly-mated queen should tolerate eggs laid by other workers. We showed that in monogyne and polygyne colonies of the ant *F. selysi*,

workers eliminate more the eggs laid by other workers than eggs laid by queens (Chapter 2). These results contrast with predictions of kin selection and therefore suggest that in this species, worker policing evolved independently from variation in the relatedness between group members. An alternative hypothesis might be that selective elimination of worker-laid eggs evolved to limit the cost of worker reproduction at the colony level (Ratnieks, 1988; Hammond & Keller, 2004; Wenseleers & Ratnieks, 2006). This hypothesis remains to be empirically tested in *Formica* species (for other species see, Cole, 1986; Dijkstra & Boomsma, 2007).

NESTMATE DISCRIMINATION

The ability of workers to recognise and selectively aggress intruders helps to prevent the invasion of the colony by unrelated conspecifics and ensures that altruistic acts are directed towards relatives (Hamilton, 1964a; Crozier & Pamilo, 1996). In *Formica selysi*, workers from polygyne colonies are selectively more aggressive toward foreign workers (Rosset *et al.*, 2006), whereas they accept both nestmate and foreign queen-laid eggs (Chapter 2). Hence, nestmate discrimination appears to vary depending on the type of intruders in polygyne colonies, whereas it did not vary in monogyne colonies (Chapter 2).

We formulated two hypotheses to explain our results. Because in ants, nestmate discrimination is generally mediated through chemical signatures (Vander Meer & Morel, 1998), we first suggested that nestmate discrimination of eggs and workers might be mediated through independent mechanisms involving different chemical signatures. In the ant *Formica fusca*, nestmate discrimination and worker policing use two independent mechanisms, whereas both consist in egg discrimination by workers (Helanterä & Ratnieks, 2009). Particularly, a preliminary experience of workers with foreign queen-laid eggs decreases the level of nestmate discrimination against other foreign queen-laid eggs, but not against foreign worker-laid eggs. In contrast, a preliminary experience of workers with foreign worker-laid eggs does not influence nestmate discrimination of queen-laid or worker-laid eggs. In *F. selysi*, our observed patterns might therefore suggest that nestmate discrimination of eggs and workers leads to different and similar responses in polygyne and monogyne colonies, respectively.

The second hypothesis we suggested is that workers response is mediated through a hierarchical use of signals (or cues) differently expressed between social forms. In the ant *Camponotus floridanus*, individuals with strong fertility signals are accepted by workers regardless of their colony membership, whereas individuals without strong fertility signals are accepted only if their cuticular hydrocarbon profile matches that of colony members (Moore & Liebig, in press). In *F. selysi*, this mechanism would suggest that a signal carried by queen-laid eggs (e.g. a fertility signal applied by the queens, see Endler *et al.*, 2004b) induces their acceptance by nestmate or foreign workers, whereas the absence of this signal on foreign workers induces nestmate discrimination process. The fact that monogyne workers discriminate between nestmate and foreign queen-laid eggs could be explained by a higher level of this signal on eggs laid by polygyne than monogyne queens. This hypothesis can be tested by egg-acceptance bioassays where monogyne workers receive queen-laid eggs from polygyne colonies and polygyne workers queen-laid eggs from monogyne colonies. Our hypothesis would predict that monogyne workers accept polygyne queen-laid eggs, because they carry a high level of the egg-signal, whereas polygyne workers reject monogyne queen-laid eggs, because the low level of the egg-signal induces nestmate discrimination by workers.

Overall, a further step in the understanding of nestmate discrimination patterns in *F. selysi* would require to directly explore chemical signatures carried by eggs and workers from monogyne and polygyne colonies (for general chemical traits of workers see, Bagnères *et al.*, 1991a; Bagnères *et al.*, 1991b).

INDEPENDENT COLONY FOUNDATION

Colony queen number is a key parameter tightly linked to colony kin structure and other fundamental life-history traits. Queen body size may play a role in determining the social structure of future colonies, because queen size is likely to affect the success of independent colony founding by single queens (Wiernasz & Cole, 2003), and may constrain small queens to found new colonies by budding (Rüppell & Heinze, 1999). In the ant *Formica selysi*, monogyne colonies produce larger queens than polygyne colonies (Rosset & Chapuisat, 2007). We showed that the association between colony queen number and size of new queens results from

genes or maternal effects transmitted to the eggs (Chapter 3). These results suggest that queens present in monogyne colonies produce large queens that could have better abilities to found new colonies independently, resulting in new monogyne colonies. However, queens produced in colonies of the two social forms did not differ in their general abilities to found new colonies independently, at least under laboratory conditions (Chapter 4). No definitive conclusion can be drawn about the success of independent colony founding by the two types of queens in the field, but our results indicate that monogyne and polygyne queens have no major intrinsic differences in their ability to found new colonies alone. The influence of environmental stress (e.g. finding a nest site, influence of predators/parasites or climatic changes) on the success of colony foundation under natural conditions remains unknown.

We suggest other experiments to complete our understanding of the relationships between queen size, colony founding and colony social structures in *F. selysi*. First, measuring the size of resident queens in colonies of the two social forms would provide indirect evidence on the link between size of new queens and foundation of new monogyne or polygyne colonies. For instance, if resident queens are larger in monogyne than polygyne colonies, this would suggest that colony social structure is transmitted by the queens. In contrast, if the size of resident queens from monogyne and polygyne colonies does not significantly differ, this would suggest plasticity in the transmission of social forms.

Another interesting experiment would be to test the acceptance of new queens in monogyne and polygyne colonies in the studied population of the ant *F. selysi*. Our results suggest that the social origin of new queens (and consequently differences in their body size) has no influence on their ability to found colonies independently. However, queen body size could be a constraint limiting their dispersal abilities (Keller & Passera, 1989) so that small queens would be more likely to stay in their original nests. Moreover, the social origin of new queens could also influence their potential acceptance of additional queens, as additional queens are generally better accepted by polygyne than monogyne workers (Bourke & Franks, 1995; but Sundström, 1997). Estimating the relatedness between resident queens would finally be an indirect estimation of the acceptance of foreign and nestmate queens in polygyne colonies of *F. selysi*.

QUEEN ACCEPTANCE IN ESTABLISHED COLONIES

We investigated the adoption of new queens in the highly polygynous ant *Formica paralugubris*. The acceptance of new queens may have large impact on colony kin structure and inclusive fitness of workers (Heinze & Keller, 2000), depending on the relatedness between queens and workers (Nonacs, 1988; Pamilo, 1991). We showed that in colonies of *F. paralugubris*, workers accept approximatively 25% of the introduced queens, independently from the nest of origin of the new queens or the type of sexual produced in the recipient colony (Chapter 5). This result suggests that workers did not evolve nestmate discrimination against foreign queens. However, it provides no evidence for the hypothesis that because of the absence of nestmate discrimination, workers should accept young queens only when queens are produced by the colony (Brown *et al.*, 2003).

CONCLUSION

As a whole, this thesis shed light on potential causes and consequences of social structure variation in colonies of social Hymenoptera. Our results indicate that evolution of social structures where queens and workers are equally related to males and females may contribute to decrease the conflict between the two castes over colony sex ratio. We also showed that social structure variation has no influence on the outcome of the conflict over male parentage in the ant *Formica selysi*. Hence, the link between conflict resolution and evolution of social structures cannot be extended to all conflicts and all species. An interesting follow-up would be to investigate the effects of other factors (e.g. other conflicts, parasitic pressure or environmental conditions) on the evolution of social structures by focusing on a single species. Socially polymorphic species, like *F. selysi*, would provide a good opportunity to address this question. To date, many studies have focused on ecological and genetic traits displayed by the population of *F. selysi*, indicating that social structures are tightly linked to many life-history traits, while a gene flow is maintained between social forms. Hence, the data gathered on this species constitute a solid background for further research on the dynamic of mixed social structures in a same population.

More generally, these species provide a good opportunity to investigate the influences of ecological, genetic or behavioural mechanisms on the evolutions and maintenance of multiple social organisations.

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