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Social evolution in the sweat bee *Halictus scabiosae*

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Département d'Ecologie et Evolution

Social evolution in the sweat bee *Halictus scabiosae*

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

par

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Jury

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Social evolution in sweat bee *Halictus scabiosae*

Lausanne, le 11 juin 2014

pour La Doyenne
de la Faculté de Biologie et de Médecine

Prof. Edward E. Farmer

Edward E. Farmer

Table of Contents

Summary

The evolution of eusociality is one of the major evolutionary transitions of life on earth. For investigating the conditions and processes that are central to the origin of such integrated social organization, it is best to study organisms in which individuals have retained some flexibility in their reproductive strategies. Halictid bees are especially well suited as model organisms, because they show huge variation in social systems, both within and between species. In this thesis, I investigated female reproductive strategies in the primitively eusocial bee *Halictus scabiosae*, with a focus on the role of helpers, in order to get insight into the mechanisms governing the evolution and maintenance of eusociality. This species produces two broods per year. The females from the first brood can stay in the natal nest to help raise a second brood of males and gynes that become the next-generation foundresses in spring. We first compared the morphology of females from the two broods, as well as the nutrition they receive as larvae. Then we conducted a helper-removal experiment in the field to quantify the effects of the presence of helpers on colony survival and productivity. Finally, we reconstructed pedigree relationships of individuals using microsatellite markers in order to detect who reproduces in the nest and how much individuals drift between nests. We found that first brood females had a uniformly small size and low fat reserves, which may be caused by the restricted pollen and nectar provisions on which they develop. Colony survival and productivity was increased by the presence of a single helper, but the effect was small and mostly limited to small colonies. By inferring parentage within and across colonies, we could determine that females from the first brood rarely reproduce in their natal nests. However, foundresses are frequently replaced, and foundresses and females from the first brood occasionally move to and reproduce in foreign colonies. As a result, colonies often contain offspring from unrelated individuals, and the relatedness of females to the brood they rear is low. Overall, this thesis shows that the reproductive system of *H. scabiosae* is highly flexible. The production of helpers in the first brood is important for colony success and productivity, but there is a high colony failure rate and part of the first brood females drift and reproduce in foreign nests. Both foundresses and helpers appear to be constrained by harsh environmental conditions or social factors limiting reproduction and independent colony founding.

Résumé

L'origine des insectes sociaux est un domaine fascinant pour la recherche. Pour comprendre les mécanismes et les conditions qui sont nécessaires pour l'évolution et le maintien de la vie en société, il est judicieux d'étudier des sociétés primitives d'insectes, où toutes les femelles ont conservé la capacité de se reproduire, même si leur rôle comportemental dans la colonie est d'aider sans se reproduire. Une des familles d'abeilles, les halictes, est idéale pour cette sorte de recherche, en raison de la grande variabilité dans leur comportement social. Dans cette thèse, j'ai étudié les stratégies reproductives des femelles de *Halictus scabiosae* pour mieux comprendre les mécanismes qui influencent l'évolution de la vie en société. Cette espèce produit deux cohortes de couvain par année. Les femelles du premier couvain restent souvent dans leur nid natal pour aider à élever le deuxième couvain, tandis que les femelles du deuxième couvain s'accouplent et hibernent pour devenir les nouvelles fondatrices au printemps suivant. Nous avons d'abord comparé la morphologie des femelles issues des deux couvains ainsi que leur nutrition au stade de larve. Puis, dans une expérience sur le terrain, nous avons quantifié l'apport d'une ouvrière pour la survie et la productivité de la colonie. Finalement, nous avons reconstruit des pedigrees en utilisant des marqueurs génétiques, pour savoir qui se reproduit dans la colonie et combien d'individus migrent entre colonies. Les résultats montrent que les femelles du premier couvain sont uniformément plus petites et plus maigres, ce qui indique que les fondatrices réduisent les provisions de nourriture pour leur premier couvain afin de les inciter à aider dans le nid au lieu de se reproduire indépendamment. Dans l'expérience sur le terrain, la survie et la productivité de la colonie augmentaient avec la présence d'une ouvrière additionnelle, mais l'effet était petit et limité aux petites colonies. Par la reconstruction de pedigrees, nous pouvions constater que les femelles du premier couvain pondent rarement dans leurs nids natals. Les fondatrices cependant sont souvent remplacées en cours de saison, et migrent fréquemment entre nids, tandis que les femelles du premier couvain pondent parfois des œufs dans des nids étrangers. De ce fait, les colonies contiennent souvent des descendants d'individus étrangers, et la parenté génétique entre les femelles et le deuxième couvain est basse. Cette thèse démontre que le système reproductif de *H. scabiosae* est très flexible. La production d'ouvrières est importante pour la survie de la colonie et sa productivité, mais le taux d'échec est élevé et une partie des femelles du premier couvain migrent et pondent dans une colonie étrangère. Autant les fondatrices que les ouvrières semblent être contraintes par des conditions environnementales ou sociales qui limitent la reproduction et les nouvelles fondations de colonie.

Zusammenfassung

Die Entstehung von sozialen Lebensformen ist eines der wichtigsten Entwicklungen in der Geschichte des Lebens. Um die Bedingungen oder Prozesse zu verstehen, welche bei der Entstehung und dem Erhalt von sozialen Merkmalen wichtig sind, sollte man Lebewesen untersuchen, welche je nach Umwelteinflüßen ihr soziales Verhalten flexibel ändern können. Furchenbienen (Halictidae) gehören dazu. Diese weisen nämlich ein breites Spektrum verschiedener sozialer Organisationsformen auf, oftmals sogar innerhalb der einzelnen Arten. In meiner Doktorarbeit befasste ich mich mit den Fortpflanzungsstrategien der Weibchen der Skabiosen-Furchenbiene *Halictus scabiosae*. Diese Art produziert zwei Bruten pro Jahr. Die Weibchen der ersten Brut bleiben dabei meist als Arbeiterinnen in ihrem Geburtsnest, wohingegen die Weibchen der zweiten Brut nach der Paarung überwintern, um im nächsten Frühling neue Kolonien zu gründen. In einem ersten Schritt verglichen wir die beiden Bruten bezüglich der Grösse und der Fettreserven der Weibchen sowie der Pollen-Nektar-Vorräte für die Larven. Dann bestimmten wir in einem Feldexperiment, wieviel eine zusätzliche Arbeiterin zum Überleben und zur Produktiviät der Kolonie beiträgt. Schliesslich ermittelten wir durch genetische Tests die Verwandtschaftsbeziehungen zwischen den Bienen, um herauszufinden, wer in den Kolonien tatsächlich die Eier legt und ob und wieviel die Bienen zwischen verschiedenen Nestern wandern. Wir stellten fest, dass die Weibchen von der ersten Brut einheitlich kleiner sind und weniger Fettreserven besitzen. Das weist darauf hin, dass die Nestgründerin die erste Brut unterernährt, um die Wahrscheinlichkeit zu erhöhen, dass diese Weibchen als Arbeiterinnen im Nest bleiben anstatt sich unabhängig fortzupflanzen. Schon eine einzelne zusätzliche Arbeiterin verbesserte die Überlebenschancen und Produktivität der Kolonie, der Effekt war allerdings klein und auf kleine Kolonien beschränkt. Die Verwandtschaftsanalysen zeigten, dass die Arbeiterinnen nur sehr selten ein Ei in ihr Geburtsnest legen. Erstaunlicherweise wanderten die Nestgründerinnen oft zwischen verschiedenen Nestern. Einige Weibchen der ersten Brut wanderten auch in ein fremdes Nest und produzierten dort Nachkommen. Diese Doktorarbeit zeigt, dass die Fortpflanzungsstrategien der Skabiosen-Furchenbiene tatsächlich sehr flexibel sind. Die Anwesenheit von Arbeiterinnen ist wichtig für das Überleben und die Produktivität der Kolonie. Die Misserfolgsraten bleiben jedoch hoch, und ein Teil der Weibchen der ersten Brut pflanzt sich in fremden Nestern fort. Sowohl die Nestgründerinnen als auch die Weibchen der ersten Brut scheinen durch Umweltsbedingungen oder durch soziale Faktoren in der Wahl ihrer Fortpflanzungsstrategie eingeschränkt zu sein.

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General introduction

General introduction

Social organisms have, since centuries, fascinated human beings. The social nature of our own species allows us to relate to behaviours that other social species show or to problems they encounter, and incites us to reflect on our own behaviour. The abundance of social organisms in the living world evidences that cooperation between conspecifics is key to great ecological success, even if this is often presented as an evolutionary conundrum (Wilson 1990; Pennisi 2005).

Most lay people would cite ants, bees or termites as typical examples of social organisms. Characteristically, these insects show a reproductive division of labour. Usually, only phenotypically specialised individuals (*queens*, and in termites also *kings*) reproduce in the colony, while others, commonly called *workers*, specialise on other tasks such as brood care, nest maintenance or defence. This observation puzzled Darwin, as according to his theory of natural selection, non-reproducing individuals couldn't pass on their traits, such as worker phenotype or behaviour, to the next generation (Darwin 1859). Hamilton provided a solution more than one century later, by showing that the worker's genes are also passed on to the next generation via the reproduction of relatives of the worker (Hamilton 1964a; Hamilton 1964b). The condition for maintaining an altruistic behaviour – a behaviour that decreases the fitness of the actor while increasing that of the receiver of the behaviour – was defined in a simple equation, called *Hamilton's rule*:

$$rb - c > 0$$

where r is the genetic relatedness between the altruist and recipient, b the benefits obtained by the recipient and c the costs to the altruist, the two latter terms being measured as the number of adult offspring gained or lost through the social behaviour (West et al. 2007). The sum of the "baseline" fitness that a focal individual would have in absence of social interactions and the benefits and costs of social behaviours, *rb* and *c,* was termed *inclusive fitness* (Hamilton 1964a). Although inclusive fitness is hard to measure in real life, it provides the theoretical framework for comparing the output of different reproductive strategies in a social context.

Evolution of eusociality in Hymenoptera

The Hymenoptera form a large insect order that comprises many ecologically important social species (Wilson 1971). It had been proposed that the haplodiploid sex determination system of Hymenotpera facilitated the evolution of eusociality, because it results in unusually high relatedness among sisters (Hamilton 1964a; Hamilton 1964b; Hamilton 1972). The central role of haplodiploidy has been questioned increasingly, however, because it became clear that it is only under specific conditions that altruism may be favoured in haplodiploid species, relative to diploid species (Bourke and Franks 1995; Crozier 2008). Moreover, many haplodiploid species are solitary, while a large diversity of diplodiploid species are eusocial (Queller and Strassmann 1998; Korb and Heinze 2008).

Altruism can only evolve when the altruist is genetically related to the receiver of help (Hamilton 1964a; Hamilton 1964b; Foster et al. 2006). Social groups of related individuals may consist of parent-offspring associations, or associations of relatives of the same generation, for instance siblings. Both kinds of association may lead to eusocial behaviour, through what has been called the subsocial or semisocial pathways to eusociality, respectively (Michener 1964). The great majority of eusocial organisms are thought to have reached eusociality via the subsocial pathway, although there are a few hymenopteran lineages (e.g. allodapine bees) where eusociality might have evolved from semisocial associations (Schwarz et al. 2007). The subsocial pathway may be predominant because in an association of a singly mated mother and her offspring, the offspring's average relatedness towards its sibs is the same as towards its own offspring, making them genetically indifferent to whether they raise their sibs altruistically, or reproduce themselves(Charnov 1978; Boomsma 2009)). In support to this view, life-time monogamy (one female mated to one male) was found to be the ancestral state for multiple independent origins of eusociality in the Hymenoptera (Hughes et al. 2008). It should be noted, however, that high relatedness among group members does not automatically lead to eusociality. Cooperation and altruistic behaviour are favoured under certain ecological conditions, such as the presence of social or brood parasites or predators, nest-site limitation, or variability in the food supply (e.g. Stark 1992; Brockmann 1997; Langer et al. 2004).

To investigate the evolution and maintenance of social behaviour, researchers have to examine which ecological factors and social processes make sociality more successful than solitary behaviour. Good systems for such investigations are species where reproductive division of labour is associated with little or no phenotypic caste differentiation. In these species, the role of each individual is flexible and depends on both social and environmental parameters, as for example in most cooperatively breeding vertebrates and many social arthropods (Reeve et al. 1998; Clutton-Brock 2002; Schwarz et al. 2007; Korb 2008). To stress this flexibility in the choice of reproductive options, in this thesis I will use the more neutral term *helper*, instead of *worker*, for individuals showing altruistic behaviour.

Halictid bees

Halictid bees are particularly interesting systems to study the evolution of eusociality, because their social system seems especially labile. Even closely related species often show completely different social organisations (Packer and Knerer 1985; Schwarz et al. 2007). Moreover, many species are socially polymorphic, in the sense that they express different types of social organisation depending on genetic or ecological factors (Eickwort et al. 1996; Richards 2000; Richards et al. 2003; Field et al. 2010). In some species, individuals can adopt solitary, social or even parasitic roles depending on conditions (Yanega 1989; Zobel and Paxton 2007). Eusociality in halictids is thought to have arisen about 35 Million years ago (Gibbs et al. 2012). The two most important genera comprising social species, *Halictus* and *Lasioglossum*, are thought to have a common social ancestor, meaning that extant solitary species in these genera are the result of reversals to solitary nesting (Danforth 2002; Gibbs et al. 2012).

The primitively eusocial bee Halictus scabiosae

H. scabiosae is a large, easily recognisable halictid bee with a body length of 13-14 mm. This species has a Western-Palearctic distribution. Because *H. scabiosae* thrives in warm habitats, its distribution range is currently expanding towards the North (LUBW 2007). In central Europe, this species is common on exposed, dry areas with little vegetation (Fabre 1903; Batra 1966; Gogala 1991). Despite its abundance, *H. scabiosae* has been relatively little studied. It forms annual colonies producing two broods per year in Europe. The first brood is female biased and the daughters often help to produce the second brood, so that the colonies are typically primitively eusocial in the second part of the season. Nevertheless, the social composition of the nest remains flexible. First, nests may be founded by one or several females in spring (haplo- or pleometrotic nest founding). Subordinate females are subsequently evicted and in most nests only one of the foundress females remains by the emergence of the first brood (Gogala 1991; Ulrich et al. 2009). Second, foundresses that had lost their nest were found to usurp foreign nests (Knerer and Plateaux-Quénu 1967). Finally, gynes seem to drift occasionally to other nests, in order to reduce competition between relatives for the dominant breeding position (Ulrich et al. 2009). Together, these previous studies suggest that the females have multiple options involving direct or indirect reproduction, which makes this species a good model for studying the evolution of eusociality.

Study site

The studies presented in this thesis were mainly conducted at a field site in Adlikon bei Andelfingen (Swiss coordinates: 693737/270926), in the Canton of Zürich, Switzerland. The field site consisted of a steep, south-exposed and dry embankment, near the highway exit A4 Adlikon/Andelfingen (Figure 1). Many ground-nesting wild bee species were found at this site, and each spring around 1000 *H. scabiosae* colonies were initiated.

Outline of this thesis

In this thesis, I investigated the female reproductive strategies in the primitively eusocial bee *Halictus scabiosae*, with a focus on the role of helpers and their direct and indirect fitness benefits. This approach will provide insights into the conditions and processes favouring the evolution and maintenance of eusociality in this species.

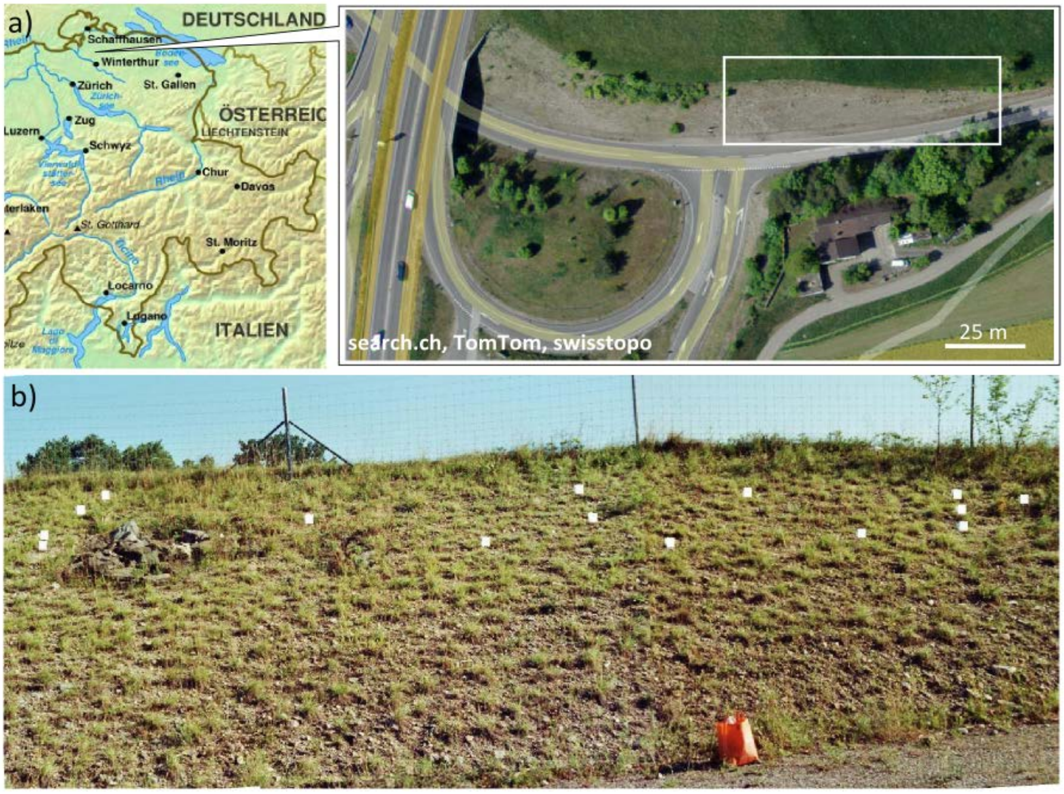

Figure 1 Field site in Adlikon. (Swiss coordinates: 693737/270926)
a) Position of the field site within Switzerland. The site at the exit of the highway is marked with a white square. b) Picture of the eastern half of the field site. The white rectangles mark aggregations of *H. scabiosae* colonies.

I first compared the nutrition of helper- and sexual destined larvae, and measured adult size differences between helpers and gynes or foundresses (Chapter 1). Then I performed a helper-removal experiment to measure the quantitative effect of one additional helper on colony survival and productivity, for various colony sizes (Chapter 2). Finally, I used microsatellite markers to detect reproduction by helpers or drift of bees between colonies (Chapter 3).

Chapter 1: Morphological caste differentiation

Primitively eusocial species are characterised by the behavioural and physiological flexibility of helpers. The individuals showing alloparental care are still able to mate and lay eggs, even if they don't always make use of this possibility. In bees, dominants are thought to suppress the ovarian development of subordinates by aggressive behaviour (Michener and Brothers 1974) or pheromones (Hogendoorn and Schwarz 1998; Bhadra et al. 2007). Moreover, mothers in primitively eusocial species may underfeed some daughters. These daughters would have reduced chances of establishing nests on their own, so that alternative options like helping become relatively more beneficial to them (Alexander 1974; Charnov 1978). Such *parental manipulation* has been well studied in primitively eusocial wasps (Hunt et al. 1996; Toth et al. 2009) and was suggested to exist in facultatively social bees (Kapheim et al. 2011). In this first chapter, I use head width data over two years, in conjonction with data on the nutritional content of larval provision masses, to explore the degree of morphological differentiation present in *H. scabiosae* and search for signs of parental manipulation.

Chapter 2: Contribution of helpers to colony survival and productivity

The indirect fitness return that a freshly eclosed bee can expect by forfeiting her own reproduction and helping in the natal nest does vary with the conditions. Besides external environmental factors such as the weather or flower abundance (Richards 2004; Rehan et al. 2011), which affect the nest as a whole, the social environment of the nest has a great importance. For instance, depending on the change in per-helper productivity with increasing group size, the helpers already present in the nest can influence the returns for a prospective helper. If the per-helper productivity decreases with colony size (Michener 1964; Bono and Crespi 2008), alternative strategies to helping may get increasingly attractive for prospective helpers in large colonies.

In chapter 2, I present an experiment for measuring the contribution of a helper to colony survival and productivity. By removing one helper in half of the colonies, I investigate the impact of this helper on the probability of colony success and the mean number of gynes and males produced.

Chapter 3: Relatedness, drift and helper reproduction

Productivity measurements do not give the full picture of the fitness benefits obtained by helpers. This is because we lack information on who actually produced the brood, and on the relatedness of the helpers to the brood they rear. By using microsatellite genetic markers, I was able to infer parentage within and across colonies, and thus identify the individuals engaging in direct reproduction. Primitively eusocial halictids show varying proportion of direct reproduction by helpers (Yanega 1988; Richards et al. 1995; Field et al. 2010; Yagi and Hasegawa 2012). Moreover, unrelated foreign individuals have been found in an increasing number of social species (Paxton et al. 2002; Lopez-Vaamonde et al. 2004; Sumner et al. 2007; Yagi and Hasegawa 2012), and some of the second brood gynes in *H. scabiosae* were found to drift to foreign nests (Ulrich et al. 2009). In chapter 3, I investigated whether and to which extent helpers in *H. scabiosae* reproduce, and whether foundresses and first brood females drift between nests. I used a nonlethal method for genetic sampling, which allowed me to monitor the changes in colony composition throughout the season.

Together, the results of the three chapters will further our understanding of the factors shaping the evolution of helper behaviour and eusociality, including maternal manipulation, ecological or social constraints, and the direct and indirect benefits associated with alternative strategies such as helping or reproducing.

Chapter 1

Born to be bee, fed to be worker? The caste system of a primitively eusocial insect

Nayuta Brand and **Michel Chapuisat**

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Abstract

Introduction

Primitively eusocial halictid bees are excellent systems to study the origin of eusociality, because all individuals have retained the ancestral ability to breed independently. In the sweat bee *Halictus scabiosae*, foundresses overwinter, establish nests and rear a first brood by mass-provisioning each offspring with pollen and nectar. The mothers may thus manipulate the phenotype of their offspring by restricting their food provisions. The first brood females generally help their mother to rear a second brood of males and gynes that become foundresses. However, the first brood females may also reproduce in their maternal or in other nests, or possibly enter early diapause. Here, we examined if the behavioural specialization of the first and second brood females was associated with between-brood differences in body size, energetic reserves and pollen provisions.

Results

The patterns of variation in adult body size, weight, fat content and food provisioned to the first and second brood indicate that *H. scabiosae* has dimorphic females. The first-brood females were significantly smaller, lighter and had lower fat reserves than the second-brood females and foundresses. The first-brood females were also less variable in size and fat content, and developed on homogeneously smaller pollen provisions. Foundresses were larger than gynes of the previous year, suggesting that small females were less likely to survive the winter.

Conclusions

The marked size dimorphism between females produced in the first and second brood and the consistently smaller pollen provisions provided to the first brood suggest that the first brood females are channelled into a helper role during their pre-imaginal development. As a large body size is needed for successful hibernation, the mother may promote helping in her first brood offspring by restricting their food provisions. This pattern supports the hypothesis that parental manipulation may contribute to promote worker behaviour in primitively eusocial halictids.

Keywords: evolution of eusociality, caste differentiation, parental manipulation, provisioning behaviour, sweat bees, halictids, *Halictus scabiosae*

Introduction

The hallmark of eusociality is reproductive division of labour between generations, a surprising social organization by which some individuals become functionally sterile helpers (Wilson 1971). Primitively eusocial species are excellent systems to study the proximate mechanisms and ultimate causes leading to eusociality, because helpers have retained the ancestral ability to breed independently and may thus obtain both direct fitness benefits through reproduction and indirect fitness benefits by helping relatives (Schwarz et al. 2007; Gadagkar 2011; Leadbeater et al. 2011).

Primitively eusocial halictids have a low degree of morphological differentiation between queens and helpers and a high degree of behavioural flexibility in both types of individuals (Schwarz et al. 2007). As a result, females have multiple reproductive options that result in diverse types of social organisation. Many primitively eusocial species live in temperate zones, where females overwinter, found nests either alone (Knerer and Atwood 1966) or in association, and raise two broods per year (Schwarz et al. 2007). The first brood daughters may become non-reproductive helpers that stay in their natal nest to assist their mother in raising a next brood of gynes and males. However, they may also gain direct fitness by reproducing in their natal nest, drifting to reproduce in other nests, or entering early diapause to become nest foundresses in the next spring (Yanega 1988; Paxton et al. 2002; Richards et al. 2003; Richards et al. 2005; Soro et al. 2009; Ulrich et al. 2009).

In eusocial halictids, the various reproductive strategies of females are generally associated with some difference in body size (Knerer and Atwood 1966; Michener 1990; Schwarz et al. 2007). Foundresses tend to be large-bodied females that have large energetic reserves enabling them to overwinter, establish nests and reproduce independently (Weissel et al. 2012). In contrast, helpers tend to be smaller-bodied daughters. For instance, across eight halictid species the proportion of helpers with undeveloped ovaries correlated with the degree of size divergence between foundresses and helpers (Packer and Knerer 1985). Body size depends in part on larval diet, which has long been recognized to play an important role in caste differentiation and sociality (Hunt and Nalepa 1994). For example, in primitively eusocial *Polistinae* wasps it has been proposed that the castes result from differential nourishment during larval development, with individuals experiencing relatively poor diet tending to become workers (Hunt and Amdam 2005; Hunt 2007; Toth et al. 2009).

An interesting aspect of body size variation and diet is that that the mother might limit the amount of resources that she provides to her offspring, thus forcing them to develop into small and lean females that are incapable of independent reproduction and are thus constrained to become helpers (Alexander 1974; Michener and Brothers 1974; West-Eberhard 1975; Charnov 1978; Craig 1979; Ratnieks and Wenseleers 2008). Moreover, small-bodied females may be easier to manipulate into a subordinate role by dominance interactions and aggression (Michener and Brothers 1974; e.g. Pabalan et al. 2000). In line with the hypothesis of parental manipulation, in *Polistes metricus* hand-fed female larvae became heavier and were more cold-resistant than those fed only by the queen (Karsai and Hunt 2002).

Maternal control of body size is likely to be particularly effective in mass-provisioning species such as social halictids, which lay a single egg on a mass of pollen and nectar deposited in a closed cell, thus providing all the food that the offspring will need to develop into adulthood. In all annual species of eusocial sweat bees studied so far, pollen provisions of gyne-destined larvae were larger than those of worker-destined larvae (e.g. Knerer and Atwood 1966; Richards and Packer 1994). Moreover, in one species the provisions provided to female offspring were more variable than the ones provided to male offspring (Kapheim et al. 2011). Overall, in various bee species the provision quality and quantity were shown to affect adult body size, as well as the sex of the egg laid (e.g. Plateaux-Quénu 1983; Danforth 1990; Roulston and Cane 2002; Burkle and Irwin 2009). Together, these data indicate that mothers can control the body size of their offspring in mass-provisioning bees. It is therefore of interest to study the relationship between pollen provisions, body size and behaviour in species that have complex social systems, and where the first generation of offspring have multiple options.

Body size variation also provides insights into the ecology, reproductive strategy and social behaviour of a species. If all offspring have similar fitness functions, a simple model predicts that there is a single optimal amount of resource that a parent should expend on each offspring (Smith and Fretwell 1974). Therefore, variations in parental expenditure and offspring body size generally reflect changes in availability of the limiting resources, in fitness expectations or in offspring role, for example switch from reproducing to helping (e.g. Rosenheim et al. 2010; Kapheim et al. 2011).

Here, we study body size variation in the sweat bee *Halictus scabiosae* (Rossi, 1790), a ground-nesting, mass-provisioning halictid that varies in social organization and relatedness among nestmates (Batra 1966; Ulrich et al. 2009). Our objectives are to examine if the mothers restrict offspring resources to promote worker behaviour in their first brood and to document the degree of body size differences between broods, which may contribute to explain behavioural specialization. In Switzerland, *H. scabiosae* forms annual colonies in which females raise two broods that are well-separated in time (Batra 1966; Ulrich et al. 2009). In spring, the foundresses – mated females that have overwintered – found new colonies, either alone or in small groups (Ulrich et al. 2009). The foundresses raise a first brood (B1) that is female-biased and emerge from the nests in June and July (Ulrich et al. 2009). Many of the B1 females do not reproduce and help their mother to raise a second brood (B2) of females and males (Batra 1966; Plateaux-Quénu 1972; Ulrich et al. 2009). However, the B1 females have retained the ability to mate and lay eggs, so they have the possibility to reproduce in their natal nest or in neighbour nests (Ulrich et al. 2009). The B2 females and males emerge from the nest in August and September. After mating, the B2 females enter diapause to pass the winter and become the next spring foundresses. Whether some of the B1 females enter early diapause, overwinter and found new colonies in the next spring, as has been documented in another species (e.g. Yanega 1989), remains to be investigated.

An interesting aspect of *H. scabiosae* is that queen turnover and drifting occur frequently, leading to low average relatedness between foundresses, B1 and B2 females (Ulrich et al. 2009; Brand and Chapuisat, Chapter 3). The fact that B1 helpers often raise unrelated brood could limit the benefits of size manipulation by foundresses and select for large-sized B1 females, if large B1 females have a higher probability to become replacement queens or found new nests.

Early reports on the degree of body size dimorphism between *H. scabiosae* foundresses and helpers (= B1 females) are somewhat equivocal, in part because of small sample sizes and variation in measurement methods. A single foundress was reported to be larger than three of her helpers (Quénu 1957). When measuring wing and abdomen length in a larger sample of bees, Knerer (1966) documented that foundresses were on average larger than helpers, but with a continuous distribution and a large overlap of sizes. In contrast, Batra (1966) found no size difference between foundresses and helpers when measuring the head width of 30 bees from seven nests. Hence, more data on body size variation among female types (foundresses, B1 and B2 offspring) are needed to better understand the social organisation, partitioning of reproduction and reproductive options in this primitively eusocial sweat bee.

In this study, we compared adult body size, weight and fat content of foundresses, first brood females and second brood females in *H. scabiosae*. We also compared the pollen and nectar provisions provided to the first and second brood, in order to evaluate if the mothers might influence the body size of their first brood offspring by limiting their food resources. Finally, we examined if body size was correlated with the probability to survive the winter. These data on the degree, origin and consequence of dimorphism between breeders and helpers will help to evaluate if parental manipulation influences body size and helping in social groups with low relatedness.

Results

Caste differentiation

The analysis of 2498 bees from 769 nests revealed that the foundresses, first brood (B1) females and second brood (B2) females differed significantly in head width (Figure 1a; effect of female type: log-likelihood ratio, LR = 11.00, $P < 0.01$). In both years, the B1 females were significantly smaller than both the B2 females (Figure 1a; Tukey's tests: 2008, $|z| = 17.51$, $P < 0.001$; 2009, $|z| = 13.33$, $P < 0.001$) and foundresses (Tukey's tests: 2008, $|z| = 7.81$, $P < 0.001$; 2009, $|z| = 26.55$, $P < 0.001$). Within the same nests, the degree of head size dimorphism between foundresses and B1 females (calculated as follows: [(foundress head width - B1 female head width) / foundress head width]) was 0.09 ± 0.07 (n = 111 nests), while the size dimorphism between B2 and B1 females was 0.05 ± 0.07 (n = 209 nests). The variance in head width also differed significantly among female types (heteroscedasticity: LR = 63.90, $P < 0.001$): the B1 females were the least variable, with the variances in head width being 1.8 and 1.3 times larger in B2 females and foundresses, respectively (Figure 1a).

Nest identity had a significant effect on head width (LR = 59.82, $P < 0.001$). The year had no main effect on head width (LR = 9.37e-7, $P = 0.99$), but there was a significant interaction between female type and year (LR = 59.91, $P < 0.001$). This is because the B2 females were significantly smaller than the foundresses in 2009 (Tukey's test: $|z| = 12.76$, $P < 0.001$), but not in 2008 (Tukey's test: $|z| = 2.63$, $P = 0.08$; Figure 1a). The B2 females of 2008 were also significantly smaller than the foundresses of 2009 (Tukey's test: $|z| = 4.91$, $P < 0.001$), which indicates that within this cohort the larger females were more likely to survive the winter.

The foundresses, B1 females and B2 females sampled in 2009 differed significantly in dry weight (Table 1; effect of female type: LR = 19.49, $P < 0.001$). In line with their smaller head size, the B1 females had a significantly lower dry weight than both the B2 females (Tukey's test: |z| = 3.99, $P < 0.001$) and foundresses (Tukey's test: |z| = 4.16, $P < 0.001$). In contrast, the weight of the B2 females was not significantly different from the one of the foundresses (Tukey's test: |z| = 0.37, $P = 0.93$).

The three female types also differed significantly in absolute fat weight (Table 1; effect of female type: LR = 15.49, $P < 0.001$). Again, the B1 females had a lower absolute fat weight than both the B2 females (pairwise Wilcoxon tests, W = 546.5, *P*

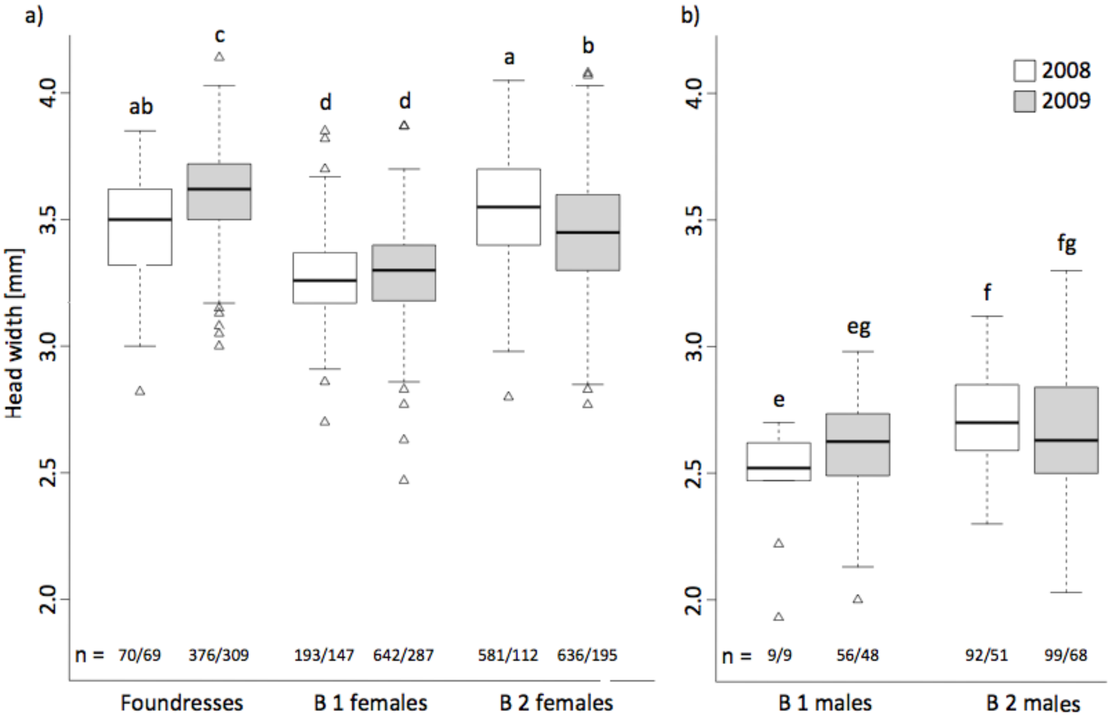

Figure 1. Head size of *H. scabiosae* bees sampled in 2008 (white bars) and 2009 (grey bars). (a) Foundresses, first brood females and second brood females. (b) First brood males and second brood males. Solid lines indicate the median for each category, boxes the interquartile range, and whiskers the most extreme values within 1.5 times the interquartile range. Sample sizes for each category (number of individuals/number of nests) are indicated above the x-axis. Different letters indicate significant differences between groups (Tukey's tests). Females and males were analysed separately.

= 0.01) and foundresses (W = 194.5, *P* = 0.02). The B2 females and foundresses did not differ significantly in fat weight (W = 546.5, *P* = 0.32). The variances in fat weight were significantly different for foundresses, B1 and B2 females (heteroscedasticity: LR = 19.08, *P* < 0.001): the B1 females were the least variable in fat weight, while the B2 females and foundresses had 5.2 and 1.8 times larger variances in fat weight, respectively.

Table 1. Adult dry weight and fat weight (2009). n = number of nests

	n	Dry weight (mg ± SD)	Fat weight (mg ± SD)	Proportion of fat
Foundresses	28	26.13 ± 4.26	1.81 ± 0.89	6.9 ± 4.1 %
First brood females	23	20.93 ± 3.80	1.23 ± 0.41	5.8 ± 3.5 %
Second brood females	34	25.94 ± 5.94	2.52 ± 1.72	9.2 ± 4.6 %
Second brood males	13	13.16 ± 4.34	0.79 ± 0.60	5.5 ± 3.9 %

The relative fat content (fat weight divided by total dry weight) did not differ significantly between foundresses, B1 and B2 females (effect of female type: LR = 7.53, *P* = 0.11), and was not explained by female head width (LR = 1.28, *P* = 0.73) nor by an interaction between female type and head width (LR = 0.77, *P* = 0.68). However, the variances in relative fat content were significantly different for foundresses, B1 and B2 females (heteroscedasticity: LR = 22.82, *P* < 0.001; Figure 2). Again, the B1 females were the least variable, and the variances in relative fat content of the B2 females and foundresses were 7.0 and 3.0 times larger, respectively. The relative fat content of the B2 females appeared to be multimodal: about 56% (19 of 34) of the B2 females had a low fat content (median at 5.3%), similar to the one of the B1 females, while the rest of the females had a larger fat content (median at 16%; Figure 2).

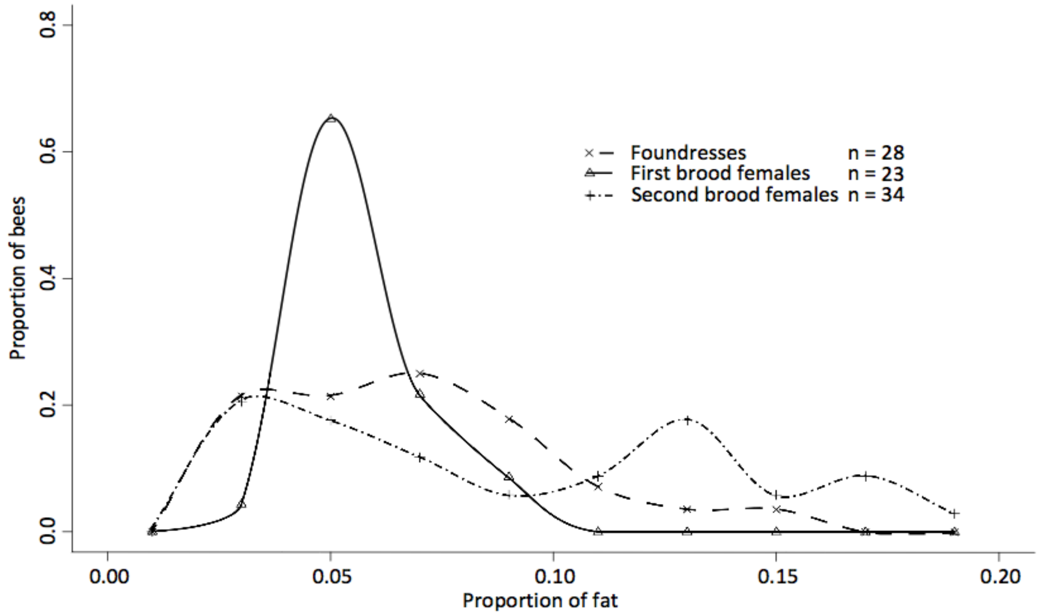

Figure 2. Relative fat content of female bees from 2009.
Distribution of bees according to their proportion of fat over total dry weight for foundresses (dashed line), first brood females (solid line) and second brood females (point-dashed line). n = number of nests.

The males from the first and second brood differed significantly in head width (Figure 1b; effect of brood: LR = 6.94, $P < 0.01$). However, the difference appeared to be small and not consistent across years (Figure 1b). The B1 males were significantly smaller than the B2 males in 2008 (Tukey's test: $|z| = 3.31$, $P < 0.01$), but not in 2009 (Tukey's test: $|z| = 2.06$, $P = 0.15$), when we sampled a much larger number of B1 males (Figure 1b). The variance in head width did not differ significantly between B1 and B2 males (heteroscedasticity: LR = 0.32, $P = 0.57$).

The males had a significantly smaller head width than the females (Figure 1a and 1b; effect of sex: LR = 29.62, $P < 0.001$). There was again a significant effect of nest identity on male and female head width (LR = 98.9, $P < 0.001$). The males were also significantly lighter than the females in terms of dry weight (Table 1; t-test: t = 6.45, df = 13.30, $P < 0.001$), fat weight (Table 1; Wilcoxon-test: W = 940, $P < 0.001$) and relative fat content (W = 741, $P = 0.049$).

Brood provisions

We sampled pollen and nectar provisions in 2009. The provisions provided to the first brood were significantly smaller than the ones provided to the second brood (Table 2; fresh weight: LR = 16.76 $P < 0.001$; dry weight: LR = 16.86, $P < 0.001$). The variance in weight was 3.6 (fresh weight) and 3.3 (dry weight) times larger for provisions of B2 offspring than for the ones of B1 offspring (heteroscedasticity: fresh weight: LR = 5.71, $P = 0.02$; dry weight: LR = 5.04, $P = 0.02$). The provisions provided to B2 offspring contained slightly more sugar (17.5% in weight) than the ones provided to B1 offspring, but this difference was not significant (Table 2; LR = 2.55, $P = 0.11$). The proportion of sugar (sugar weight divided by total dry weight) was on average higher and more variable in provisions of B1 offspring than in the ones of B2 offspring (Table 2; effect of brood: LR = 6.8, $P = 0.01$; heteroscedasticity: LR = 12.21, $P < 0.001$).

Table 2. Pollen provisions provided to the first and second brood (2009). n = number of pollen balls

	n	Fresh weight (mg ± SD)	Dry weight (mg ± SD)	Sugar weight (mg ± SD)	Proportion of sugar
First brood	16	125.5 ± 19.9	77.5 ± 12.9	34.3 ± 11.3	43.6 ± 8.4 %
Second brood	16	177.4 ± 37.5	112.3 ± 23.4	40.3 ± 9.4	36.0 ± 4.0 %

Discussion

The females of *H. scabiosae* were clearly dimorphic. On average, females originating from the first brood (B1) were significantly smaller, lighter and had lower absolute fat reserves than both foundresses and females produced in the second brood (B2). The relative mean size difference between the B2 and B1 females at the population level amounted to 6%, 24% and 105% for head width, dry weight and fat weight, respectively.

In insects, the head size of adults doesn't change after the cuticle of the head capsule has fully sclerotized. Adult head size generally depends on the genotype and on food quality and quantity during development (Roulston and Cane 2002). These factors are likely to vary among colonies, which is in line with the finding that nest identity had a significant effect on head width in our and other studies (e.g. Richards and Packer 1996; Roulston and Cane 2002). The degree of head size dimorphism between B2 and B1 females within nests of *H. scabiosae* (5%) was slightly lower than the one recorded in other socially polymorphic and weakly eusocial halictids, such *H. sexcinctus* (7.5%), *H. ligatus* (8%) and *H. poeyi* (10%) (Packer and Knerer 1986; Richards and Packer 1994; Richards and Packer 1996; Richards 2001). Moderate dimorphism in *H. scabiosae* is consistent with the finding that females have flexible reproductive strategies (Ulrich et al. 2009). Interestingly, in *H. rubicundus* the degree of wing length dimorphism between foundresses and B1 females was very low (0.3%) compared to wing length dimorphism between B2 and B1 females (4.3%), because many of the foundresses were B1 females that had overwintered (Yanega 1988; Yanega 1989). In comparison, the high degree of head size dimorphism between foundresses and B1 females (9%) suggests that most B1 females do not over-winter in our study population of *H. scabiosae*.

Within each category of females (foundresses, B1 and B2), the head width, dry weight and fat weight showed a large amount of variation, and the size distributions of the three categories partially overlapped. Size variation may reflect changes in the number of foragers (e.g. Kapheim et al. 2011), in resource availability (e.g. Richards and Packer 1996), or in parental allocation. Importantly, the variance in head width and fat weight was significantly and consistently larger in B2 females (gynes) and foundresses than in B1 females (workers), even after controlling for differences in means. The reverse pattern was found in advanced eusocial insects: the variance in size was greater for workers than for queens in formicine ants and vespine wasps (Bargum et al. 2004; Kovacs et al. 2010), suggesting lower selection pressure on castes that are no longer capable of direct reproduction (Kovacs et al. 2010). In contrast, the low size variability in B1 females of *H. scabiosae* is consistent with the parental manipulation hypothesis (Alexander 1974): it suggests that foundresses constrain the food resources to rear uniformly small B1 females that will behave as workers. Conversely, if the survival and fecundity of reproductive females (gynes) increase gradually with body size and energetic reserves (e.g. Roulston and Cane 2002; Shreeves and Field 2008; Weissel et al. 2012), variation in resources or brood number might result in high size variability in B2 females.

The pollen and nectar provisions provided to the B2 offspring were much larger and more variable in size than the ones provided to the B1 offspring. The difference amounted to 45% in terms of dry weight. Such differential provisioning of the first and second brood has been documented in several eusocial halictine bees (Knerer and Atwood 1966; Boomsma and Eickwort 1993; Richards and Packer 1994). It would be interesting to investigate provisioning in species that are facultatively social (Field et al. 2012), as well as in eusocial and parasocial colonies exhibiting split sex-ratio (Mueller 1991). Somewhat surprisingly, in *H. scabiosae* there was no significant difference between B1-destined and B2-destined provisions in terms of total sugar weight, due to the higher average sugar concentration in spring provisions. Our study is the first to find that provisions fed to B1 offspring have a higher but more variable concentration of sugar. In contrast, in *H. ligatus* the sugar concentration was higher in gyne-destined than in male-destined and B1 female-destined provisions (Richards and Packer 1994). Variation in sugar content may reflect differences in sex ratio, variation in the number of foragers (Kapheim et al. 2011), or temporal and seasonal variation in nectar quality and availability, for example due to weather conditions (Richards and Packer 1994; Richards and Packer 1996).

The smaller pollen provisions provided to the first brood are consistent with the idea of parental manipulation (Alexander 1974). Indeed the foundresses may force their first offspring to behave as helpers by restricting their food provisions in such a way that they become small, lean females unable to establish independent colonies, particularly if large energetic reserves are needed to survive the winter or to nest independently (Michener and Brothers 1974; West-Eberhard 1975; Richards and Packer 1994; Weissel et al. 2012). It is somewhat surprising to find signs of parental manipulation in a species that has high rates of queen turnover and high incidence of drifting, which leads to a low relatedness between foundresses, B1 and B2 females in part of the nests (Ulrich et al. 2009; Brand and Chapuisat 2014). If colony relatedness becomes very low, the B1 females should be selected to resist manipulation and claim their share of reproduction (Charnov 1978; Craig 1979).

In *H. scabiosae*, first brood females occasionally replace foundresses in orphaned nests, forming semisocial colonies (Ulrich et al. 2009; Brand, 2014 #6587). Overall, the first females appear to be sufficiently large to become replacement queens in existing colonies, but to lack the energetic reserves that are necessary for independent colony founding and overwintering (Weissel et al. 2012).

Parental manipulation is hard to distinguish from seasonal variation in resource availability and resource acquisition, which are influenced by vegetation, weather, photoperiod, number of colony members foraging (Kapheim et al. 2011), as well as parasitism and predation risks (Lienhard et al. 2010). Annual weather variation appeared to have had some impact on body size in our population, since B2 females were smaller in 2009, a year with frequent rainfalls during the period of B2 provisioning (late June to mid July). Similarly, foundresses were larger in 2009, after a harsh winter with a temperature drop towards the end of hibernation (late February). Interestingly, B1 female size was very similar over the two years despite pronounced differences in weather conditions in spring, which is consistent with the hypothesis that the mothers control and restrict the provisions destined to the B1 offspring.

Foundresses had significantly larger head size than gynes of the previous year, which suggests that small females were less likely to survive the winter. A similar pattern has been documented in *Bombus terrestris* introduced to Japan (Inoue 2011). As *H. scabiosae* has expanded its range to the north in recent years (Frommer and Flügel 2005), it is possible that the body size of gynes is not yet adapted to the winter of Switzerland. More importantly, the higher size of foundresses sampled in spring than gynes sampled in the previous autumn, combined with the small size of first brood females, suggest that first brood females are unlikely to survive the winter. The relative fat content of gynes (9.4%) was surprisingly low compared to other studies (e.g. H. ligatus, 17.8%,Richards and Packer 1994; Weissel et al. 2012). It seems likely that gynes continue to build up fat stores after their first exit from the nest. This may contribute to explain the low difference in fat weight and fat content between gynes and foundresses that have overwintered, along with the fact that the foundresses were caught at an early stage of colony founding (Weissel et al. 2012).

The males were smaller than females, as commonly observed in insects (Roulston and Cane 2002; Shreeves and Field 2008). They also had very low overall fat content and a proportion of fat comparable to the one of B1 females, consistent with the idea that fat reserves are for overwintering and colony founding (Weissel et al. 2012). In contrast to females, males from the first and second brood showed no clear and consistent differences in size and size variances, which is the expected pattern if variation in female size is due to parental manipulation rather than environmental variation (Kapheim et al. 2011). This result should however be interpreted with caution, because sample sizes were smaller for males than for females.

Conclusion

The marked size dimorphism between females produced in the first and second brood and the consistently smaller pollen provisions provided to the first brood suggest that the first brood females of the sweat bee *H. scabiosae* are channelled into a helper role during their pre-imaginal development. As a large body size is needed for successful hibernation, the mother may promote helping in her first brood offspring by restricting their food provisions. This pattern, which is common to many primitively eusocial halictids, supports the hypothesis that worker behaviour is in part be enforced by parental manipulation of the brood resources in mass-provisioning bees.

Methods

Sampling and measurement of bees

Our study site is located in Adlikon, near Zürich, in northern Switzerland. It consists of a dry, south-exposed and sparsely vegetated embankment. *H. scabiosae* is abundant at this site, with more than 1000 nests per breeding season over an area of ca. 30 x 10 meters. We marked nests with numbered nails and flags. We captured the bees by posing net traps on the nest entrance in the early morning (6-8 am), before the bees became active (8:30-10 am). We sampled most foundresses in May and June, most adult bees originating from the first brood (B1) in July, and all adult bees from the second brood (B2) in August and early September. In spring, we detected multiple foundress associations in 16% of the nests, but the vast majority of these associations appeared to be transient and were not resampled later in the season. As the season progressed, bees from earlier cohorts (foundresses or B1 females) could easily be distinguished by the wear of their wings, mandibles and hairs (Mueller and Wolfmueller 1993).

Head width is commonly used as a proxy for overall adult body size in halictid bees and other insects (e.g.Richards and Packer 1996; Schwander et al. 2005; Zobel and Paxton 2007). In 2008 and 2009, we measured the head width of 2754 live bees originating from 791 nests. We briefly immobilized the bee on a sponge and measured its largest head width across the eyes, using a precision calliper (SPI 2000 dial calliper, SPI, CA). To diminish measurement errors, we measured each bee three times and used the mean value for subsequent analysis. The coefficient of variation across the three measures was 0.01. To avoid double measurements, we marked the bees on the thorax with a dot of honeybee-marking enamel paint (Apicolori, Bienen-Meier Künten) before releasing them.

In 2009, we measured the weight and fat content of a sub-sample of 109 adult bees originating from 98 nests. At the start of the period of activity of bees, between May 17th and June 6th, we captured 28 foundresses from 28 nests. Later in the season, we captured first brood females (25 individuals from 23 nests, June 25–29), second brood females (41 individuals from 34 nests, August 11–31) and second brood males (15 individuals from 13 nests, August 11–September 8). For the weight analysis, we used bees that were captured upon their first exit from the nest. We froze the bees, dried them for five days at 65°C, and measured their dry weight with a microbalance (Mettler Toledo MT5). To measure their fat content, we extracted the lipids by soaking the bees in petroleum ether for 10 days, replacing the ether once. After this extraction, we dried the bees again, re-weighed them, and estimated their fat weight as the dry weight loss between the two measures.

Brood provisions

To compare the provisions provided to B1 and B2 offspring, we excavated nests and collected the contents of brood cells. In the early morning, we humidified the soil around nest entrances and blew starch into the burrows to follow them more easily while digging. A complete provision consisted of an intact ball of pollen and nectar, enclosed in a sealed brood cell containing a bee egg. On May 21st, 2009, we collected 16 complete provisions prepared for B1 offspring, from eight nests. Between July 22nd and August 11th, 2009, we collected 16 complete provisions prepared for B2 offspring, from nine nests. These provisions were frozen until further analysis. We could not get any information on the ploidy of the collected eggs.

We measured the fresh weight, dry weight (after 48 h at 65°C) and sugar content of complete provisions. We estimated the sugar content by refractometry, using the method described by Richards and Packer (1994) and Kapheim *et al.* (2011). In short, we re-suspended the provisions in 200 µl of H_2O, estimated the sugar concentration in Brix degrees using a refractometer (Abbe-Refraktometer B, Zeiss, Germany), and converted this into total sugar weight per provision, measured in sucrose equivalents.

To estimate annual weather variation, we used data from the weather station Aadorf/Tänikon, available at http://www.meteoswiss.admin.ch/web/en/services/data_portal.html.

Statistical analysis

We investigated size differences among female types with linear mixed models (LMM, see Pinheiro and Bates 2000 for a review). We used stepwise log-likelihood tests and controlled for heteroscedasticity between categories by estimating the variance of the residuals modelled as a linear function of the predictor variables (Harvey 1976; Pinheiro and Bates 2000; Kapheim et al. 2011). This approach permits us to compare variances after controlling for differences in means (Kapheim et al. 2011). To test for size differences between foundresses, B1 and B2 females, we included the female type as a fixed effect in the model. In order to control for the non-independence of bees sampled from the same nests and for the effect of the year, we also included the nest identity and year of sampling as random effects. We used Tukey's HSD post-hoc tests to examine which type of female differed from one another. We used similar models to examine size differences between male broods and between sexes.

To examine variation among adult bees in dry weight, fat weight and relative fat content (fat weight divided by total dry weight), we included the type of female or the sex as a fixed effect in a generalized least square model (GLS, Pinheiro and Bates 2000). For the analysis of the relative fat content of females, we also included head width as a covariate. For these weight data, as we had measured a single bee for most of the nests (90 out of 98), we used one mean value per nest to ensure the independence of the data. We log-transformed the weight data to have randomly distributed residuals.

We used linear mixed-effects models to compare the pollen provisions provided to the first and second brood. We included the brood (B1 or B2) as a fixed effect. To control for the non-independence of pollen balls sampled from the same nest, we included the nest identity as a random effect. All statistical analyses were carried out with the software R 2.14.0 (R Development Core Team 2011) using the R packages nlme 3.1 (Pinheiro et al. 2012) and multcomp 1.2 (Hothorn et al. 2008).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NB and MC designed the study. NB performed the field work, chemical analyses and statistical analyses. MC provided guidance and advised on data analysis. NB and MC wrote the manuscript. All authors read and approved the final manuscript.

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Chapter 2

Impact of helpers on colony productivity in a primitively eusocial bee

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Abstract

Small societies of totipotent individuals are good systems in which to study the costs and benefits of group living that are central to the origin and maintenance of eusociality. For instance, in eusocial halictid bees, some females remain in their natal nest to help rear the next brood. Why do helpers stay in the nest? Do they really help, and if yes is their contribution large enough to voluntarily forfeit direct reproduction? Here, we estimate the impact of helpers on colony survival and productivity in the sweat bee *Halictus scabiosae*. The number of helpers was positively associated with colony survival and productivity. Colonies from which we experimentally removed one helper produced significantly fewer offspring. However, the effect of helper-removal was very small, on average. From the removal experiment, we estimated that one helper increased colony productivity by 0.72 additional offspring in colonies with one to three helpers, while the increase was smaller and not statistically significant in larger colonies. We conclude that helpers do actually help in this primitively eusocial bee, particularly in small colonies. However, the resulting increase in colony productivity is low, which suggests that helpers may be constrained in their role or may attempt to reproduce.

Keywords: social evolution; cooperative breeding; altruism; eusociality; halictid bees

Introduction

How eusociality arises and is maintained is a major question in evolutionary biology, because eusocial groups contain individuals that do not reproduce and instead help others. The transition from solitary to social breeding and the emergence of a non-reproducing helper caste are best studied in small societies where all individuals are still able to mate and reproduce, such as those of many bees and wasps (e.g. Schwarz et al. 2007; Chapuisat 2010; Yagi and Hasegawa 2012). Such species show great lability in social organization and behaviour – subordinate individuals may increase their indirect fitness by helping relatives, but may also obtain considerable direct fitness, for example when they inherit the nest (Hamilton 1964a; Hamilton 1964b; Bourke 2011; Leadbeater et al. 2011; Schwarz et al. 2011).

In primitively eusocial insects, as in cooperatively breeding birds, young individuals may either leave the parental nest or stay in it in order to help, reproduce personally, or wait for breeding opportunities. The payoff of each option depends on multiple ecological and social factors that jointly influence the opportunities for independent breeding, the probability to inherit the nest, the efficacy of help in increasing the fitness of relatives, and the possibility to get a share of reproduction (e.g. Keller and Chapuisat 1999; Barclay and Reeve 2012; Hatchwell et al. 2013). Breeding cooperatively or helping also provides head start benefits, as well as an insurance-based advantage – if an individual dies, other group members will finish rearing the brood (Queller 1989; Gadagkar 1990; Field et al. 2000). Moreover, the mother or other members of the social group can influence the behavioural trajectories of potential helpers, for example by influencing their development or restricting their reproduction (Alexander 1974; Ratnieks and Wenseleers 2008; Brand and Chapuisat 2012).

The presence of multiple cooperating adults can greatly increase colony survival and productivity compared to lone breeding females, particularly when the risk of predation, parasitism or usurpation is high or when ecological conditions are harsh (Hogendoorn and Zammit 2001; Dunn and Richards 2003; Rehan et al. 2011; Yagi and Hasegawa 2011; Yagi and Hasegawa 2012). Moreover, division of labour between breeders and helpers can further increase colony success, as specialists become more efficient (Bourke 1999; Thomas and Elgar 2003). Ultimately, the evolution of a specialized helper caste will depend on the indirect fitness benefits generated by helping, as compared to the direct fitness benefits obtained by breeding in the group or independently (worker efficiency, Crozier and Pamilo 1996). Measures of the costs and benefits of helping are therefore essential to gain insights into the evolution of a helper caste.

The average productivity *per helper* has often been estimated by collecting entire colonies in primitively social Hymenoptera (e.g. Michener 1964; Hogendoorn and Zammit 2001; Smith et al. 2007; Yagi and Hasegawa 2012). However, with this correlational approach some third factor may jointly affect helper number and overall colony productivity. Moreover, each helper is weighted equally, while it seems likely that the first helpers will have a greater influence on colony survival and productivity. To measure the marginal contribution of each additional helper to colony survival and productivity, one could remove helpers experimentally. Removal experiments in wasps have demonstrated the benefits of foundress associations (Clouse 2001;

Tibbetts and Reeve 2003; Sumner et al. 2010), and in particular the importance of insurance-based advantage (Field et al. 2000; Lucas and Field 2011). To our knowledge, the impact of single helpers on the success of obligately but primitively eusocial colonies has not yet been investigated experimentally.

Here, we estimated the effect of helpers on colony survival and productivity in the primitively eusocial bee *Halictus scabiosae*. In this species, foundresses rear a first brood consisting principally of relatively small females that help their mother to rear a second brood of males and gynes (Brand and Chapuisat 2012). The first brood females have the possibility to mate and reproduce, either in their natal nests or in other nests (Ulrich et al. 2009). Hence, it is interesting to examine whether helpers really increase colony survival and productivity, and by how much. The contribution of a helper to colony success may be sufficient to select for complete reproductive altruism (self-restrained due to kin selection when nestmate relatedness and worker efficiency are high Crozier and Pamilo 1996; Wenseleers et al. 2004). Alternatively, the first brood females may be constrained to a helper role, for example if their mothers restrict their pollen provision (Brand and Chapuisat 2012). We do not know if first brood females can establish their own nests – due to their small size and late emergence, this seems unlikely. First brood females may thus be doing the best of a bad job, either by helping relatives, or by reproducing in their natal nest or in foreign nests (e.g. Richards et al. 1995; Lopez-Vaamonde et al. 2004; Ulrich et al. 2009; Leadbeater et al. 2011).

In a field experiment, we removed one helper (first brood female) from half of the colonies, and examined the effects of the number of helpers remaining in the colony and of the removal treatment on colony success. The quantitative estimate of the effect of helpers on productivity provides insights into the likelihood of voluntary reproductive altruism, versus enforced altruism or direct fitness gain. Our results thus contribute to the understanding of the principles governing the evolution and maintenance of eusociality.

Materials and Methods

Study species and study site

H. scabiosae is a primitively eusocial halictid bee forming annual colonies. In May, overwintered females establish underground nests. They lay eggs in individual cells stocked with provisions of pollen and nectar (mass-provisioning Brand and Chapuisat 2012). The foundresses rear a first brood (B1) that emerges from the nests in June and July (Ulrich et al. 2009; Brand and Chapuisat 2012). The first brood consists primarily of small-sized females that generally stay in their natal colony to help raise a second brood (B2) of gynes and males (Batra 1966; Ulrich et al. 2009; Brand and Chapuisat 2012). Helpers excavate new cells, forage to provision the offspring and occasionally defend the nests, for instance against predators like ants, parasitic flies or intraspecific usurpers. The gynes and males from the second generation emerge from the nests in August and September. After mating, the gynes overwinter and become the next generation of foundresses in the following spring (Ulrich et al. 2009; Brand and Chapuisat 2012).

Our study site is a dry, south-exposed embankment located in Adlikon, near Zürich, in northern Switzerland (Brand and Chapuisat 2012). It contains a large population of *H. scabiosae*, with more than 1000 nests per breeding season over an area of ca. 30 x 10 meters. In spring 2009, we marked the nests with numbered nails and flags, and selected 245 colonies that successfully produced one or more B1 helpers. Throughout the breeding season, we captured the bees by placing net traps on the colony entrance in the early morning (6-8 am), before the bees became active (8:30-10 am). We captured the foundresses between May 6th and June 4th, and marked them with individual colour codes, using quick-drying honeybee-marking paints (Apicolori, Bienen-Meier Künten).

Helper removal experiment and measures of colony productivity

We randomly allocated 114 colonies to the control treatment (no helper removal) and 131 colonies to the removal treatment, which consisted in removing one B1 female helper before it started to help. Between June 25th and July 1st, when the first B1 females started to emerge, we trapped one B1 female per colony, on its first exit from the natal nest. At our study side, brood emergence was synchronised and started on this week for all studied colonies. We released this B1 female near her natal colony in the controls, and removed her definitively in the removal treatment. We subsequently estimated the post-removal number of helpers by trapping, marking and releasing all other B1 females emerging from the colonies, on every clear day, until August 24th.

We estimated colony productivity by trapping, marking and releasing all individuals produced in the second brood (gynes and males). Individuals from the second brood emerged from the nests between August 11th and September 8th. Within each nest, the two broods are separated in time, and we could easily distinguish B2 from B1 females based on previous marking, head width (Brand and Chapuisat 2012), wing wear and mandibular wear (Mueller and Wolfmueller 1993).

To check that the trapping method provided accurate estimates of the number of B1 females and of colony productivity (number of B2 gynes and males), we excavated a sample of colonies that were not included in our control or removal colonies, but were treated in the same way as control colonies with respect to bee capture and marking. We blew starch into the burrows to follow them more easily while digging, carefully excavated the entire nest, and counted the number of offspring in brood cells. The average number of B1 females estimated by excavating colonies (2.54±0.61, *n*=13) was not significantly different from the one estimated by trapping (2.45±0.12, *n*=245; Wilcoxon text, *W*=1627.5, *P*=0.89). Similarly, the average colony productivity estimated by excavating colonies (7.69±1.69, *n*=13) was not significantly different from the one estimated by trapping in successful control colonies (4.81±0.61, *n*=48; Wilcoxon text, *W*=220.5, *P*=0.11).

Statistical analysis

We used Generalised Linear Models (GLM) to test the effect of potential explanatory variables (number of B1 females, removal treatment, and their interaction) on colony survival (characterized by the successful production of B2 offspring) and colony productivity (measured as the number of B2 offspring). In a first analysis, we used the "post-removal number of helpers", in order to estimate the influence of the actual number of B1 females remaining in the control and removal colonies after the removal treatment. In a second analysis, we included the removal treatment, the "pre-removal number of helpers" and their interaction, in order to examine the effect of removing one helper and assess if this effect varied with respect to the total number of B1 females produced by the colony.

We used a binomial GLM for analysing colony survival. For analysing colony productivity, we used a hurdle model with negative binomial and binomial error distributions, in order to appropriately handle the excess of zeros and overdispersion in the response variable (Zeileis et al. 2008). Adding quadratic and cubic polynomials to the model did not improve the fit. We assessed the significance of explanatory variables using stepwise log-likelihood-ratio tests, and sequentially removed non-significant terms. We performed post-hoc pairwise comparisons using a simultaneous inference procedure (Hothorn et al. 2008).

We obtained qualitatively similar statistical results when we took the sex ratio and differential investment in each sex into account for measuring productivity (the dry weight of females is twice the one of males in H. scabiosae, Brand and Chapuisat 2012), or when we restricted the analysis to the production of gynes only. All statistical analyses were carried out in R 2.10.1, using the packages multcomp, pscl, VGAM and lmtest (R Development Core Team 2011).

Results

Colony size, productivity and sex ratio

H. scabiosae formed very small colonies, which had low productivity. On average, the foundresses reared 2.58±0.12 (mean±*s.e.*) offspring in their first brood, and the number of B1 females per colony ranged from one to 11, with a mean of 2.45±0.12 (*n*=245 colonies; Figure 1). Colony survival from first to second brood was moderate, as only 42% of the 114 control colonies successfully raised a second brood. The 48 successful control colonies produced 4.81±0.61 gynes and males on average, and the mean productivity across all control colonies, including the ones that failed, was

2.03±0.34. The sex ratio was female-biased in both broods, with 5%±1% males in the first brood (*n*=245 colonies) and 15%±4% males in the second brood (*n*=48 control colonies). Across all colonies, the sex ratio in the second brood did not vary significantly with the pre-removal number of helpers (χ_1^2=0.22, *P*=0.64), nor with the removal treatment (χ_1^2=1.46, *P*=0.23).

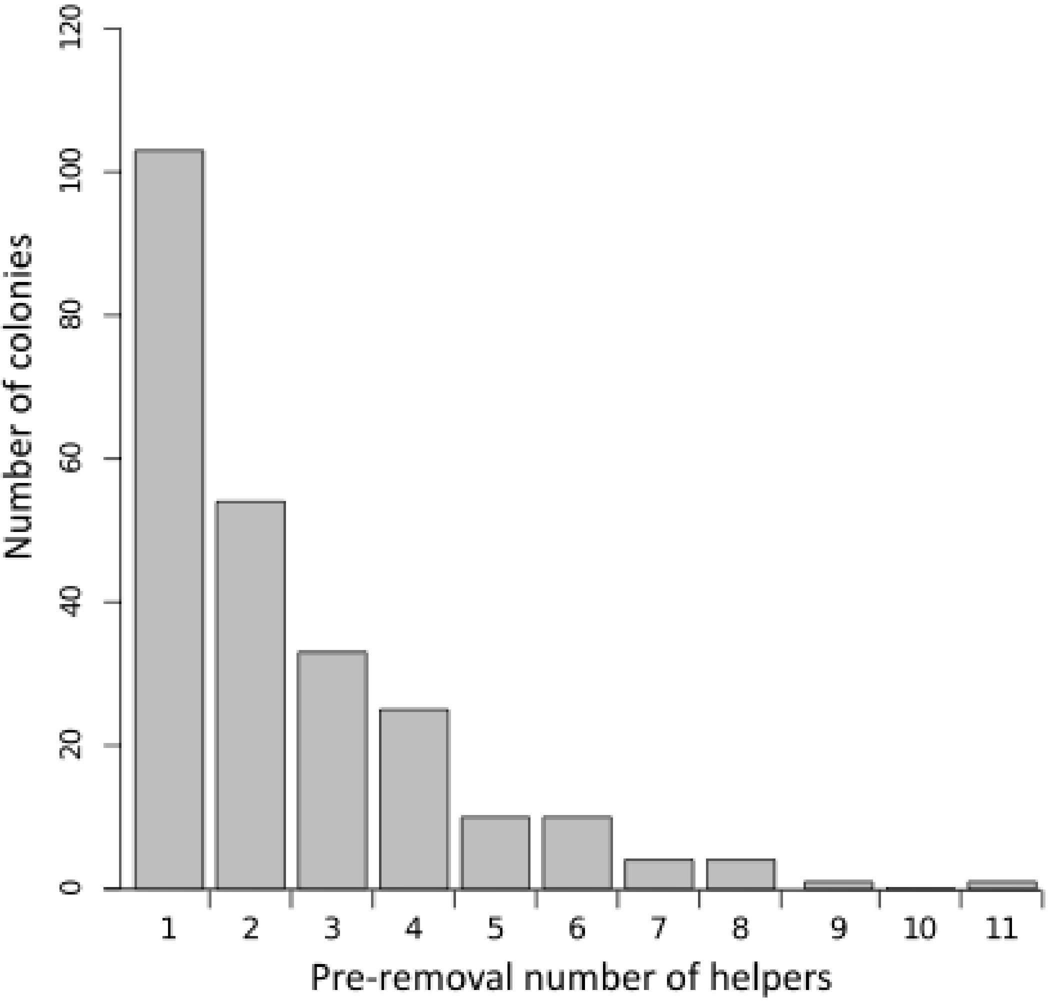

Figure 1. Distribution of the colonies according to the total number of helpers (B1 females) produced per colony.

Influence of the post-removal number of helpers

The post-removal number of helpers (i.e. the number of B1 females remaining in control and removal colonies after the removal treatment) showed a strong positive association with colony survival (Figure 2a; stepwise log-likelihood ratio test: χ_1^2=48.27, *p*<0.001; *n*=245 colonies). Only 20% of the colonies headed by foundresses with no helper left after removal successfully produced B2 offspring, and the survival rate gradually increased to more than 85% for colonies with six or more helpers (Fig. 2a). In pairwise comparisons between colonies differing by one helper, colony survival increased significantly between one- and two-helper colonies (|z|=2.9, *P*=0.02), but not between zero- and one-helper colonies (|z|=2.24, *P*=0.13). According to the coefficients of the GLM, on average each helper increased the odds ratio of successful against failed colonies by a factor of 1.8. The post-removal number of helpers was positively associated with the productivity of colonies that produced a second brood (Figure 2b; χ_1^2= 19.43, *P*<0.001; *n*=101 colonies). Based on the coefficients of the GLM, each helper in successful colonies increased colony productivity by a factor of 1.2 on average.

Across all colonies, including the ones that failed to produce any B2 offspring, the post-removal number of helpers was correlated with productivity (Figure 2c; χ_1^2=65.93, *P*<0.001). Colonies headed by foundresses with no helper left produced on average 0.28±0.10 B2 offspring, and the productivity rose to 7.00±1.95 in colonies with seven helpers (Figure 2c). In pairwise comparisons, colony productivity increased significantly between zero- and one-helper colonies (|z|=2.68, p=0.05), as well as between one- and two helper colonies (|z|=3.22, *p*=0.01).

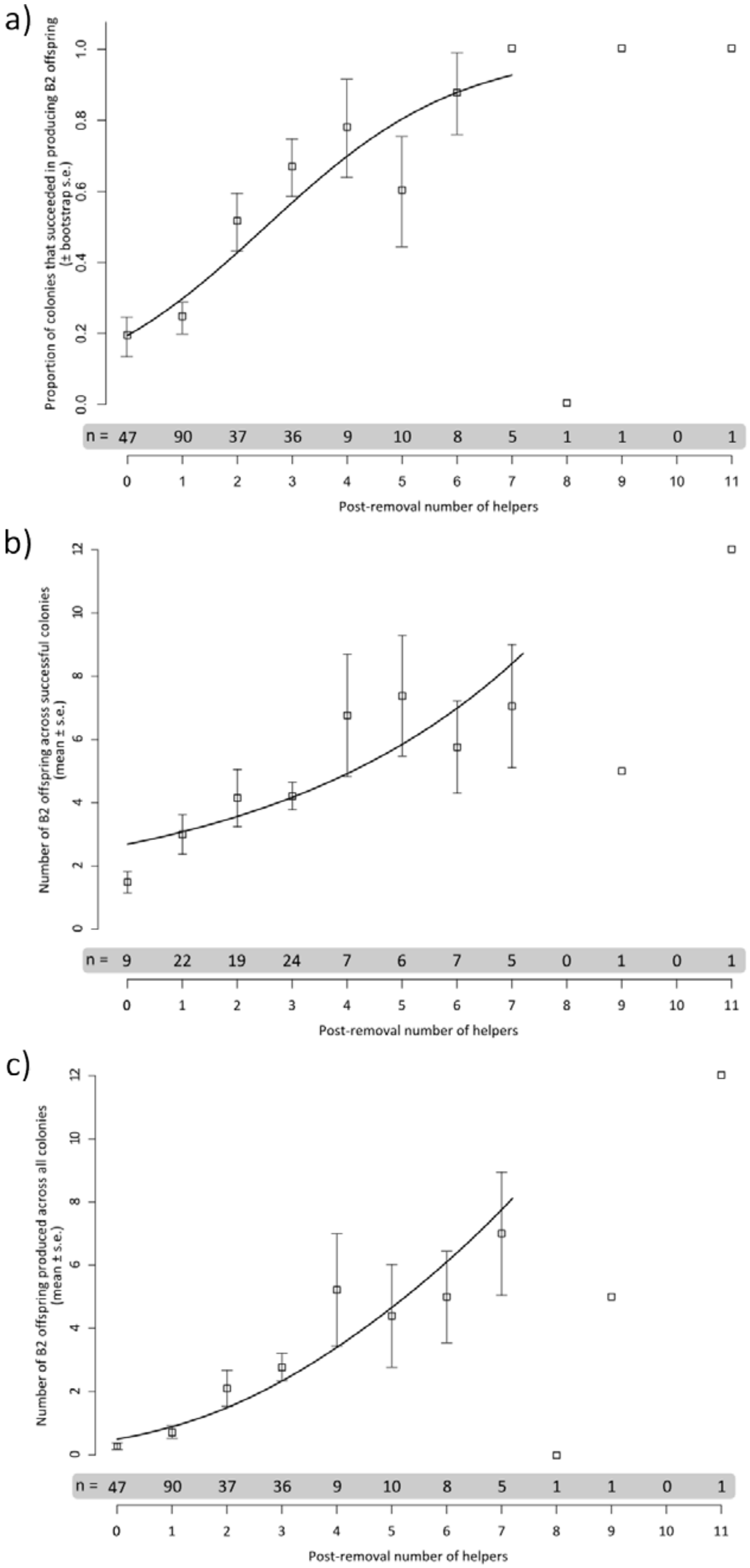

Figure 2. Influence of the number of B1 helpers remaining in control and removal colonies after the removal treatment on **a)** colony survival (= proportion of nests that succeeded in producing B2 offspring ±bootstrap *s.e.*), **b)** colony productivity (= number of gynes and males ±*s.e.*) of successful colonies, i.e. colonies producing B2 offspring and **c)** colony productivity across all colonies, including the ones that failed to produce any B2 offspring. The solid line fits predicted values from the model, for sample sizes of five or more. Sample sizes are indicated above the x-axis (*n*=number of colonies).

Effect of helper removal

Colonies from which we removed one B1 helper (removal treatment) produced 1.59±0.24 males and gynes in the second brood, on average (n=131). Across all colonies, including the ones that failed to produce any B2 offspring, the removal treatment had a significant impact on colony productivity (χ_1^2=4.22, P=0.04), as did the pre-removal number of helpers (χ_2^2=67.31, P<0.001), and there was a significant interaction between the two variables, indicating that the effect of removing one helper depended on the total number of helpers produced by the colony (χ_1^2=5.7, P=0.02; Figure 3). Indeed, the negative effect of helper removal on colony productivity decreased with the number of helpers (Figure 3).

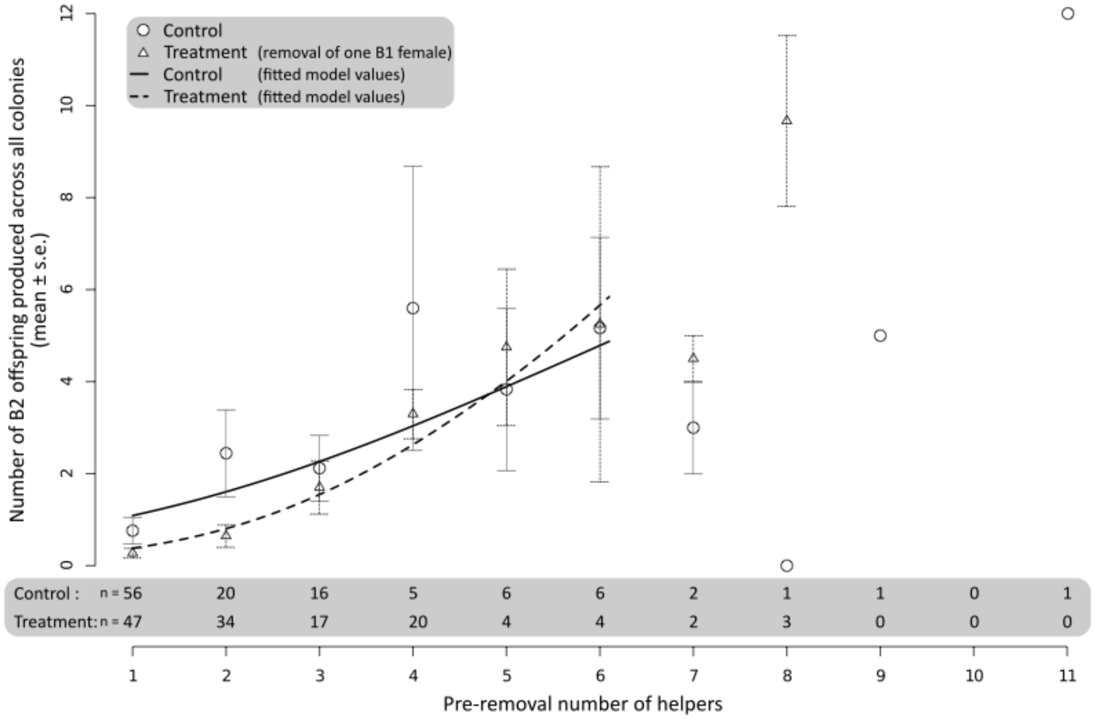

Figure 3. Effect of helper removal on colony productivity across all colonies, including the ones that failed to produce any B2 offspring, in function of the total number of B1 helpers produced by the colony (x-axis). The average (±*s.e.*) number of gynes and males produced are shown for control colonies (circles) and for colonies in which we had removed one B1 helper (triangles). The solid line and dashed line fit predicted values from the model for control colonies and helper-removal colonies, respectively, for sample sizes of four or more. Sample sizes are indicated above the x-axis (n=number of colonies).

We estimated the *per helper* productivity by comparing the productivity of control colonies to the one of colonies in which we removed one helper. In small colonies, with one to three helpers before removal, control colonies produced 0.72±0.33 additional B2 offspring (mean±bootstrap *s.e.*), as compared to removal colonies, a difference that was significant (*P*=0.02, permutation test with 2000 permutations). In large colonies, with four or more helpers, control colonies produced 0.41±1.19 additional offspring, as compared to removal colonies, a difference that was not significant due to large variance in productivity (*P*=0.37). Together, our removal experiment demonstrates that each individual helper contributes to increase colony productivity, but that this increase is very small and variable, particularly in large colonies.

Discussion

Halictid bees are excellent model systems to study the evolutionary transitions between solitary and social life, because their social organization and reproductive strategies vary widely (Chapuisat 2010; Field et al. 2010; Yagi and Hasegawa 2012). Females from the first brood generally have multiple options, allowing researchers to investigate the costs and benefits of helping versus reproducing (e.g. Richards et al. 2005; Yagi and Hasegawa 2012). Here, we estimated the impact of helpers on colony survival and productivity in an obligately but primitively eusocial sweat bee, *H. scabiosae*.

Colonies with more helpers had a higher probability of surviving from the first to the second brood, and produced more gynes and males. Overall, more than half of the colonies failed between the emergence of the first B1 helper and the production of the second brood, and the rate of colony failure dropped steadily as the number of helpers increased. The second helper appeared to have the strongest influence on colony success. High rates of colony failure are common in primitively eusocial species, in particular due to predation or usurpation, which are likely to be leading selective pressures for the evolution of sociality (Wenzel and Pickering 1991; Hogendoorn and Zammit 2001; Strohm and Bordon-Hauser 2003; Zammit et al. 2008).

In our removal experiment, we were interested in the actual contribution of one extra helper to colony survival and productivity, in relation to the number of helpers in the colony. We therefore removed one helper upon emergence in half of the colonies, before it provided any help. In contrast, other studies have looked at the effect of help already provided before helper removal. Colony members that die before the end of brood rearing have "assured fitness returns" if other individuals finish rearing their brood (Gadagkar 1990). Assured fitness returns have been documented in wasps and allodapine bees that are progressive provisioners (Field et al. 2000; Hogendoorn and Zammit 2001; Lucas and Field 2011), as well as in some mass provisioning halictid species (Kukuk et al. 1998; Smith et al. 2003).

The experimental removal of one helper upon emergence reduced colony productivity, but the effect depended on the number of helpers in the colony. The decrease in colony productivity was more pronounced and more consistent in small colonies than in large ones (Figure 3). When examining colony survival and productivity in function of the post-removal number of helpers, the second helper tended to have the largest influence, particularly for the survival of the colony, while the influence of the first helper was smaller (Figure 2a and pairwise tests). Overall, the impact of helpers peaked at the second helper and became less pronounced in larger colonies. The small mean helper number in *H. scabiosae* (2.45±0.12) is in line with the finding that supplementary helpers had insignificant additional effect on colony productivity in colonies with more than three helpers.

A very small contribution of helpers to colony productivity seems common in primitively social halictid bees. So far, the *per capita* productivity had been estimated by regressing colony productivity over the total number of foundresses and helpers present in the colony, using entire colonies sampled at the end of the season. Typically, the *per helper* productivity ranged between 0.5 and four in social halictids, usually based on successful colonies only (Michener 1964; Boomsma and Eickwort 1993; Richards et al. 2005; Yagi and Hasegawa 2012). However, in these correlational data, it is hard to disentangle the influence of helpers from that of other factors linked to colony characteristics or foundress quality. Moreover, the influence of helpers on colony survival is not taken into account when only successful colonies are analysed.

In our experimental study, the average contribution of one helper to the final productivity of colonies was less than one additional offspring, when measured across all colonies, including the ones that failed. Why do foundresses bother to produce a helper, if it contributes to less than one extra individual, on average? Part of the answer may lie in the fact that gynes are larger than helpers, and require larger pollen and nectar provisions. Foundresses may restrict the pollen and nectar provisions of their first-brood daughters in order to force them to help rearing larger B2 offspring, rather than reproducing independently (the parental manipulation hypothesis;

Alexander 1974; Kapheim et al. 2011; Brand and Chapuisat 2012). In *H. scabiosae*, the size difference between gynes and helpers amounts to 24% and 105% for dry weight and absolute fat weight measured by lipid extraction, respectively (Brand and Chapuisat 2012). Hence, the pollen and nectar provisions provided to the second brood are 45% heavier in terms of dry weight than those provided to the first brood (Brand and Chapuisat 2012). If we take this differential provisioning into account, the average *per capita* productivity of a helper becomes close to one in small colonies.

Overall, our data demonstrate that helpers in *H. scabiosae* do actually help and increase colony survival and productivity, particularly in small colonies. However, the average contribution per helper remained very small, and became statistically undetectable in large colonies. It thus appears that helpers are doing the best of a bad job. They may have no chance for independent breeding, either because they lack the energetic reserves required for founding nests independently, due to restricted pollen provisions provided by their mothers (Brand and Chapuisat 2012), or because of adverse ecological conditions (Yagi and Hasegawa 2012). Yet, putative helpers may attempt to reproduce in their natal nest or in other nests, and thus gain direct fitness (Lopez-Vaamonde et al. 2004; Ulrich et al. 2009). More generally, a small impact of helpers on colony productivity is consistent with the frequent bidirectional transitions between solitary and eusocial organization that have been documented in halictid bees (Eickwort et al. 1996; Hirata and Higashi 2008; Field et al. 2010; Gibbs et al. 2012).

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Chapter 3

Low relatedness and frequent drifting in a sweat bee

Nayuta Brand and Michel Chapuisat

This chapter is a manuscript being prepared for publication

Abstract

Primitively eusocial halictid bees are good systems for studying the evolution and maintenance of helping behaviour, because they form small societies of totipotent individuals that have multiple behavioural options (stay or leave, reproduce or help). Here, we investigate colony organization, inter-colony movements and patterns of reproduction in *Halictus scabiosae,* a species where foundresses rear a first brood of females that often behave as helpers to rear a second brood of gynes and males. Using non-destructive sampling and microsatellite genotyping, we monitored the genotypic composition of a large sample of colonies over the entire reproductive season, which allowed us to reconstruct sibships and infer parentage within and across colonies. We detected that foundresses and females from the first brood often move to foreign colonies. Moreover, foundresses are frequently replaced, and at least 5% of the females from the first brood reproduce, most often in foreign colonies. As a result, colonies often contain offspring from unrelated individuals, and the genetic relatedness between foundresses, first brood females, and gynes and males produced in the same colony is consistently low, with an average estimate of 0.18. High rates of colony failures may contribute to explain why females from the first brood generally behave as helpers.

Keywords: social evolution, helping behaviour, inclusive fitness, drifting, *Halictus scabiosae*, sweat bees

Introduction

How helping behaviour evolved and is maintained is still a major question in evolutionary biology. There is phylogenetic evidence that eusociality originally evolved in simple mother-offspring associations (Hughes et al. 2008), and ample evidence that kin selection is a key process explaining the evolution of workers' altruism (Abbot et al. 2011; Bourke 2011). However, some primitively eusocial insects have complex colony structures, with multiple breeders per nest (Richards et al. 1995; Reeve et al. 1998; Richards et al. 2003). Moreover, individuals may move between colonies and helpers are sometimes unrelated to the reproductive individuals, which is surprising, as they will gain no indirect fitness by helping (e.g. Queller et al. 2000; Leadbeater et al. 2010; Blacher et al. 2013). Importantly, unrelated helpers may also reproduce and/or inherit the nest, and helpers may maximize their inclusive fitness by combining some degree of helping with some amount of direct reproduction, depending on constraints and opportunities (e.g. Richards et al. 1995; Queller et al. 2000; Lopez-Vaamonde et al. 2004; Leadbeater et al. 2011; Hatchwell et al. 2013).

Primitively eusocial bee and wasp species are ideal model systems to study the evolution of helping and eusociality, because all females have retained a great flexibility in their behavioural and reproductive options (Schwarz et al. 2007). For instance, bee helpers may work to increase colony survival and productivity (Brand and Chapuisat 2014), obtain some reproductive share while the dominant breeder is still alive (Richards et al. 1995), wait to inherit the nest (Richards 2000; Hart and Monnin 2006; Schwarz et al. 2011), leave to found a nest independently (Yanega

1988; Rehan et al. 2013), or drift to foreign nests (Ulrich et al. 2009). Bidirectional transitions between solitary nesting (one female rearing her own brood) and eusociality appear frequent in halictid bees, both across and within species (Chapuisat 2010; Field et al. 2010). Eusociality was inferred to be the ancestral state for the two largest genera comprising solitary and eusocial species, *Halictus* and *Lasioglossum*, which suggests that extant solitary species in these genera result from reversals from eusociality to solitary nesting (Danforth 2002; Gibbs et al. 2012).

Whether an individual stays or leaves, and helps or reproduces, will depend on ecological and social factors that jointly influence the pay-offs of alternative behaviours (e.g. Keller and Chapuisat 1999). Helping will be favoured when ecological conditions make solitary nesting difficult or risky, for example because of high predation rate, high parasite pressure, harsh climatic conditions or limited nest sites (e.g. Lin 1964; Eickwort et al. 1996; Arnold and Owens 1999; Hatchwell et al. 2013). Helping should also be favoured when the relatedness of the helper to the brood is high, whereas direct reproduction becomes more attractive in conditions of low relatedness (e.g. Gadagkar et al. 1993; Paxton et al. 2002; Yagi and Hasegawa 2012). The payoff for staying may depend on colony size, because the effect of helpers often decreases with colony size (e.g. Brand and Chapuisat 2014), and helpers in small colonies have a higher chance to replace the dominant breeder (Field and Cant 2009). Finally, whether individuals help or reproduce will also depend on social interactions, and particularly on coercion or policing by reproducers or helpers (Michener and Brothers 1974; Ratnieks and Wenseleers 2008; Leadbeater et al. 2010). For example, in halictid bees the foundress may coerce her daughters into a helper role by limiting their food resources during development, which reduces their chances of independent reproduction (Alexander 1974; Charnov 1978; Brand and Chapuisat 2012; 2014).

Here, we investigate the social organization and behavioural options of females in the primitively eusocial bee *Halictus scabiosae*. This species exhibits interesting variation in social behaviour. Foundresses establish nests and rear a first brood composed mostly of females that often stay at the nest to help rearing a second brood of gynes and males (Brand and Chapuisat 2012; 2014). Due to their small size and low fat reserves, these first brood females are probably unable to found their own nest or to overwinter (Brand and Chapuisat 2012), but they may mate and reproduce in their natal nest or in foreign nests. Moreover, occasional cases of co-founding by multiple females, evictions of subordinates, nest usurpation and drifting of gynes late in the season have been documented (Knerer and Plateaux-Quénu 1967; Gogala 1991; Ulrich et al. 2009). We monitored a large number of nests over a full breeding season, using non-destructive sampling and microsatellite genotyping to document patterns of reproduction, relatedness and nest drifting in the foundresses, first and second brood cohorts. These data will reveal to which degree first brood females behave altruistically or get a share of reproduction, and will shed light on the alternative behaviours of females that are central to the evolution and maintenance of eusociality.

Materials and Methods

Life-cycle and study population of H. scabiosae

H. scabiosae is a primitively eusocial halictid bee forming annual colonies. In spring, overwintered females establish underground nests. They lay eggs in individual cells stocked with provisions of pollen and nectar (mass-provisioning, Brand and Chapuisat

2012). The foundresses rear a first brood (B1) that emerges from the nests in June and July (Ulrich et al. 2009; Brand and Chapuisat 2012; 2014). The first brood consists primarily of small-sized females, with only 5% of males (Brand and Chapuisat 2014). Females from the first brood tend to stay in their natal colony to help raise a second brood (B2) of gynes and males (Batra 1966; Ulrich et al. 2009; Brand and Chapuisat 2012; 2014). However, these females are able to mate and reproduce, and can do so in their natal or in foreign nests. Helpers excavate new cells, forage to provision the offspring and occasionally defend the nests, for instance against predators like ants, parasitic flies or intraspecific usurpers. The gynes and males from the second brood emerge from the nests in August and September. After mating, the gynes overwinter and become the next generation of foundresses in the following spring (Ulrich et al. 2009; Brand and Chapuisat 2012).

Our study site is a dry, south-exposed embankment located in Adlikon, near Zürich, in northern Switzerland (Brand and Chapuisat 2012; 2014). It contains a large population of *H. scabiosae*, with more than 1000 nests per breeding season. In spring 2009, we marked the nests with numbered nails and flags. We recorded the position of each nest using a global positioning system (GPS1200 Leica, horizontal and vertical accuracy of 10 and 20 mm, respectively). The average pairwise distance between the sampled nests was 16.39 ± 14.30 (SD) m.

Throughout the breeding season, we captured the bees by placing net traps on the colony entrance in the early morning (6-8 am), before the bees became active (8:30-10 am). We captured the foundresses between May 6th and June 4th, and marked them with individual colour codes, using quick-drying honeybee-marking paints

(Apicolori, Bienen-Meier Künten). We captured B1 offspring between June 25th and August 24th and B2 offspring between August 11th and September 8th. We marked each captured offspring with one dot of paint, to avoid double counting. Within each nest, the two broods are produced at different times, and we could easily distinguish B2 from B1 females based on previous marking, head width (Brand and Chapuisat 2012), wing wear and mandibular wear (Mueller and Wolfmueller 1993).

For DNA analysis, we sampled the tip (about two mm) of the tarsus from one hind-leg, and immediately stored it in 99% non-denatured EtOH. We then released each bee near its nest hole. This sampling procedure appeared to have minimal impact on the bees. First, a similar removal of the terminal part of one tarsus had no effect on the survival and foraging efficiency of bumblebees (Holehouse et al. 2003). Second, in a pilot study in 2008, we found that *H. scabiosae* colonies that had been sampled for DNA analysis as described above (n = 46) did not differ significantly from control, non-manipulated colonies (n = 153) in terms of colony productivity ($F_{1,199} = 0.018$, $P = 0.89$) and colony survival ($F_{1,199} = 0.025$, $P = 0.88$).

DNA extraction and microsatellite analysis

In total, we obtained the microsatellite genotype of 471 bees from 73 nests and additional 8 bees with no nest attribution. We extracted DNA by a standard proteinase K digestion followed by phenol-chloroform purification and ethanol precipitation (Hoy 2003). We rinsed each tarsus sample in ddH_2O, froze it in liquid nitrogen, and crushed it with a pestle. Each sample was digested overnight at 56 °C in 50 µl of buffer containing proteinase K (0.05 M Tris-HCl pH8, 1% SDS, 1 mM EDTA, DTT 8 mg/ml, 0.25 M NaCl, 0.4 mg/ml proteinase K). After phenol-chloroform purification and ethanol precipitation, we re-suspended the DNA in 50 µl ddH$_2$O. We amplified 11 microsatellite loci in three multiplex PCR reactions, using the protocol described by Ulrich et al. (2009) with minor modifications in the PCR cycle (15 min at 95°C, 35 x {30 s at 94°C, 90 s at Ta 58/60°C, 60 s at 72°C}, 30 min at 60°C), PCR mix (4 µl DNA template, 2.5 µl Qiagen Multiplex PCR Mastermix, 0.5 µl of multiplex primers, 1 µl ddH$_2$O), and thermocycler (T1, Biometra, Goettingen, Germany). We mixed amplification products of the second and third multiplex PCR in 1:1 proportion, and analysed them separately from products of the first multiplex PCR, on an ABI Prism 3100 Genetic Analyzer (see Ulrich et al. 2009). Alleles were scored with Genemapper® software v 4.0. The 11 microsatellites had from six to 25 alleles per locus, with an average of 13.7 alleles per locus and a mean expected heterozygosity of 0.69 (Table S1). Using the equations in Soro et al. (2009), the probability of non-detection of a second father among the offspring of a sibship (d_p , i.e. the probability that two males had the same genotype at all loci) was on average 5×10^{-7} and the probability of non-detection of an additional matriline among a set of putative daughters (d_m) ranged between 3×10^{-7} and 0.025

Two B1 males and five B2 males were diploid. These seven diploid males were included in the pedigree analyses because they give useful information on drifting, but were excluded from relatedness estimates.

Nestmate relatedness and pedigree relationships

We estimated genetic relatedness among nestmates (*r*) using the algorithm of Queller & Goodnight (1989), as implemented in the computer program Relatedness 5.0.8.

This coefficient is the "life-for-life relatedness", which takes into account the lower reproductive value of haploid males (Hamilton 1972). We obtained relatedness estimates for 66 nests from which we had sampled multiple nestmates (428 females and 25 males in total). Allele frequencies in the background population were estimated weighing nests equally. Standard errors and 95% confidence intervals were obtained by jackknifing across loci (Queller and Goodnight 1989).

To get insight into which female reproduced and to detect cases where bees had drifted between nests, we inferred close pedigree relationships among all sampled bees, independently of nest membership. For this aim, we used the maximum likelihood approach implemented in the computer program COLONY 2.0, which sorts individuals belonging to an "offspring sample" into most probable sibships (= full-sib groups), and infers parentage to potential parents (Jones and Wang 2010). We analysed the 428 females, 25 males and 7 diploid males from 66 nests used in the relatedness analysis, plus 11 females sampled from 7 additional nests and 8 females with no nest attribution. We included all genotypes of B1 and B2 females in the "offspring sample" (n = 196 and 206 for B1 and B2 females, respectively), and all genotypes of foundresses (n = 52) and B1 females (n = 196) in the sample of candidate mothers. After each run, we inspected the inferred pedigree relationships, marked stable sibships as “known sibs”, and repeated the analysis until we obtained fully consistent and stable sibships and mother-offspring relationships. After the female sibships had been established, we added the haploid genotypes of B1 and B2 males (n = 7 and 18, respectively) to the offspring sample, and re-analysed the dataset with COLONY 1.2, as this earlier version of the program handles haploid offspring genotypes. Except for the relatedness and pedigree analyses described above, all statistical analyses were carried out in R 2.10.1 (R Development Core Team 2011).

Results

Productivity and relatedness among nestmates

Out of approximately 1000 nests founded in spring, about 600 produced offspring in the first brood (B1) and 200 produced gynes and males in the second brood (B2). The productivity per successful colony was low, amounting to only 3.26 ± 4.07 B2 females and 0.42 ± 0.91 B2 males, on average. These are minimal estimates, as some bees might have eluded capture, particularly males that tend to leave the nest once and rarely return to it (Sakagami and Fukuda 1972).

The relatedness estimates among nestmates were generally low (Table 1). These low values indicate that multiple matrilines coexist in the same nest, which may be due to polygyny, foundress replacement, egg dumping, or drifting. Co-foundresses sampled from the same nest in spring were unrelated, as shown by their relatedness estimate very close to zero, with a small confidence interval overlapping with zero (Table 1). The foundresses that we captured in spring were only moderately related to the B1 females and B1 males later produced in the same nests, and even less related to the B2 gynes and males emerging in autumn (Table 1). This indicates that foundresses move among nests and/or are frequently replaced.

Table 1. Average relatedness among nestmates, ± standard errors, with 95% confidence intervals in square brackets. *N* indicates the number of nests.

Actor	Recipient				
	Foundresses	B1 females	B1 males	B2 females	B2 males
Foundresses	-0.02 ± 0.06 [-0.08 ; 0.04] $N = 10$	0.17 ± 0.03 [0.10; 0.24] $N = 29$	0.26 ± 0.05 [0.15; 0.37] $N = 6$	0.12 ± 0.04 [0.02; 0.22] $N = 17$	0.16 ± 0.06 [0.04; 0.28] $N = 6$
B1 females	-	0.41 ± 0.02 [0.37; 0.45] $N = 47$	0.17 ± 0.06 [0.03; 0.31] $N = 5$	0.21 ± 0.03 [0.14; 0.28] $N = 40$	0.24 ± 0.05 [0.14; 0.34] $N = 12$
B2 females	-	-	-	0.43 ± 0.03 [0.37; 0.49] $N = 35$	0.22 ± 0.02 [0.17; 0.27] $N = 12$

The relatedness among nestmate females from the same brood was 0.41 and 0.43 for B1 females and B2 gynes, respectively (Table 1). These values are significantly lower than the relatedness expected for full sisters (0.75), but are still quite high, indicating that few females successfully reproduce in each nest. Interestingly, the relatedness between B1 females (the potential helpers) and B2 gynes from the same nest was moderate (0.21) and significantly lower than the relatedness among nestmate females belonging to the same brood (Table 1). The fact that the between-brood relatedness is lower than the within-brood relatedness indicates that B1 and B2 females from the same nests are frequently produced by different mothers. In contrast, the average relatedness of females to nestmate males was relatively high, with values not significantly different from the one expected from sisters to brothers (0.25), or from an equal mix of unrelated males (0) and sons (0.5).

Pedigree relationships and partitioning of reproduction

By sorting the bee genotypes with the computer program COLONY, we obtained 134 full-sib groups (=sibships). In one nest, two sibships were likely maternal half-sibs of a doubly mated foundress. We did not identify any other clear case of multiple mating, which indicates that females generally mate once.

Each of the 134 sibships consisted of one to 20 females and males from the B1 and B2 cohorts (mean ± SD: 3.2 ± 3.6). For 36 of these sibships, an inferred maternal genotype matched the one of a sampled foundress (26 cases) or B1 female (10 cases), respectively. Hence, about half of the foundresses and 5.1 % of the B1 females had offspring within our sample of B1 and B2 individuals. The foundresses had on average 2.35 ± 1.26 B1 and 2.04 ± 4.05 B2 offspring, whereas reproducing B1 females had on average 2.40 ± 2.50 B2 offspring. These are minimal estimates, as part of the offspring might have remained undetected.

We further investigated the partitioning of reproduction in the 66 nests from which we had sampled multiple nestmates. In line with the low relatedness estimates, 70% of the nests (46 out of 66) contained more than one sibship, indicating polygyny, foundress replacement, egg-dumping, or drifting. For example, in one of these nests the foundress produced two B1 females and one B2 gyne, and one of these B1 females produced two B2 gynes and a male. The remaining 20 nests (30%) contained a single sibship, consistent with monogyny. On average, in each nest we detected 1.74 ± 1.03 and 1.86 ± 1.01 sibships in the first and second brood, respectively, and 2.58 ± 1.54 sibships when considering both broods together. The rate of failure (no B2 production) was significantly higher for monogynous than polygynous nests ($\chi^2 = 13.47$, $P < 0.001$).

Drifting

We detected that bees frequently moved between nests. Many of the foundresses had offspring in other nests than the ones in which we captured them for the first time. Such cases of nest switching occurred for 12 (46%) of the 26 foundresses that matched a sibship in our sample of B1 and B2 individuals. The situations were diverse, as either the B1, B2 or both broods were distributed in foreign nests (Table S2). Similarly, eight of the ten B1 females that reproduced (i.e. were mothers of some of the B2 individuals we sampled) had offspring in another nest than the one in which they were first captured. The average distance between the nest of origin and the nest in which they had offspring was 9.7 ± 10.6 m and 10.6 ± 8.6 m for foundresses and B1 females, respectively.

Further evidence of inter-nest movements is provided by the fact that sibships were often distributed in multiple nests (Figure S2, Table S3). Specifically, 29 sibships were sampled in more than one nest, representing 36.3 % of all sibships with more than one individual. Across the entire sample, sibships occupied 1.30 ± 0.64 nests on average (range: 1-4 nests). The mean distance between nests containing split brood was 12.7 ± 15.3 m. As many as 44 out of the 66 genotyped nests contained part of a split brood, and 17 nests contained more than one and up to five split broods.

Discussion

We used non-destructive sampling coupled with microsatellite analysis to monitor the genotypic composition of colonies of the halictid bee *H. scabiosae* over the entire reproductive season. We found that colonies were extremely labile and that bees frequently moved between nests. First, foundresses commonly switched nests during the solitary nest founding phase, as shown by the fact that 46% of the foundresses had offspring in other nests than the ones in which we captured them for the first time. Second, and most importantly, as many as 70% of the nests contained multiple sibships, and more than a third of all sibships with more than one individual were distributed in multiple nests. Such full-sib groups distributed in multiple nests attest drifting – either of the mother or of the offspring (e.g. Packer 1986; Ulrich et al. 2009; Peso and Richards 2011).

Why do bees frequently drift to other nests? Nest failure during the founding phase was frequent in *H. scabiosae*, with an estimate of 40% in our study population, and of 93 % in another population (Ulrich et al. 2009). Foundresses from failed or usurped nests, as well as evicted co-foundresses, may drift to other nests (Knerer and Plateaux-Quénu 1967; Gogala 1991; Nonacs and Reeve 1993; Zobel and Paxton 2007). This may explain the very dynamic colony composition and frequent drifting early in the season.

Later in the season, egg laying in foreign nests, or permanently drifting to foreign nests, may serve to reduce competition among related females (Ulrich et al. 2009). We found 10 cases where each of multiple nests contained two or more members of the same sibship (Table S3). In such cases, it seems likely that the mother has drifted between nests, either temporarily or permanently. This is because joint drifting of multiple full-sibs to the same nest appears unlikely, given the high number of nests in the population and large mean distance between nests containing split broods. When a single member of a sibship is found in a foreign nest, mother drift, egg dumping (e.g.

Packer 1986) or offspring drift (Ulrich et al. 2009) are possible. We found 24 such cases (Table S3).

The labile colony structure, with occasional polygyny, foundress replacement and egg dumping, coupled with extensive drifting of the original foundresses, B1 and B2 females, resulted in low degrees of relatedness among nestmates. In particular, the relatedness of foundresses to B2 gynes and males was only 0.14, on average. Hence, foundresses are far from monopolizing reproduction in the original nest where they were first captured, and they frequently move to other nests and/or are replaced by other foundresses. The relatedness of B1 females to B2 gynes and males was also moderate, with an estimate of 0.225 on average, which further indicates that unrelated bees often drift to foreign nests. Such low values of relatedness between nestmates and the brood they rear are surprising in primitively eusocial insects, because simple family structure and high relatedness play a central role in facilitating the evolution of eusociality (Hughes et al. 2008; Boomsma 2009). Low intra-colony relatedness should typically be associated with lower incentive for helping and higher competition to get a share of reproduction (Ratnieks et al. 2006).

The presence of multiple egg-layers or multiple sibships in the same nest may also provide some colony-level benefits. In this study population of *H. scabiosae*, monogynous colonies had a higher failure rate than polygynous colonies. This is a common pattern in species with facultative polygyny, which might be due to a better division of labour in polygynous colonies, or a better ability to cope with predation, nest usurpation, or parasitism (Tibbetts and Reeve 2003; Smith et al. 2007; Rehan et al. 2011; Yagi and Hasegawa 2012). Hence, the higher success of polygynous colonies may contribute to explain why multiple females often reproduce in the same nest.

The majority of the females from the first brood behaved as helpers and did not reproduce. However, our pedigree reconstruction indicates that at least 5% of the B1 females had B2 offspring, and that at least 10% of the B2 gynes and males were the offspring of B1 females. Most reproducing B1 females did not have offspring in their natal colony, but in foreign nests. By drifting, B1 females may avoid being coerced by the dominant foundress (Michener and Brothers 1974; Hogendoorn and Schwarz 1998), or may decrease local competition with relatives (Ulrich et al. 2009). In other social insect species, workers that drift to foreign colonies often show higher rates of reproduction than workers staying in their natal colony (Paxton et al. 2002; Lopez-Vaamonde et al. 2004; Yagi and Hasegawa 2012).

To sum up, *H. scabiosae* forms very labile colonies, with high failure rates, extensive bee movements between colonies, and a combination of helping and reproduction. The colonies have low productivity, and due to frequent drifting to foreign nests, the foundresses and helpers are only moderately related to the brood they rear. The majority of the B1 females behave as helpers, which may be in part due to high rates of colony failure and constrains associated with their small body size (Brand and Chapuisat 2012). However, a small proportion of B1 females reproduce, occasionally in their own nest, but most often in foreign nests. Overall, the low degree of relatedness in colonies of *H. scabiosae* is surprising, because eusociality typically evolved in simple, monogamous families (Boomsma 2009). The labile structure, low relatedness and low productivity in colonies of *H. scabiosae* may be indicative of an unstable system that could revert to solitary nesting.

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Data Accessibility

The sampling data and microsatellite genotypes will be deposited on Dryad.

Author Contributions

N.B. performed the field work, molecular work and data analysis. N.B. and M.C designed the study and wrote the paper.

Supporting information

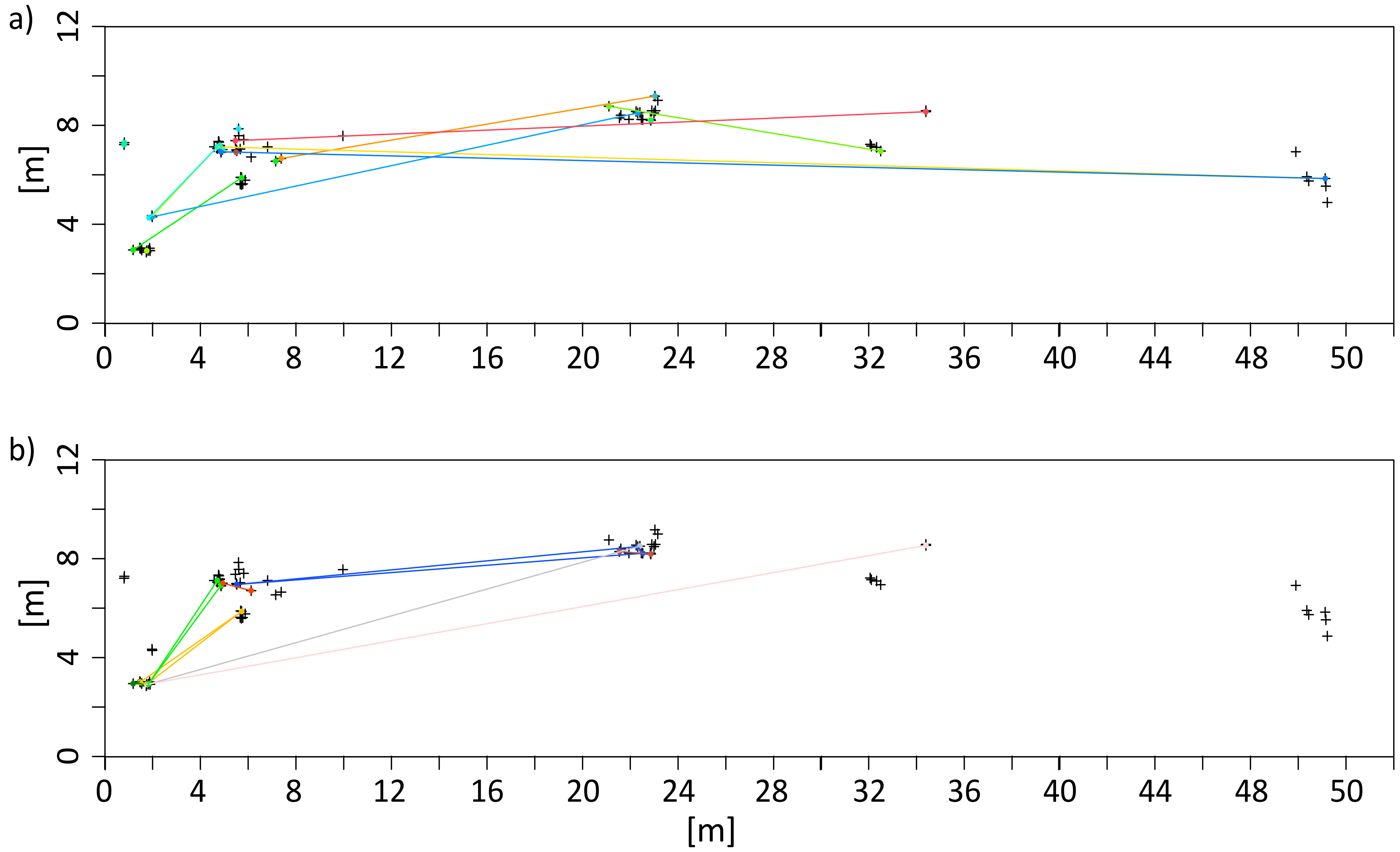

Fig. S1. Maps of nests sharing sibships (= full-sib groups). The scale on axes indicates the geographic distance in meters. The position of each genotyped nest is marked with a cross. Lines of the same colour connect nests that share individuals of the same full-sib group. To minimize overlap, sibships occupying two nests are shown on map a), while sibships occupying three or four nests are shown on map b)

Table S1. Summary statistics for the 11 microsatellite loci: number of alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e)

Locus	nb of alleles	H_o	H_e
LHMS10	6	0.635	0.681
rub02	9	0.549	0.629
rub06	19	0.702	0.814
rub35	24	0.812	0.896
rub37b	8	0.556	0.712
rub55	25	0.729	0.861
rub59	10	0.712	0.718
rub60	17	0.83	0.828
rub72	9	0.625	0.745
rub73	8	0.411	0.418
rub80	16	0.256	0.311
Overall	13.73	0.620	0.692

Table S2. Distribution of offspring for the 12 foundresses that had offspring in other nests (= foreign) than the one in which they were first captured (original nest).

Pattern description	Number of cases
Produced only B1 offspring, in foreign nest	5
Produced only B1 offspring, in original and foreign nest	1
Produced B1 and B2 offspring, B1 in foreign nest, B2 in original nest	1
Produced B1 and B2 offspring, B1 split between original and foreign nest, B2 in original nest	1
Produced B1 and B2 offspring, B1 in original nest, B2 in foreign nest	1
Produced B1 and B2 offspring, both broods in foreign nest	1
Produced B1 and B2 offspring, both broods split between original and foreign nest	2

Table S3. Distribution of sibships in multiple nests. The ID of nests occupied by each sibship, the number of B1 and B2 offspring present in each nest, and the geographic distance between occupied nests are indicated. The first ten rows show cases where multiple members of the same sibship are present in each of multiple nests.

Sibship ID	Mother generation	Nest ID				Offspring numbers								Distance between Nests [m]					
		Nest 1	Nest 2	Nest 3	Nest 4	Nest1		Nest 2		Nest 3		Nest 4		1-2	1-3	2-3	1-4	2-4	3-4
						B1	B2	B1	B2	B1	B2	B1	B2						
110	B1	116	409	-	-	-	2	-	7	-	-	-	-	0.24	-	-	-	-	-
020	Foundress	416	457	-	-	3	1	0	6	-	-	-	-	4.25	-	-	-	-	-
024	Foundress	472	983	-	-	1	1	0	5	-	-	-	-	0.08	-	-	-	-	-
102	Foundress	844	124	-	-	0	2	3	0	-	-	-	-	0.09	-	-	-	-	-
071	Unknown	571	688	-	-	0	2	0	2	-	-	-	-	17.39	-	-	-	-	-
116	Foundress	416	660	571	-	1	0	0	2	3	11	-	-	17.30	16.14	1.17	-	-	-
115	Foundress	195	983	534	-	1	0	2	0	0	13	-	-	5.35	0.30	5.48	-	-	-
119	Foundress	646	688	660	-	1	0	1	1	2	3	-	-	1.34	1.28	0.15	-	-	-
003	Foundress	472	983	470	176	1	0	0	2	0	3	4	0	0.08	0.08	0.05	33.90	33.98	33.90
022	Foundress	431	472	457	416	1	0	1	0	0	5	0	10	5.51	1.56	4.19	0.26	5.61	4.25
004	B1	125	416	-	-	-	2	-	1	-	-	-	-	4.38	-	-	-	-	-
008	Foundress	698	151	-	-	1	0	1	3	-	-	-	-	16.27	-	-	-	-	-
107	Foundress	287	284	-	-	1	0	3	0	-	-	-	-	0.32	-	-	-	-	-
110	Foundress	116	409	-	-	1	0	1	0	-	-	-	-	0.24	-	-	-	-	-
119	Foundress	698	166	-	-	5	12	1	0	-	-	-	-	0.21	-	-	-	-	-
004	Foundress	434	005	-	-	1	0	2	0	-	-	-	-	45.57	-	-	-	-	-
032	Foundress	631	035	-	-	1	0	5	2	-	-	-	-	11.58	-	-	-	-	-
118	Foundress	300	575	-	-	3	0	0	1	-	-	-	-	17.17	-	-	-	-	-
010	Foundress	005	408	-	-	1	0	1	0	-	-	-	-	46.25	-	-	-	-	-
079	Foundress	176	908	-	-	1	0	0	1	-	-	-	-	29.39	-	-	-	-	-
082	Foundress	431	210	-	-	0	1	0	1	-	-	-	-	46.91	-	-	-	-	-
116	Foundress	162	587	-	-	1	0	1	0	-	-	-	-	15.60	-	-	-	-	-
013	Foundress	210	416	-	-	1	0	1	0	-	-	-	-	47.05	-	-	-	-	-
034	Unknown	533	195	-	-	-	1	-	6	-	-	-	-	5.74	-	-	-	-	-
081	Unknown	687	457	-	-	-	1	-	1	-	-	-	-	21.39	-	-	-	-	-
016	Foundress	386	519	116	-	1	0	1	0	0	4	-	-	0.68	1.24	0.58	-	-	-
062	Foundress	131	533	851	-	1	0	1	0	0	1	-	-	0.36	0.30	0.11	-	-	-
122	Foundress	472	832	981	-	1	0	1	0	1	0	-	-	22.14	22.10	0.11	-	-	-
029	Foundress	670	687	967	-	1	0	1	0	1	0	-	-	0.36	17.23	17.40	-	-	-

General discussion

General discussion

Halictus scabiosae is an interesting model system for investigating the flexibility and determinants of individual reproductive strategies in primitively eusocial systems, and thus gain insights into the evolution and maintenance of eusociality. In this thesis, I investigated the role of helpers in *H. scabiosae,* with a focus on their direct and indirect fitness benefits. I first compared the adult morphology of first and second brood females and the nutrition they receive as larvae (Chapter 1). Then I performed a helper-removal experiment to measure the quantitative effect of one additional helper on colony survival and productivity (Chapter 2). Finally, I used microsatellite genotyping to detect reproduction by helpers or drift of bees between colonies (Chapter 3).

Integrating the results of the three chapters, it appears that first brood females in *H. scabiosae* do not have the same reproductive options as the second brood gynes. This is likely to be caused by the manipulation of nutrition of the first brood larvae by the colony foundress (Chapter 1). First brood females help in the colony or, rarely, replace the foundress as the main egg layer. But they may also drift to other nests and at least 5% of them produce some offspring (Chapter 3).

Since the first brood also contains males (5%, Chapter 2), many of the first brood females that lay eggs are mated and produce female offspring (Chapter 3). One could wonder whether the early production of males that predominantly mate with first brood females, who will mostly stay helpers, is advantageous for the foundresses. This might be a bet-hedging strategy of the foundress, because helpers – especially the first two – are essential for colony success (Chapter 2), but the high frequency of colony failure and foundress replacements lower the probability that the original foundress will produce a second brood of males and gynes in her own nest (Chapter 3). For this reason, the foundress might profit from also investing in males that will mate with first brood females from her own or other colonies. In case she dies or the nest gets usurped by a foreigner, she has at least some probability that a mated first brood female will reproduce directly and transmit her genes to the next generation. It is also possible that mating triggers ovary development (Plateaux-Quénu and Packer 1998).

One may also wonder what prevents the foundress from reverting to a solitary, bivoltine life cycle, and produce gynes and males in the first brood. This might be linked to the competition and instability of nests in spring. Indeed, foundresses may have to minimise the foraging time for preventing nest usurpation by a foreign foundress, and hence might be constrained in their time to produce competitive daughters (Chapters 1, 2, 3). This problem could be alleviated by pleometrotic nest founding, yet in our population the nests that contain multiple foundresses are also eusocial. This supports the view that the small size of first brood daughters result from foundress manipulation (Chapter 1) and that ecological constraints to independent nesting in mid-summer (e.g. the dryness and the heat at the earth surface) promote the production of helpers in the first brood (Chapter 3).

To conclude, the production of helpers and thus an eusocial colony organization is important for colony success in this species, but colony failure rate is high, and bees often drift to other nests. Both foundresses and helpers appear to be constrained by harsh environmental conditions and social factors limiting reproduction and independent colony founding. These constraints may impede *H. scabiosae* from reverting to solitary behaviour, because of the extremely low survival rate of foundresses without helpers (Chapter 2), on the other hand also hinder the species from evolving to advanced eusociality with exclusively altruistic helpers, because the contribution by one helper is also low. It would be interesting to compare colony characteristics and drifting frequency across the distribution range of this species, and to perform common garden experiments for investigating the flexibility of these characteristics.

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