Social Polymorphism and Social Behaviour in Sweat bees (Hymenoptera: Halictidae)

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SUMMARY

I use field observations, experiments and molecular techniques to describe the social biology of the sweat bee *Lasioglossum calceatum*, and to investigate the mechanisms underlying social polymorphism and body size in this species. I also investigate environmental constraints on sociality, and the impact that workers have on productivity, in the obligate social species *L. malachurum*.

Chapter 1 introduces sweat bees as a study system, and reviews social behaviour within the group. I then provide concise introductions to the study species.

In Chapter 2 I show that *L. calceatum* exhibits latitudinal social polymorphism in the UK, with only bees in the south expressing primitive eusociality. I then describe the social life cycle from continuous field observations, with reference to genetic data.

In Chapter 3 in I examine environmental and genetic components of social phenotype in *L. calceatum* by conducting a field transplant of bees from the north of the UK to the south. Social phenotype is likely to be predominantly determined by fixed genetic differences between social and solitary populations.

Chapter 4 examines whether the transition between social and solitary nesting results in saw-tooth size clines in *L. calceatum* and *Halictus rubicundus*. Overall, both species exhibit converse-Bergmann clines but not saw-tooth clines.

In Chapter 5 I transplant the obligate social sweat bee *L. malachurum* to the north of the UK, to test whether sociality is constrained by season length. Phenology was considerably delayed such that the life cycle could not be completed.

In Chapter 6 I investigate queen quality, productivity and costs of worker production in *L. malachurum*, by manipulating the number of workers per nest. I show that queens probably incurs costs from producing more workers, and that a possible mechanism is that workers from larger groups may be of lower quality.

In Chapter 7 I bring together key findings of the thesis, and comment on future directions.

Declaration

I hereby declare that this thesis has not been and will not be submitted in whole or in part to another University for the award of any other degree.

Signature:

Paul James Davison

Acknowledgements

Finally I would like to thank my parents for their constant and loving support, I could not have completed this thesis without them.

Below I briefly outline key practical contributions made to the outcome of each chapter, by people to be acknowledged in papers arising from each chapter. All papers arising from chapters 2-6 will be authored by Davison and Field. I conducted all statistical analyses and wrote all chapters. Percentage practical fieldwork undertaken personally is given in brackets.

Chapter 1. Thomas Wood kindly provided a photograph.

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To Dick Loxton

Music is on a higher plane than everything else in this world.

Jean Sibelius

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Chapter one

Introduction

Advanced eusocial insects such as honeybees, ants and termites have evolved extreme forms of altruism (Wilson, 1971). The origin of eusociality from solitary ancestors is considered to be a major evolutionary transition (Maynard Smith and Szathmáry, 1995; Bourke, 2011a), and as such has been a subject of longstanding interest in biology (Dugatkin, 2006). The transition from solitary living to eusociality requires that some altruistic offspring become dedicated workers and forgo personal reproduction (Box 1; Wilson, 1971). This apparent evolutionary paradox has been successfully explained by inclusive fitness theory: given appropriate ecological conditions, altruistic individuals gain fitness by reproduce collaterally via helping to rear relatives with whom they share genes (Hamilton, 1964; Bourke, 2011b). However, until more recently, the underlying mechanisms driving the transition to eusociality have received comparatively less attention (Kapheim et al. 2012; Field et al., 2012). The highly complex forms of eusociality in well-studied groups such as honeybees, ants and termites are ancient in origin (Cardinal and Danforth, 2011). Although such groups can tell us much about the maintenance and elaboration of eusociality, its early stages are lost in the distant past (Bourke and Franks, 1995). Therefore, to understand how eusociality first evolved from solitary ancestors, it is necessary to study taxa in which it is possible to observe the mechanisms driving this transition.

The globally distributed sweat bees (Hymenoptera: Halictidae) provide an unparalleled opportunity to investigate the transition from solitary nesting to eusociality. The family Halictidae comprises behaviourally eclectic lineages ranging from solitary or communal nesting to primitive eusociality, with myriad intermediate facultative states (Box 1.1; Wcislo, 1997; Schwarz et al., 2007). Within sweat bees primitive eusociality has been repeatedly lost such that

behaviour is independent of phylogeny, and the 830[1] or so primitively eusocial species are widely distributed among solitary lineages (Danforth, 2002; Danforth et al., 2003; Gibbs et al., 2012a). With more than 4300 species Halictidae is the second most speciose bee family after Apidae (Danforth et al., 2013), and excluding honeybees, sweat bees are often the most frequently encountered bees in temperate zones (Michener, 2007). Taken together these properties make sweat bees accessible and uniquely suitable for investigating the origins of eusociality. I now provide an overview of sweat bee biology, evolution and social behaviour.

1.2 The classification of sweat bees

Sweat bees are distantly related to the more familiar corbiculate honeybees and bumblebees (family Apidae) (Fig. 1.2a; Danforth et al., 2013). Halictidae comprises four subfamilies, although primitive eusociality is currently restricted to the largest, Halictinae (Fig. 1.2b; see Appendix D for a brief review of the other halictid sub-families). Halictidae is composed of five tribes: Augochlorini, Caenohalictini, Thrinchostomini, Sphecodini and Halictini (Fig. 1.2c; Danforth et al., 2013). Eusociality is known from only Augochlorini and Halictini, with solitary

Figure 1.1
Lasioglossum calceatum: A typical sweat bee female resting on a
daisy. The thorax has been marked white for identification. Photo by
Louise Hislop.

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[1] A definite underestimate, eusociality is being described from a continually growing list of species
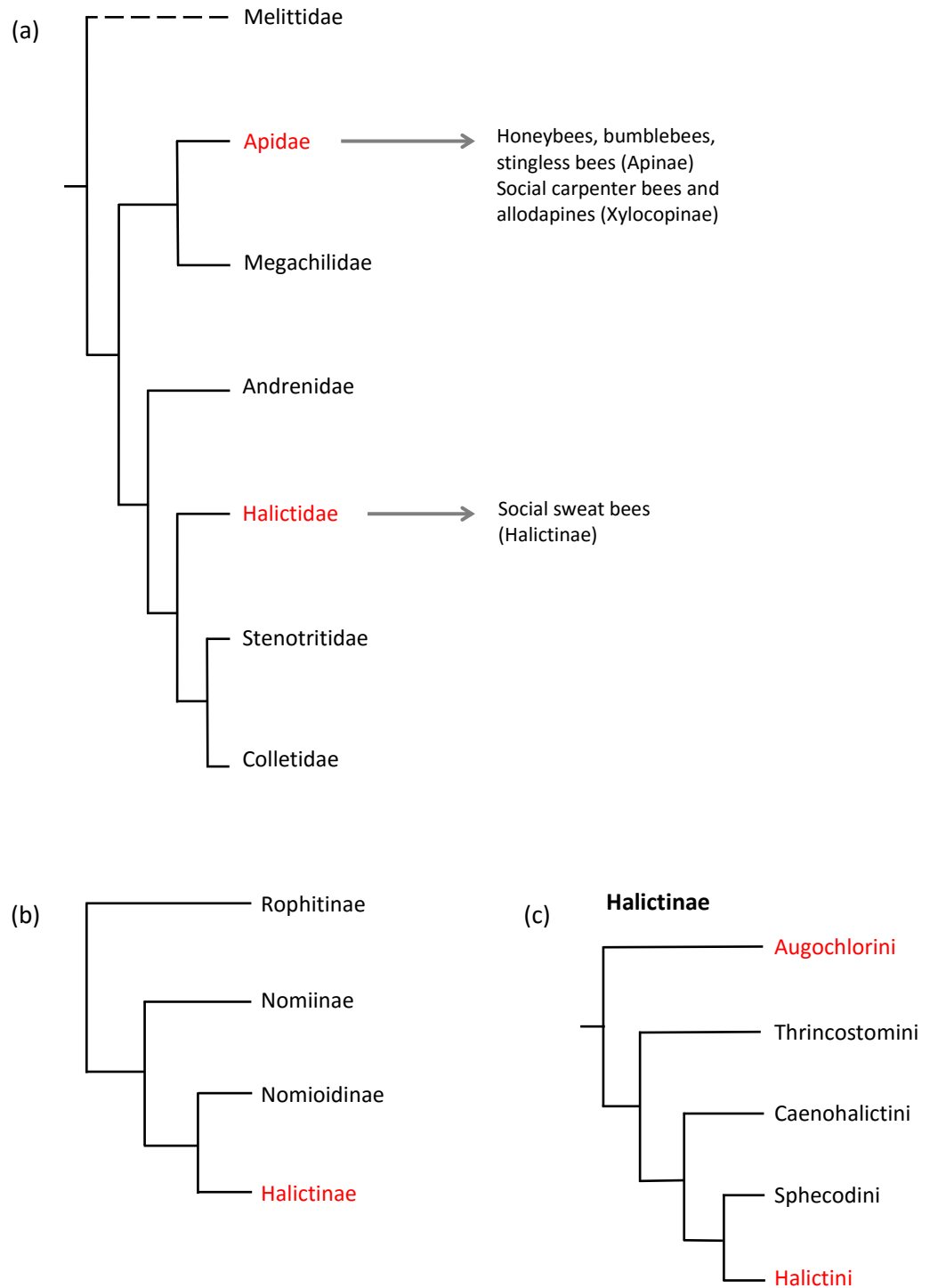
Figure 1.2

(a) Phylogeny showing the family-level classification of bees, and distribution of social behaviour among families (modified from Danforth et al., 2013). The two families highlighted in red contain lineages in which bees nest socially, with details given to the right of the arrow. Sweat bees form the family Halictidae.

(b) Phylogeny of Halictidae showing the placement of Halictinae within Halictidae (modified from Danforth et al., 2008). Only Halictinae (highlighted red) contains social lineages.

(c) Phylogeny showing the placement of Halictini within Halictinae (modified from Danforth et al., 2013). Social lineages occur within Augochlorini and Halictini (highlighted red; see text for details)

Glossary (following Wilson, 1971)

Social- any form of social behaviour listed below, excluding communality.

Eusocial- condition where reproductive division of labour, cooperative care for young and overlapping generations are present simultaneously within the same nest.

Primitively eusocial- the conditions for eusociality are met but there is no morphological caste differentiation, and caste switching may be possible.

Advanced eusocial- the conditions for eusociality are met, and there is morphological caste differentiation.

Semisocial- reproductive division of labour and cooperative care for the young are present, but all adult individuals are of the same generation.

Communal- a single nest comprises multiple related or unrelated independently reproducing females.

Box 1.1 Glossary of terms.

and communal behaviour together with forms of parasitism found in the remaining tribes (Danforth et al., 2008). At nearly 2200 described species Halictini is the most speciose, behaviourally diverse and geographically widespread tribe (Gibbs et al., 2012a). Within Halictini, however, most of the behavioural diversity is found within the large genus *Lasioglossum* (Michener, 2007; Danforth et al., 2008). The species within the tribe Halictini will be the primary focus of the introduction.

1.3 Life cycle

1.3.1 Nests

Sweat bee life cycles can be loosely categorised as either solitary, semisocial, communal or primitively eusocial (see Box 1.1). Nearly all species construct subterranean nests in soil, occasionally in dense aggregations, but some Augochlorini and a few *Lasioglossum* nest in rotting wood or vines (Sakagami & Michener, 1962; Danforth and Eickwort, 1997). Nest architecture is highly variable but typically comprises a vertical burrow with separate brood cells arranged either in a cluster, connected to the main burrow by a short lateral offshoot, or individually with each connected to the main burrow separately (Fig. 1.7; see Sakagami and Michener, 1962 for a review).

1.3.2 Solitary life cycle

The life cycle of a typical solitary, univoltine temperate-zone species is as follows (Fig. 1.3 with red arrow (b)). Mated females (foundresses) emerge from hibernation in spring and excavate a nest in the soil. Females typically found nests solitarily, although co-founding by two or more females can occur to varying degrees in different species (Packer, 1993). All sweat bees are mass provisioners (Michener, 2007), and nest foundresses forage to separately provision each brood cell with a ball of pollen and nectar, on which they lay a single egg before closing the cell. Hatched larvae then consume the pollen ball entirely before pupating into adults. Unlike

many other bees, sweat bees complete development and emerge in the year they are born[2] (Michener, 2007). Newly eclosed adults mate soon after emergence and females enter hibernation to found their own nest in the following year. Males die before winter and do not survive to the following year. Females frequently overwinter beneath their natal nest (e.g. Bonelli, 1968), and deep enough to avoid freezing (Sakagami et al., 1984). Where the season is sufficiently long offspring may emerge and directly found new nests instead of entering hibernation, and two or more solitary generations may occur (Plateaux-Quénu et al., 1989).

1.3.3 Primitively eusocial life cycle

Nests become primitively eusocial when at least some first generation females (B1) that remain and help their foundress mother, now queen, rear a successive brood of offspring (B2) as workers (Figs. 1.3, 1.4). Primitively eusocial sweat bees almost always exhibit caste-size dimorphism (Schwarz et al., 2007), where workers are smaller than nest foundresses. Caste-size dimorphism can range from only 2-3% up to around 20% in the most socially specialised species (Sakagami and Munakata, 1972; Packer and Knerer, 1985; Wyman and Richards, 2003). Once workers emerge, queens remain in the nest and workers forage for pollen and nectar to provision reproductive brood. However, queens sometimes die before the end of the season, in which case a worker assumes the role of replacement queen (e.g. Eickwort 1986; Soro et al., 2009; Field et al., 2010). Species are therefore termed 'primitively eusocial' even though some nests may meet the full definitional requirements for only a short period of time, and in reality nesting aggregations may often contain a mix of eusocial and semisocial nests near to the end of the season (Eickwort, 1986). Foundresses are frequently singly mated and relatedness among workers is often high (Crozier et al., 1987; Packer and Owen, 1994; Mueller et al., 1994; Field et al., 2010), although some degree of multiple mating, worker drifting or nest usurpation can reduce relatedness among workers (Paxton et al., 2002; Soro et al., 2009; Ulrich et al., 2009; Brand and Chapuisat, 2016). The replacement of a dead queen by a worker can mean that workers are not always sisters to all reproductive brood females (Packer and Owen, 1994; Field et al., 2010; Brand and Chapuisat, 2016), and workers occasionally reproduce directly (Richards et al., 2005; Brand and Chapuisat, 2016). Communality occurs where multiple independently reproducing females share a common nest entrance, although complex interactions may occur between individuals (Kukuk et al., 1998).

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[2] In contrast, both sexes of most other bees overwinter as adults or pupae inside their cell, and emerge for the first time in the following spring to mate. Also, see Appendix for overwintering males in Nomioidinae

April
Foundresses emerge from hibernation and initiate nests

Winter
B2 females hibernate in a deep burrow

(a)

May
Provision first brood (B1)

(b)

August
B2 emerge and mate before entering hibernation

July
B1 emerge and become workers (in some species some B1 females found new nests or enter hibernation)
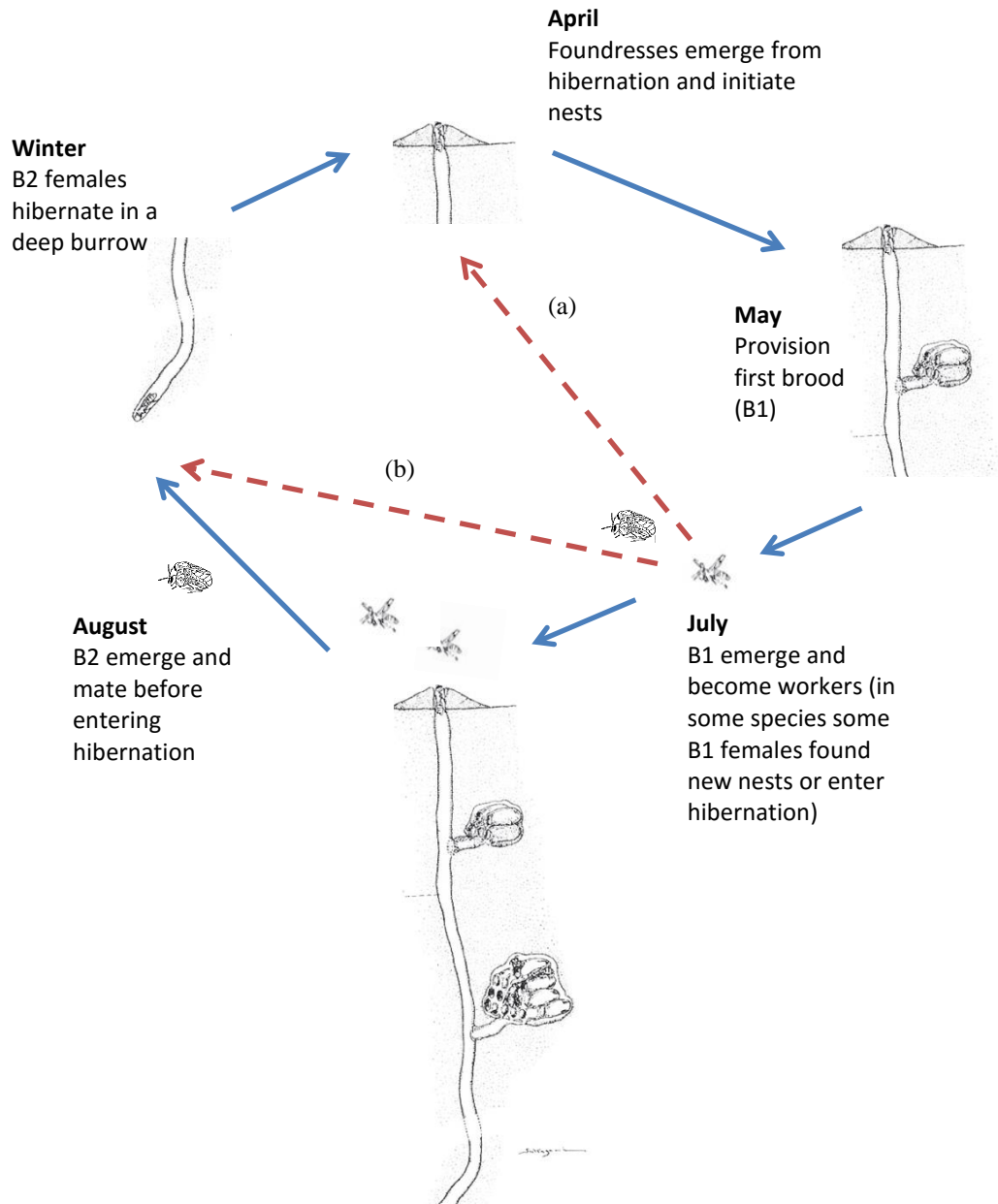
Figure 1.3
Diagram depicting the life cycle of a typical primitively eusocial temperate zone sweat bee (blue arrows). The red dashed arrows show the alternative reproductive options exhibited by first brood females in some species: females can mate and either (a) initiate a new summer nest or (b) enter directly into hibernation. Workers may also occasionally reproduce independently in their own nest, or become replacement queens. After Sakagami and Hayashida (1960) and Sakagami and Fukuda (1972).

1.3.4 Reproductive options for worker brood offspring

In addition to becoming a replacement queen, worker brood offspring may eschew work altogether. Detailed field studies of *Halictus rubicundus* Christ by Yanega (1988, 1989, 1997)

Figure 1.4
Photographs showing sweat bee nests and behaviour. Females often found new nests in spring (a) by digging down into the soil and excavating the contents, which form a mound above the nest entrance or "tumulus" (b). Before leaving the nest on cooler mornings in spring, foundresses often sit in their nest entrance (c), presumably to warm up before emerging to forage for pollen and nectar to provision brood cells (d). Also shown clearly (d) is the "rima", or hairless patch on the fifth tergite that is a distinguishing feature sweat bees. Panel (a) shows *L. malachurum*, and (c)-(d) *L. calceatum*.

in North America revealed that only some first brood female offspring became workers, while others entered directly into hibernation and founded their own nests in the following year. Such brood divalency appears to be widespread among sweat bees (Smith et al., 2007; Yagi and Hasegawa, 2012), and may also include the option to reproduce independently within the same year, or enter foreign conspecific nests to lay eggs (Yagi and Hasegawa, 2012).

The studies cited above are of species known to also express purely solitary behaviour, but brood divalency is also recorded from so-called obligate primitively eusocial species such as *Halictus ligatus* (Richards et al., 2015). Moreover, worker brood *H. ligatus* that emerge into an empty or destroyed nest can reproduce independently in their natal nest or found a new nest within the same year (Richards and Packer, 1994; Rehan et al., 2013), thus adopting a life cycle similar to a solitary bivoltine species. Therefore, even obligate social species retain latent behavioural plasticity, which may become evident only in very specific or unusual circumstances (Rehan et al., 2013). In light of this, it important to expose sweat bees to novel environments or scenarios not typically observed under natural nesting conditions (Wcislo, 1997). In particular, so-called obligate social species may express novel behaviours if transplanted far beyond their natural range (Sexton et al. 2009), where foundresses and offspring could then experience environmental cues usually experienced only by bees in solitary populations.

1.3.5 Parasites and predators

Sweat bees face a range of threats throughout the life cycle such as predation by solitary wasps and spiders (Evans and O'Neill, 1988; O'Neill, 2001; Robertson and Maguire, 2005), and predation and nest destruction by ants (Sakagami and Fukuda, 1989; Smith et al., 2003; Yagi and Hasegawa, 2012). Interspecific cuckoo parasitism is also ubiquitous (Bogusch et al., 2006; Michener, 2007), and conspecifics frequently usurp nests instead of constructing their own (Zobel and Paxton, 2007).

1.4 Evolutionary gains and losses of eusociality within Halictidae

The advent of robust molecular phylogenetic reconstructions has considerably reduced the number of proposed origins of sociality within Halictinae. The prevalence of closely related social and solitary species lead earlier authors to conclude that primitive eusociality had evolved many times within sweat bees (Michener, 1974; Seger, 1991), and even "perhaps more than in all other insects combined" (Seger, 1991 pg. 346). Subsequent studies suggested there might in fact have been only three independent origins of sociality within Halictinae around 20mya: once in Augochlorini and once each for the Halictini genera *Halictus* and *Lasioglossum* (Danforth et al., 1999; Danforth et al., 2003; Brady et al., 2006). However, a more recent large-scale analysis of the tribe Halictini has pushed the origin back to 35mya, prior to the splitting of *Halictus* and *Lasioglossum + Patellapis*, suggesting eusociality may have as few as two origins within Halictinae (Gibbs et al., 2012a). Nevertheless, some uncertainty remains over the taxonomic distribution and phylogenetic relationships of eusocial lineages among halictids, particularly in

Augochlorini (Danforth et al., 2008; Gibbs et al., 2012a), and current hypotheses are certain to be revised in the future.

A key outcome of phylogenetic reconstructions has been the discovery of rampant secondary loss of eusociality (Danforth et al., 2002; Gibbs et al., 2012a). Secondary loss has occurred at least twelve times within Halictinae, and includes reversals to full solitary nesting, social polymorphism (see below) and social parasitism (Wcislo and Danforth, 1997; Danforth et al. 1999; 2003; Gibbs et al., 2012b). At least two reversals to solitary nesting were accompanied by the transition from polylecty to oligolecty (Danforth, 2002). Floral specialisation typically shortens the length of the season during which bees can provision brood cells (Michener, 2007, but see McGinley, 2003), which precludes the longer life cycle required for sociality (Fig. 1.5). Reversals to social polymorphism may have been a response to the invasion of unpredictable environments (Danforth, 2002; Field et al., 2010). Together this illustrates that during its early stages eusociality may be hard to evolve but is easily lost, in contrast to advanced eusocial taxa where reversals to solitary may be more difficult (e.g. Wilson and Hölldobler, 2005). These findings parallel the higher-level loss of social behaviour in other groups of bees (Cardinal and Danforth, 2011; Rehan et al., 2012), although primitive sociality may not always involve frequent reversions to solitary nesting (Chenoweth et al., 2007). In contrast, sociality appears to have been only rarely lost in wasps (e.g. Fucini et al., 2009), and in ants is limited to the loss of the queen caste (Wilson and Hölldobler, 2009). Therefore, the evolutionary lability of social behaviour in sweat bees has uniquely given rise to extensive intra-generic and intra-specific
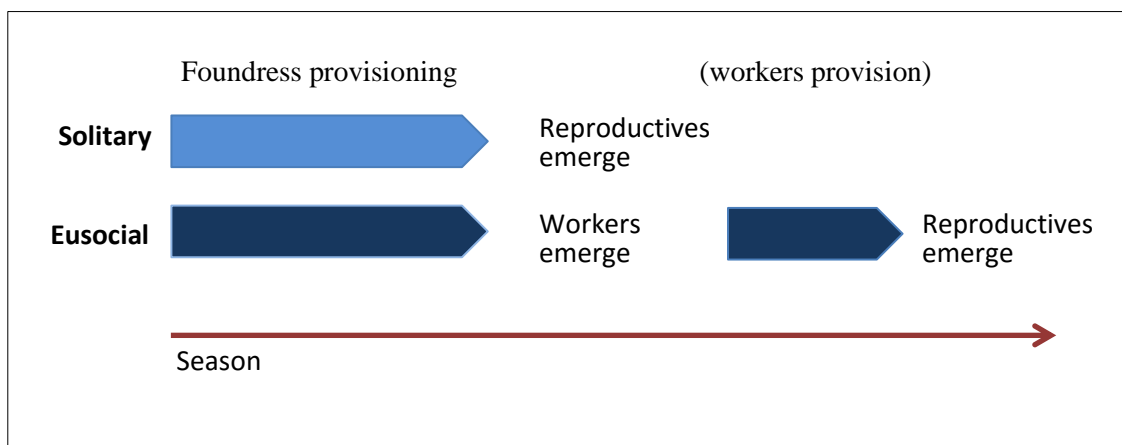
Figure 1.5
Being social takes longer than being solitary. Brood rearing in the solitary life cycle is completed when offspring provisioned by the foundress emerge, but in the social life cycle offspring must provision a second brood as workers. In both cases the life cycle must be completed before the end of the season.

variation in social behaviour, providing ideal opportunities to test hypotheses for the evolution of eusociality (Chapuisat, 2010; Rehan and Toth, 2015).

1.5 Social polymorphism

1.5.1 Overview

Social polymorphism is one of the most striking forms of social variation exhibited by sweat bees, and occurs where the same species expresses both social and solitary behaviour. In temperate regions, the geographic distribution of social and solitary phenotypes is closely linked with the length of the active season (Hirata and Higashi, 2008; Kocher et al., 2014). By omitting the worker brood, solitary populations are able to persist much further north and at higher altitudes than social populations (Eickwort et al., 1996). This pattern is found in a number of temperate zone halictids, but has been studied in only a small number of species: *Halictus rubicundus* (Soucy and Danforth, 2002; Field et al., 2010), *Lasioglossum calceatum* (Sakagami & Munakata, 1972), *L. albipes* (Plateaux-Quénu et al., 2000), *L. baleicum* (Cronin and Hirata, 2003; Yagi and Hasegawa, 2012), *L. apristum* (Miyanaga et al., 1999) and *Augochlorella aurata* [3] (Packer, 1990). In most cases solitary nests occur where the climate probably temporally precludes consecutive broods because the season is too short, although both phenotypes may occur in sympatry where conditions are marginal (Soucy, 2002; Hirata and Higashi, 2008). It is important to note that all known cases of social polymorphism in sweat bees involve the secondary loss of sociality rather than its gain (Danforth et al., 1999, 2003; Gibbs et al., 2012a), as this may help explain the existence of social plasticity in some species (see section 1.5.2 below).

The relationship between social phenotype and season length may not be simple. A striking case is the coexistence of primitive eusociality and communal nesting in a population of *H. sexcinctus* Fabricius in southern Greece (Richards et al., 2003; Richards, 2003). These two distinct nesting strategies differ in the length of the colony cycle (Richards et al., 2003), but in this case cannot be ascribed to proximate constraints of season length. Similarly, in France, *L. albipes* nests solitarily in areas where its sister species *L. calceatum* nests socially, suggesting there may be intrinsic differences between species such as spring phenology (Plateaux-Quénu et al., 2000). Miyanaga et al. (1999) showed that season length, calculated as degree-days, was the same in two populations expressing alternative phenotypes, again suggesting that some additional genetic or environmental factor might be important in determining which phenotype

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[3] *A. striata* is an earlier synonym (Coelho, 2004)

is expressed (Keller, 2003). In general the precise geographic extent of social and solitary phenotypes in polymorphic sweat bees is poorly known (Wcislo, 1997). Other groups such as carpenter bees (Stark, 1992; Rehan et al., 2010), as well as some tropical sweat bees (Kapheim et al., 2015a), can exhibit extensive within-population social polymorphism. Nevertheless, between-population social polymorphism is a propitious trait of temperate-zone socially polymorphic sweat bees. Solitary and social populations of the same species represent lineages in the midst of the transition to eusociality, and has only rarely been reported from other taxa (e.g. Fucini et al., 2009). This distinction does not exclude the existence of within-population polymorphism in temperate zone sweat bees; rather, sympatric social and solitary nesting may occur at high frequency only in marginal environments closer to the transition zone between social and solitary nesting.

1.5.2 Mechanisms

A key challenge is to determine the extent to which polymorphism represents phenotypic plasticity or fixed genetic differences between populations (Keller, 2003); however, relatively few studies have examined mechanisms underlying social polymorphism in sweat bees. Reciprocal transplants of *H. rubicundus* between social and solitary populations in the UK have revealed that social phenotype is plastic and conditional on the environment (Field et al. 2010). Nests in which offspring emerged earlier in the season were more likely to become social (Field et al., 2010), suggesting that cues such as photoperiod might be critical in determining social phenotype (Hirata and Higashi 2008). Field et al. (2010) further showed that nests where the foundress was alive at offspring emergence contained significantly more workers, indicating that the presence of the foundress might also influence whether offspring become workers (Packer, 1990). Moreover, in a subsequent transplant Field et al. (2012) showed that *H. rubicundus* foundresses are capable of adjusting the size of first brood offspring dependent on anticipated social phenotype. Solitary-population foundresses transplanted to a social site by Field et al. (2010) did not provision smaller workers, and the effect of nutritional biasing of offspring behaviour by queens remains to be seen (but see Kapheim et al., 2015a).

In North America, however, indirect phylogeographic evidence suggests that social phenotype may have a considerable fixed genetic component. Soucy and Danforth (2002) discovered considerable mitochondrial differentiation between populations in which native bees express alternative social phenotypes. This was true irrespective of geographic distance, signifying a lack of gene flow between social and solitary populations (Soucy and Danforth, 2002). In contrast there appear to be no barriers to gene flow among social and solitary UK populations (Soro et al., 2010), further suggesting there may be interesting differences between North

American and European *H. rubicundus*). Non-plasticity has been experimentally indicated only in a laboratory common garden study of *Lasioglossum albipes* (Plateaux-Quénu et al. 2000). Bees tended to retain their phenotype of origin even when reared under conditions simulating those where the alternative phenotype occurs (Plateaux-Quénu et al. 2000). Large-scale field transplants are, however, essential to determine the extent to which sociality is plastic because bees can be exposed to natural temperature and photoperiod regimes (Field et al., 2012).

1.6 Environmental effects on behaviour and sociality

Environmental conditions strongly influence the distribution of social behaviour across a broad range of taxa (Jetz and Rubenstein, 2011; Purcell, 2011). However, these affects are not consistent, even within taxonomic groups, and ecology can play a key role in determining which environmental factors are important in either promoting or selecting against sociality (e.g. Gonzalez et al., 2013; Kocher et al., 2014). Below I briefly outline the ways in which social behaviour in temperate zone sweat bees can be shaped by environmental conditions.

1.6.1 Seasonality

All social sweat bees have an annual life cycle[4]. In contrast to perennial species such as ants (Bourke and Franks, 1995), annual social species must produce workers and reproductives sequentially within one season (Reeve, 1991; Schwarz et al., 2007). In temperate zones the season is defined by the number of days on which it is possible for bees to be active, and often by dry and wet seasons in the tropics (Forrest and Miller-Rushing, 2010). Interestingly, in seasonal environments, sweat bees may be pre-adapted to maximise the time available. Unlike almost all other primitively eusocial bees (bumblebees are an exception) and solitary sphecid wasps, sweat bee females overwinter as mated adults (Matthews, 1991; Michener, 2007). However, although this means nest foundresses might get a head start in spring, the early advantage may be offset by the need to fully complete development, emerge and mate before hibernating at the end of the season.

At a broad scale, season length sets a physical limit on the duration of the annual colony cycle because bees must pass the unfavourable part of the year in hibernation (Schwarz et al., 2007). The effect of season length is also shown by the geographic distribution of social or solitary behaviour among sweat bees: sociality is never recorded from the highest latitudes or altitudes where sweat bees occur, and socially polymorphic species often transition from social to

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[4] *Lasioglossum marginatum* is uniquely perennial: a single worker brood is reared each year until reproductives are produced in the fifth or sixth year (Plateaux-Quénu, 1962).

solitary behaviour along gradients of decreasing season length (e.g. Soucy and Danforth, 2002). Some socially polymorphic species express only solitary nesting in areas where others are social (Plateaux-Quénu et al. 1992; Miyanaga et al., 1999), and therefore intrinsic factors other than just season length per se must play a role in determining the geographic limits of sociality.

Within a species, nests in more southern areas may be initiated more than a month earlier than further north (Albert and Packer, 2013; Richards et al., 2015). This can have important consequences for foundresses, because those enduring a longer colony cycle must lay considerably more eggs and in many cases survive for longer (Richards et al., 2005, 2015). In seasonal environments organisms seek to maximise reproduction within the limited time available (Roff, 1980). Where the season is sufficiently long, some obligate social sweat bees achieve this by incorporating additional worker broods into the life cycle, which significantly boosts productivity (Richards et al., 2005). Although a longer season may facilitate the interpolation of additional worker broods, it is unclear what mechanisms underlie this behaviour (Weissel et al., 2006). Intermediate populations can exist where neighbouring nests of the same species alternatively produce either one or two worker broods (Strohm and Bordon-Hauser, 2003; Weissel et al., 2006), and therefore foundresses probably use cues associated with season length coupled with factors such as their own condition (Weissel et al., 2012).

The removal of any form of season constraint can have important consequences for the colony life cycle. For example, western Costa Rica has a distinct wet and dry season and, thus seasonally constrained, nests of *L. umbripenne* exhibited a life cycle typical of that depicted in Figure 1.3 (Wille and Orozco, 1970). By contrast, eastern Costa Rica is almost aseasonal; there, *L. umbripenne* were active nearly all-year-round and nests were smaller with a much less clearly defined social structure (Eickwort and Eickwort, 1971). Similarly, nests of *H. poeyi* in sub-tropical Florida are unconstrained by seasonality and brood can be found in nests at any time of year (Packer and Knerer, 1987).

Development time in social sweats bees is relatively long (Kocher et al., 2014), and is strongly influenced by temperature (Kamm, 1974; Weissel et al., 2006). Therefore, not only do sweat bees at higher latitudes or altitudes experience a shorter season, but also they develop more slowly in the cooler temperatures (Field et al., 2012). Bumblebees and honeybees have evolved mechanisms to actively regulate nest temperature above ambient (Heinrich, 1979; Seeley, 1985). Sweat bees, however, can only attempt to elevate temperatures experienced by developing brood through locating their nests in sunny areas that maximise exposure to the sun (Potts and Wilmer, 1997). In consequence the physical environment can play a considerable role in social organisation in sweat bees (see below).

1.6.2 Variation in weather conditions

Sweat bees are particularly sensitive to inter-year variation in weather conditions. Like other ground-nesting Hymenoptera such as sphecid wasps (O'Neill, 2001), developing sweat bee larvae are vulnerable to excessive rainfall, which can cause brood cells to waterlog and pollen to become mouldy (Richards and Packer, 1995; Soucy, 2002). However, annual variation in rainfall and temperature can also have important effects on colony social organisation. Poor weather may limit resource availability and provisioning opportunities for adults, resulting in the production of smaller offspring (Richards and Packer, 1996; Richards, 2004). One consequence is that caste-size dimorphism and the number of workers produced can vary significantly between years (Richards and Packer, 1996; Albert and Packer, 2013), with reproductive skew greatest when large foundresses produced small workers (Richards and Packer, 1996).

1.7 Experimental field studies of sweat bees

The vast majority of experimental studies conducted using sweat bees have taken place in laboratory cages or circle tube arenas. Many have focussed on interactions between colony members and kin recognition (e.g. Michener and Brothers, 1974; Kukuk and May, 1991; Soro et al., 2011; Polidori et al., 2012), as well as determining key aspects of social biology (Plateaux-Quénu, 1992; Plateaux-Quénu et al., 1998; 2000). Comparatively few studies have actively manipulated sweat bees in the field. This is possibly because unlike the open aerial nests of paper wasps (Reeve, 1991; Jandt et al., 2013), the subterranean nests of sweat bees can be difficult to locate (Wcislo, 1997; Richards et al., 2015), and collecting brood requires painstaking and lengthy excavations of nests (Michener, 1974).

A number of sweat bee studies have excavated nests to uncover details of the colony cycle and describe colony sociogenetics (e.g. Crozier et al., 1987; Paxton et al., 2002; Richards et al., 2005). Studies have used sweat bees to test specific hypotheses regarding sex ratios and resource allocation (Boomsma and Eickwort, 1993; Packer and Owen, 1994; Brand and Chapuisat, 2012). To my knowledge, however, there have been only five studies that have performed direct manipulations in the natural environment[5]. Three of these involved the removal of individual bees from nests to investigate (i) sex ratios in semisocial versus eusocial nests of *Augochlora aurata* (Mueller, 1991), (ii) intraspecific nest usurpation pressure in *L. malachurum* (Zobel and Paxton, 2007) and (iii) the contribution of workers to colony

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[5] Other studies have experimentally investigated responses to parasitism and predation, (Wcislo, 1997; Wcislo et al., 2003)

productivity in *H. scabiosae* (Brand and Chapuisat, 2013). The remaining two studies are large-scale field transplants of *H. rubicundus* to investigate the mechanisms underpinning social polymorphism (Field et al. (2010, 2012; see section 1.4). Sweat bees currently therefore hold a largely untapped potential for experimental manipulation in the field, and a key aim of this thesis is to further the use of sweat bees for experimental fieldwork (see section 1.8 below).

1.8 An introduction to the study species: *Lasioglossum* (*Evylaeus*) *calceatum* and *L.* (*E.*) *malachurum*

Lasioglossum is a speciose genus of "morphologically monotonous" but behaviourally diverse and cosmopolitan Palaearctic bees within the tribe Halictini (Michener, 2007 pg. 354; section 1.2; Fig. 1.2c). Both *L. calceatum* and *L. malachurum* are placed within the subgenus *Evylaeus*, which includes species that exhibit solitary nesting, social polymorphism, and primitive eusociality (Schwarz et al., 2007).

1.8.1 The subgenus *Evylaeus*

The subgenus *Evylaeus* comprises typically medium-sized, non-metallic *Lasioglossum* characterised by the presence of a vertical carina separating the lateral faces and declivity of the propodeum (Michener, 2007). *Evylaeus* does not constitute a monophyletic group, and is paraphyletic with respect to the subgenera *Dialictus, Sphecodogastra* and *Sellalictus* (Gibbs et al., 2012a). Although variable, *Evylaeus* nest architecture is typically characterised by the placement of all the cells of each brood clustered together and surrounded by a cavity (Fig. 1.7; Packer, 1991, see Sakagami and Hayashida, 1960 for a detailed description). The cavity is thought to aid drainage and help prevent the cell cluster from becoming waterlogged during periods of prolonged rainfall (Packer, 1983). Bees within *Evylaeus* exhibit a broad range of social behaviour, from solitary nesting and social polymorphism to some of the most specialised primitively eusocial sweat bees (Michener, 1974; Packer and Knerer, 1985).

1.8.2 An overview of *Lasioglossum* (*Evylaeus*) *calceatum*

Lasioglossum (*Evylaeus*) *calceatum* is a common, medium-sized sweat bee (Fig. 1.6) with a cosmopolitan Palaearctic distribution (Pesenko et al., 2000; Michener, 2007). Within the UK, *L. calceatum* is common and widely distributed throughout (Falk and Lewington, 2015). The majority of information regarding the life cycle of *L. calceatum* comes from bees reared in cages (see Plateaux-Quénu et al., 1992 for an English summary). Few studies of wild nests are in English (see Pesenko et al., 2000 for an English summary). Together with the laboratory

Figure 1.6
Lasioglossum calceatum nest foundress, resting on my forefinger.

studies of Plateaux-Quénu et al. (1992), Bonelli (1965, 1968) provides a description of the life cycle from the field.

Most *L. calceatum* populations studied have shown a typical primitively eusocial halictid life cycle (see section 1.2.3; Fig. 1.4). A solitary foundress phase is followed by a single social worker phase, and a small proportion of nests may be co-founded (Vleugel, 1961). Sakagami and Munakata (1972) identified the same life cycle in Japan, where prior to 1972 *L. calceatum* was not known, but also discovered a solitary population at around 1000masl on Mount Yokotsu. Field (1996) has since reported probable solitary nests from Dartmoor (UK), and *L. calceatum* is therefore socially polymorphic. No further attempts have been made to study social polymorphism in *L. calceatum*, and it is currently unknown whether alternative phenotypes represent fixed genetic differences or a plastic response to environmental conditions (Field et al, 2010, 2012).

As in other *Lasioglossum*, a low frequency of co-founded nests seems common (Packer and Knerer, 1985). In *L. calceatum* the cell clusters are connected to the main vertical burrow by a short horizontal tunnel entering in the upper portion of the cluster, and one or more pillars support the cluster itself (Sakagami and Munakata, 1972; Fig. 1.8a, b). In common with other social sweat bees, *L. calceatum* is polylectic, foraging on a wide range of open flowers up to 1km away from the nest (Beil et al., 2008). No continuous detailed observational studies have been undertaken at nesting aggregations.

Many key behavioural characteristics remain poorly understood in *L. calceatum* under natural conditions. Details are scant or absent on foundress longevity, mating status of workers, brood sizes, sex ratios and caste-size dimorphism. Further, it is unknown whether there is brood divalency (see Yanega, 1989), and nothing is known about colony sociogenetics except that Sakagami and Munakata (1972) recorded only a small proportion of workers with developed ovaries. Laboratory studies suggest that few males are produced in the first brood (but that 100% of first brood offspring mated) and that caste-size dimorphism can reach up to 20% in warm conditions (Plateaux-Quénu, 1992): both characteristics of more socially advanced sweat bees (Packer and Knerer, 1985). However, caste-size dimorphism data from phenological studies ranges from 3-5.5% in Japan (Sakagami and Munakata, 1972) up to 8.3% and 13% in France (Plateaux-Quénu, 1992), and 85% of first brood females were mated in field samples (Plateaux-Quénu, 1992). The disparity between figures reported from cage and field studies indicates that cages may be unreliable for investigating these characteristics.

Laboratory-reared larvae excavated from solitary nests in Japan exhibited a 1:1 sex ratio (Sakagami and Munakata, 1972), but sex ratios from the reproductive or worker broods of

(a)   (b)

Figure 1.7
(a) Internal structure of a *L. calceatum* nest containing one brood (modified from Sakagami and Munakata, 1972), showing the cells clustered and surrounded by a cavity. The main tunnel is approximately 6cm in length. In a social nest, the pictured cluster would be the worker brood with a second, larger cluster constructed below for the second brood of reproductives. The arrow points to one of the three depicted earth pillars used to support the cell cluster within the cavity. Cell clusters can often be removed intact (b) from nests during excavation.

social nests are unknown from the wild. Other behavioural observations show that *L. calceatum* foundresses, together with workers, engage in extended brood care by opening sealed cells and cleaning the pollen ball, brood, and the cell itself (Plateaux-Quénu, 2008).

From data gathered so far, the primitive eusociality expressed by *L. calceatum* appears to be less specialised than in other species of *Evylaeus* where, for example, caste dimorphism is greater and fewer workers appear to be mated (Packer and Knerer, 1985). Key characteristics, however, such as sex ratios and brood sizes remain to be investigated before direct comparisons can be made.

1.8.3 An overview of *Lasioglossum* (*Evylaeus*) *malachurum*

Lasioglossum malachurum (Fig. 1.8a) is a small to medium sized sweat bee that is common and widespread throughout central and southern Europe, with a range that extends into North Africa and the desert regions of southwest Asia (Pesenko et al., 2000). In contrast with *L. calceatum*, the social biology of *L. malachurum* has been studied extensively in the field for almost 100 years (e.g. Stöckhert, 1923; Knerer, 1992; Richards et al., 2005). Although once considered a scarce, coastal species in the UK (Falk, 1991), over the past 20 years *L. malachurum* has become "one of the most frequent *Lasioglossum* species of southern England" (Falk and Lewington, 2015 pg. 204).

Nesting frequently occurs in dense aggregations (Fig. 1.8b) numbering thousands of individual nests that can persist for decades (Knerer and Atwood, 1967), making *L. malachurum* highly amenable to study. Nest structure is similar to that of *L. calceatum*, except only a partial cavity may surround the cluster of first brood cells, and is absent altogether from subsequent broods (Michener, 1974). *L. malachurum* is highly polylectic (Westrich, 1989; Polidori et al., 2010).

The life cycle is typical for a primitively eusocial sweat bee (see section 1.2.3), except for variation in the number of worker broods produced. In northern Europe a single worker brood is followed by the production of reproductives (Packer and Knerer, 1985). Further south, in central Europe, some nests have an additional helper brood (Weissel et al., 2006), while in southern Europe where the season is longest there are always two helper broods and some nests may even produce a partial third (Wyman and Richards, 2003). In consequence, colonies vary greatly in size: in northern colonies reproductive brood are provisioned by on average only 4-5 workers (Strohm and Bordon-Hauser, 2003), whereas in

(a)

Figure 1.8
(a) *Lasioglossum malachurum*
foundress adjacent to the entrance
of her nest, and (b) a dense
aggregation of *L. malachurum*
nests in southern England (Photo
Thomas Wood).

(b)

southern Europe more than 30 workers are present at the time the reproductive brood is provisioned (Richards et al., 2005).

Nests are always social and singly founded, except for in southern Europe where a small number can be co-founded (Richards et al., 2005). Nest usurpation by conspecifics in spring can be common when nest foundresses are foraging for pollen to provision the first brood (Smith and Weller, 1989; Zobel and Paxton, 2007). Sociality in *L. malachurum* is likely to be much more specialised than in *L. calceatum*: workers and queens consistently show a

non-overlapping bimodal size distribution (see data presented in Wilson, 1971; Knerer, 1980) of between 14-18%, and a tiny fraction of males (<2%) are produced in the worker broods (Packer and Knerer, 1985).

Foundresses frequently survive long enough to monopolise the laying of reproductive brood eggs (Paxton et al., 2002; Richards et al., 2005). However, workers in northern European nests are frequently mated and in larger nests display considerable ovarian development (Strohm and Bordon-Hauser, 2003), although it is not clear that this frequently translates into oviposition (Paxton et al., 2002; Richards et al., 2005). In southern Europe many workers have developed ovaries but few oviposit or are mated, probably because almost no males are produced in the worker broods at all (Richards et al., 2005).

1.9 Aims of the thesis

The general aim of this thesis is to use long-term field observations and field manipulation experiments to investigate social polymorphism and social behaviour in sweat bees. A key goal is to describe, and examine the mechanisms underlying, social polymorphism in *Lasioglossum calceatum* in the UK, as well as to explore some wider implications of social polymorphism for other life history traits. A second goal is to address the extent to which social nesting is influenced by environmental conditions, and whether social behaviour in obligate social sweat bees is responsive to novel environmental cues. Finally, in this thesis I aim to explore the often-reported relationship in sweat bees that productivity increases with group size.

The biology of *L. calceatum* is not well known from the wild (see section 1.7.2), and has only been cursorily investigated in the UK (Field, 1996). A key first step towards understanding social polymorphism in *L. calceatum* is to determine the geographic distribution of social and solitary phenotypes, and to investigate details of nesting and social biology. This is the focus of Chapter 2, where I establish social phenotype at field sites within the UK and provide a description of the social life cycle.

A fundamental question concerning social polymorphism is the extent to which alternative social phenotypes represent environmentally mediated plasticity, or fixed genetic differences among populations (e.g. Field et al., 2010 vs. Plateaux-Quénu et al., 2000; Soucy and Danforth, 2002). Field transplants between environments in which populations

exhibit social or solitary phenotypes are the most effective way to test these alternative hypotheses. A central goal of this thesis is to perform a field transplant of *L. calceatum*, from a population where only solitary behaviour is expressed to where native bees nest socially. In Chapter 3 I aim to utilise the study populations established in Chapter 2 for this purpose.

The environment is thought to affect key life history traits in sweat bees such as body size (section1.7; Richards and Packer, 1996). In Chapter 4 I aim to further investigate the wider implications of social polymorphism by examining how transitions in social phenotype might interact with previous findings that body size in sweat bees probably decreases with latitude. Studies of solitary taxa with free-living larval forms show that, in some cases, body size can follow a saw-tooth cline when populations transition from univoltinism to bivoltinism (Mousseau and Roff, 1989). Continuous body size clines have never been investigated in sweat bees, and the focus of Chapter 4 is how the environment and polymorphism interact to produce size clines in *L. calceatum* and *H. rubicundus*.

The environment is also thought to exert considerable influence over the geographic limit of social nesting, partly due to constraints set by season length (Kocher et al., 2014), and is correlated with life cycle variation in obligate social species (Weissel et al., 2006; Richards et. al., 2005). However, the idea that shorter season lengths can preclude social nesting has never been demonstrated experimentally, and the extent to which obligate social species can express behavioural plasticity in response to novel environmental conditions remains unknown. The principal aims of Chapter 5 are to experimentally address these ideas by transplanting the obligate social *L. malachurum* from the south of the UK where it is common, to the north of the UK where it never occurs (see section 1.7.2).

Chapter 6 focuses on addressing the frequently reported positive relationship between group size (i.e. the number of workers) and productivity in sweat bees. Many reported data are incidental or from studies that either did not attempt manipulations (Strohm and Bordon-Hauser, 2003), or where manipulations were limited in scope (Brand and Chapuisat, 2013). Comprehensive experimental manipulations are required, however, to separate the effects of group size and potential confounding variables such as the quality of nest foundresses and workers. In Chapter 6 I aim to examine the effect of group size on productivity in *L. malachurum*, whist simultaneously controlling for potentially confounding variables.

Each chapter is presented as a largely self-contained piece of work.

Chapter two

Social polymorphism in the sweat bee

Lasioglossum calceatum

Abstract

Temperate-zone socially polymorphic sweat bees (Hymenoptera: Halictidae) are ideal model systems for elucidating the origins of eusociality, a major evolutionary transition. Bees express either social or solitary behaviour in different parts of their range, and social phenotype typically correlates with season length. Despite their obvious utility, however, socially polymorphic sweat bees have received relatively little attention with respect to understanding the origins of eusociality. *Lasioglossum* (*Evylaeus*) *calceatum* is a widespread sweat bee that is thought to be socially polymorphic, with important potential as an experimental model species. We first determined the social phenotype of *L. calceatum* at three sites located at different latitudes within the UK. We then investigated sociality in detail across two years at the southernmost site. We found that *L. calceatum* exhibits latitudinal social polymorphism within the UK; bees were solitary at our two northern sites but the majority of nests were social at our southern site. Sociality in the south was characterized by a relatively small mean of two and 3.5 workers per nest in each year respectively, and a small to medium mean caste-size dimorphism of 6.6%. Foundresses were smaller in our more northern and high altitude populations. Sociality is clearly less specialized than in some closely related obligate social species but probably more specialized than other polymorphic sweat bees. Our research provides a starting point for future experimental work to investigate mechanisms underlying social polymorphism in *L. calceatum*.

2.1 Introduction

Understanding why an individual gives up its own reproduction to help others is a central goal of evolutionary biology (Maynard Smith and Szathmáry, 1995). Despite this, accounting for how the decision to help is made at the individual level has received comparatively little attention (Field et al., 2012). Primitively eusocial sweat bees (Hymenoptera: Halictidae) lack fixed castes and vary greatly in their social organisation, making them model organisms for studying the origins of eusociality (Schwarz et al., 2007). Of particular interest is social polymorphism, where both solitary and social phenotypes are expressed within the same species (Soucy and Danforth, 2002). In social nests, at least some first brood offspring are workers that help rear a second brood of reproductives. In contrast all offspring in solitary nests are reproductives, and these nests tend to occur where the season is probably too short to complete the social life cycle (Hirata and Higashi, 2008; Kocher et al., 2014). Socially polymorphic sweat bee lineages therefore offer unique opportunities to understand the mechanisms underlying the origin of eusociality, because it is possible to directly investigate the environmental and genetic processes mediating the decision to become a worker or a reproductive (Field et al., 2010, 2012).

The Palearctic sweat bee *Lasioglossum* (*Evylaeus*) *calceatum* Scopoli is socially polymorphic (Sakagami and Munakata, 1972; Field, 1996). Originally this species was thought to be only primitively eusocial (e.g. Bonelli, 1965, 1968). Then, more than 40 years ago, Sakagami and Munakata (1972) discovered that *L. calceatum* was socially polymorphic in Japan: nests were found to be solitary at more than 1000 masl on the summit of Mt Yokotsu, but a social life cycle was inferred in the surrounding lowlands. Similarly, Field (1996) reported solitary nests from Dartmoor, an upland area in the southern United Kingdom (UK). Since these studies, however, there has been no attempt to further understand the underlying causes of social polymorphism within *L. calceatum*. Moreover, details of the social life cycle and the degree of social complexity relative to other primitively eusocial sweat bees remain poorly understood in colonies from the wild (Packer and Knerer, 1985; Plateaux-Quénu, 1992).

From studies to date, the life cycle of *L. calceatum* can be summarised as follows (Bonelli, 1965, 1968; Sakagami and Munakata, 1972; Plateaux-Quénu, 1992 and references therein). Mated females (foundresses) emerge from hibernation in spring and each initiates a subterranean nest. Foundresses mass provision a first brood (B1) of ≈4-6 offspring, providing each with a ball of pollen and nectar in a series of separate, sealed brood cells, which are arranged in a cluster adjacent to the main nest burrow. In solitary nests offspring emerge, mate, and females enter directly into hibernation. In social nests, however, B1 females are typically

slightly smaller than their mothers and become workers. Workers help to provision a second brood (B2), during which time nests become primitively eusocial. B2 offspring emerge at the end of summer to mate, and females enter hibernation before emerging as foundresses the following spring. Males are produced in both broods but die before winter and play no role in nesting. In some primitively eusocial halictids a proportion of B1 females enter directly into hibernation, or found new summer nests by themselves (Yanega, 1988; Yagi and Hasegawa, 2012). It is currently unknown whether either of these behaviours occurs in *L. calceatum*.

More advanced halictine sociality is generally associated with traits such as larger colony size, and a greater degree of caste-size dimorphism between workers and foundresses (Packer and Knerer, 1985). A population level comparison between foundresses and presumed B1 females in Japanese *L. calceatum* found a size difference of 3.5-5.5% (Sakagami and Munakata, 1972), whereas in France foundresses have been reported as being up to 13% larger than workers (see Plateaux-Quénu, 1992). It is unclear whether the latter two figures were also measured at the population level or directly between mothers and daughters within nests, but these data do indicate there may be geographic variation in caste-size dimorphism. Colony size in wild nests has been reported from only a single location in Italy, where Bonelli (1965) excavated nests with 4-6 B1 brood cells.

Characteristics such as caste-size dimorphism, number of workers and bee size may vary temporally as well as spatially, and can be influenced by fluctuating environmental conditions. In a multiyear study of *Halictus ligatus* Say, such characteristics largely depended upon weather conditions from year to year (Richards and Packer, 1996). For example the sizes of a foundress and her workers are determined in separate years. A large foundress may be produced in a dry, warm year but then raise small workers if the subsequent year is cool and wet (Richards and Packer, 1996). Consequently it is not only necessary to study geographically disparate populations, but also individual populations over multiple years to achieve an accurate description of social phenotype (Wcislo, 1997).

The geographic distribution of social and solitary phenotypes within polymorphic species is closely associated with the length of the active season. Nests are typically social in southern and low altitude areas where the season is long enough to facilitate rearing two broods (Soucy, 2002; Field et al., 2010), but solitary at higher latitudes or altitudes where multiple broods are likely to be temporally precluded (Eickwort et al., 1996; Field, 1996). Recent work on *Halictus rubicundus* Christ in the United Kingdom (UK) has shown that social phenotype was plastic, and that time of first brood emergence could be an important factor influencing whether offspring become workers (Field at el. 2010). However, because foundresses are capable of

varying the size of B1 offspring with respect to expected social phenotype (Field et al., 2012, but see Field et al., 2010), they may use a reliable cue such as time of nest initiation to inform whether or not they provision smaller, worker-sized offspring. The timing of nest initiation could therefore be an important factor determining social phenotype if earlier-provisioned offspring are themselves likely to emerge earlier, and foundresses could therefore use time of provisioning to anticipate social phenotype (Field et al., 2010). Nevertheless, the generality of this pattern in other species has not been tested.

In this chapter I determine whether *L. calceatum* is socially polymorphic in the UK, by establishing social phenotype at three different latitudes (Table 2.1; Fig. 2.1). I also investigate in detail the social phenotype of *L. calceatum* over two years at the southernmost site (Sussex). At Sussex I investigate bee size, caste-size dimorphism, and the number of workers, and test for a relationship between the date of initiation of foundress provisioning and offspring emergence time. I also compare bee size among sites, additionally using specimens from a population on Dartmoor that is thought to be solitary (Fig. 2.1; Field, 1996).

2.2 Methods

2.2.1 Study sites

Three nesting aggregations of *L. calceatum* in the UK where social phenotype was unknown were studied between 2012-2014 (Table 2.1; Figs. 2.1, 2.2). Details of each site are given in Table 2.1. The Sussex site was a narrow, west-facing strip of grass 5.8 m long and 1.3 m wide on the University of Sussex campus, bordered on the eastern side by a single storey brick building (Fig. 2.2a). Hexham was a small section of a much larger south-facing recreational grassland area approximately 5 m long and 3 m wide, bordered on the northern side by a row of mature trees (Fig. 2.2c). At Inverness nests were situated in the grassy centre and to the sides of a 5m section of stone track (Fig. 2.2a).

2.2.2 Method of observation at Sussex

Detailed observations were made to establish and characterise the social phenotype of *L. calceatum* at Sussex. Behaviour was observed directly by continuously standing or sitting in front of the aggregation for the duration of activity on every day of suitable weather (Observation days; 2012, n=120, 2013 n=50). In the early spring of 2012 and 2013 the aggregation was checked daily on sunny days for activity by newly emerged foundresses. The

Figure 2.1
(a) Map of the UK showing the locations of the four
Lasioglossum calceatum study sites mentioned in the text;
University of Sussex campus (Sussex), Hexham, Dartmoor and
Inverness. Circles denote sites where bees are solitary, and the
square where bees exhibit primitive eusociality.

first *L. calceatum* foundress was seen on 29 February and 20 April in 2012 and 2013 respectively, and activity continued until October in both years (Fig. 2.3). Continuous observations in 2012 and 2013 proceeded from these dates in each year respectively, and we therefore observed the first date of provisioning for each foundress. New nests were individually marked with numbered nails inserted adjacent to each nest, as soon as they appeared in spring.

In 2012 a subset of 50 foundresses from 47 nests were marked and measured during the foundress-provisioning phase, and in 2013 23 foundresses from 17 nests within the observation area were marked and measured. Foundresses were caught with an insect net as they emerged from their burrows after a provisioning event had been observed. Each was given a unique combination of enamel paint spots (Revell® and Humbrol™ enamel model paints) applied to the thorax with a pin. Wing length was measured to the nearest 0.1mm with digital callipers, as

the distance between the outer edge of the tegulum and the end of the forewing. The aggregation was divided into two sections, and these were observed on alternate days.

Sociality was confirmed by the presence of workers. These were initially identified as unmarked bees observed provisioning the nests (where the foundress or foundresses had been marked) after the short period of inactivity between foundress provisioning and offspring emergence (Fig. 2.3). Workers were caught and measured on departure from their nest after a provisioning event had been recorded. Within each nest, workers were given a single unique colour spot. A bee was then designated as a worker only if it was observed provisioning again after marking, and the total number of workers for each nest was counted as all such bees. Provisioning events within each area were recorded, and where possible the colour of the provisioning worker noted.

Halictid aggregations are notoriously difficult to find (Richards et al., 2015), and therefore we did not destructively sample adult bees or nests because we wished to preserve the nesting aggregation for future experimental work.

2.2.3 Methods of observation at Hexham and Inverness

Aggregations at Hexham and Inverness were first visited during the foundress-provisioning phase on 19-20 June 2012 and 3-4 June 2013 respectively. Foundresses were caught in flight at Hexham (n=17), and marked with a single colour spot until no unmarked provisioning bees remained. At Inverness ten foundresses were marked and their nest locations noted. Wing length for all marked specimens was measured. A second visit to Hexham was made on 27 June

Table 2.1 Details of the sites used in the study

Location	Latitude/ longitude	Temperature $^\circ$C[a]	Altitude (masl)	Year studied	Number of nests at start of spring[b]	Number of observation days
Sussex	50.864 N/-0.084 W	17.4	82	2012-2013	>100 [16] (2012) <40 [17] (2013)	120 (2012) 50 (2013)
Hexham	54.978 N/-2.100 W	14.4	37	2012	≈20 (17)	3
Inverness	57.554 N/-4.456 W	13.4	5	2013-2014	>100 both years (10 in 2013)	8 (2013) 6 (2014)
Dartmoor	50.5 N/-3.8 W	16.0	>300	1992[c]	50 [4]	NA

[a] Mean land surface temperature 1981-2006 (Hay et al., 2006)
[b] Number of foundresses initially marked is given in the text. Numbers in square brackets denote number of nests that successfully produced B1 female offspring, and at which it was possible to determine social phenotype. Numbers in round brackets show the number of foundresses marked in spring, for where the number of nests with B1 offspring was unavailable
[c] See Field (1996)

(a)

(b)

(c)
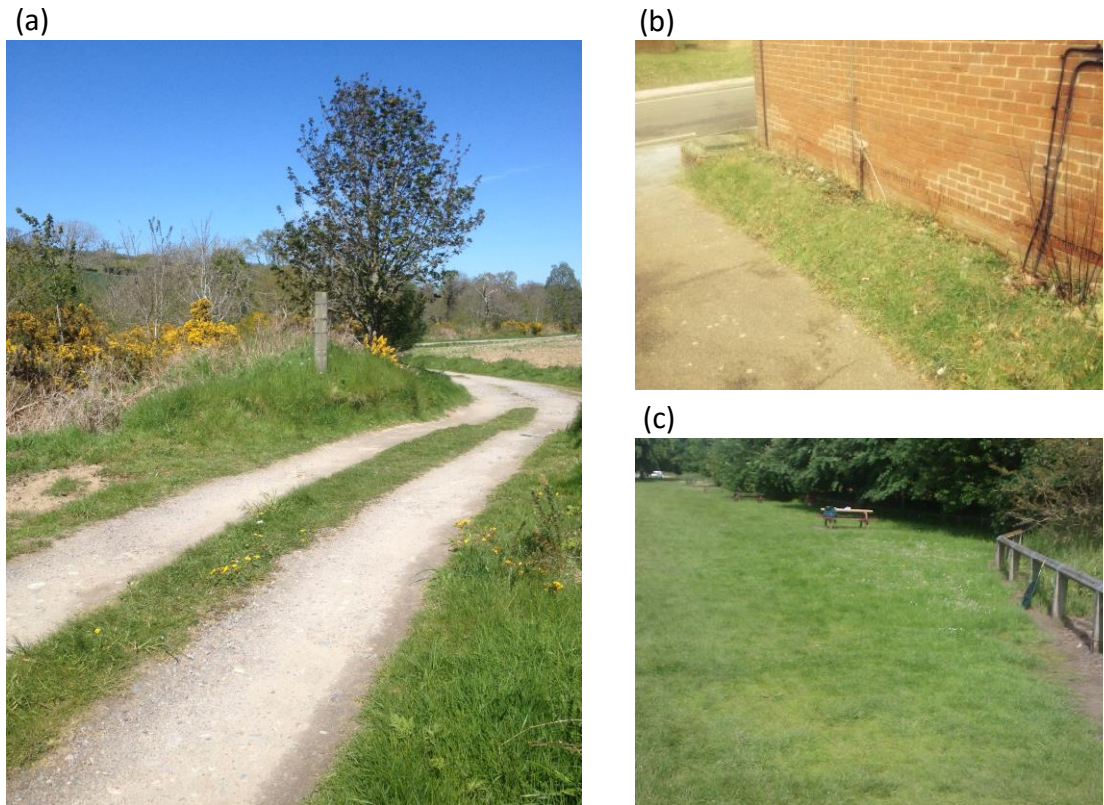
Figure 2.2
The three principal field sites: (a) Inverness, (b) Sussex and (c) Hexham. See Methods, section 2.2.1 for details.

2012, and repeat visits to Inverness on 10-11 July 2013**,** 20-21 August 2013, and 10-11 September 2013 to check for the presence of workers. Foundresses were not marked at Inverness in 2014, but visits were made throughout August to check for provisioning workers. All visits were made on days of suitable weather, and when bees were active Note that logistical constraints meant that systematic observations were not conducted at Hexham and Inverness as at Sussex (see Table 2.1). Therefore, visits did not explicitly consider individual nests but checked for evidence of social behaviour within the two aggregations as a whole (see section 2.3.2). The number of nests in which B1 offspring were observed entering was not recorded; however, B1 activity at both Hexham and Inverness was observed at multiple nests.

2.2.4 Dartmoor foundresses

Wing lengths of specimens from a population on Dartmoor caught in 1992 by J. Field were also measured. Dartmoor is an area of upland generally >300 masl in the southwest UK (50.58 N, -3.91 W), where bees are thought to nest solitarily (Field, 1996; Fig. 2.1).

2.2.5 Climate and weather data

Weather data at each site were taken from nearby web-based weather stations (approximately the same altitude as the study sites) located on Weather Underground (www.wunderground.com). For Sussex the nearest station was in Lewes, 5.8 km away (station IDNS52). For Hexham the nearest station was in Hexham (station INORTHUM28), approximately 2 km from the site. For Inverness the nearest station was less than 1 km away (Station IROSS-SH1) in Maryburgh. Temperature data presented in Table 2.1 are the 1981-2001 annual mean land surface temperature derived from the satellite-mounted Advanced Very High Resolution Radiometer (AVHRR) sensor (see Chapter 4; Hay et al., 2006).

2.2.6 Data analysis

Unless stated otherwise all analyses incorporate data from both 2012 and 2013. Interaction terms between other explanatory variables and year were initially included in maximal models. These were never significant and are not reported. I generally report the main effect of 'year' as a covariate where significant only. All analyses were conducted in the *R* environment (R Development Core Team, 2013), using the lme4 package (Bates, 2015) for generalised linear mixed models (GLMMs). Results are presented ±1 standard error.

Foundresses might use date of first provision in spring as a cue for offspring emergence time if earlier-provisioned offspring emerge earlier in the year (Field et al., 2012). I use a generalised linear model (GLM) with normal errors to test for a relationship between a foundress' first provisioning date and the date of her first B1 offspring emergence. Earlier-starting foundresses may produce more workers because they have more time during which to provision, and/or larger foundresses may produce more workers because they are better at foraging. I therefore use a GLM with Poisson errors to test the effect of foundress size and date of first provision on the number of workers produced. Co-founding may also increase B1 productivity and I therefore also used Pearson's chi-squared test to examine whether co-founded nests produced more workers than singly founded nests.

I used a GLMM with normal errors to test for significant differences in wing length between foundresses and workers, with 'caste' and 'year' as fixed factors and 'nest' as a random factor. I include only those foundresses that produced workers. Within-nest caste-
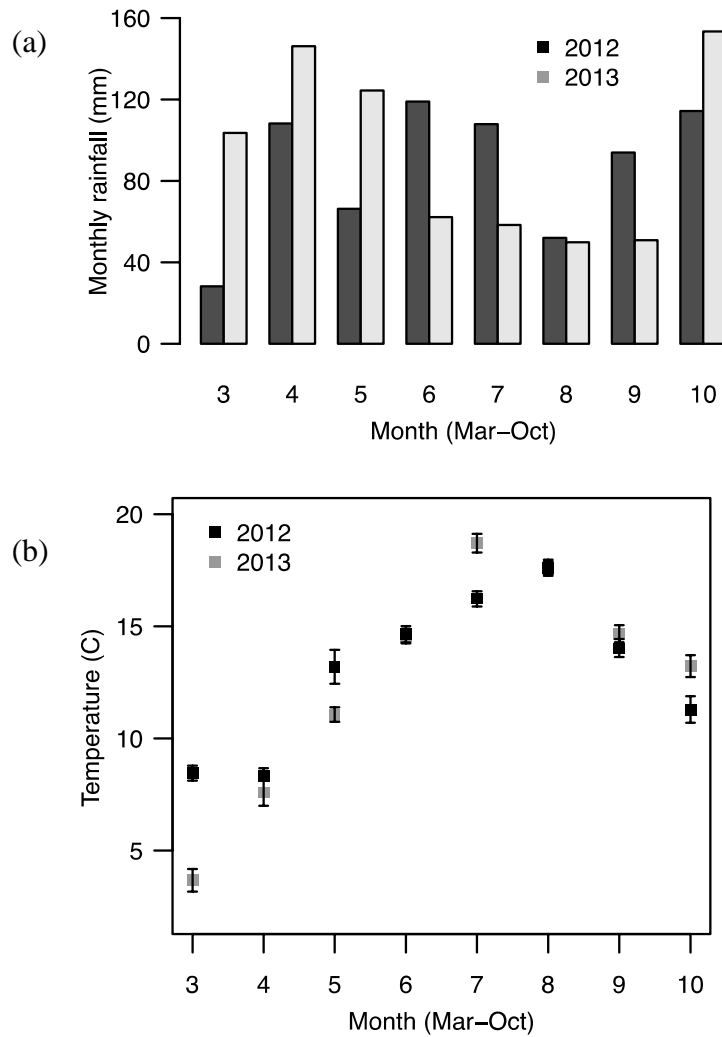
Figure 2.3
(a) Total monthly rainfall between March and October at Sussex in 2012 and 2013. Dark bars are 2012 and light bars are 2013.
(b) Mean daily temperature per month at Sussex in 2012 and 2013. Dark squares are 2012, light squares are 2013. Temperatures are presented ±1SE.

size dimorphism was calculated after Packer and Knerer (1985) as [((F-W)/F)*100)], where F is foundress wing length and W is worker wing length. I used a one-way ANOVA to test for differences in foundress wing length between sites, and Tukey's HSD test to determine significant differences between sites. Foundresses from 'Inverness' include additional samples from other nearby aggregations that were not studied. All foundresses from both years at Sussex were included regardless of whether they produced offspring. Analyses of foundress and worker size, foundress size and the number of workers, and caste size dimorphism excluded co-founded nests, because it was not known which bee was mother to the offspring. In 2013 three bees that had previously been co-foundresses later initiated their own nests independently, and began provisioning during a week where observations were not being made. These three nests were therefore assigned a provisioning start date of the first day of that week.

Environmental variation is known to affect the nesting success of ground-nesting Hymenoptera, with excessive rainfall causing elevated brood mortality (Richards and Packer, 1995; Soucy, 2002). Patterns of rainfall were different between years (Fig. 2.4), and I used this opportunity to examine the effect of weather on nesting success and B1 productivity. Nest co-founding has been shown to reduce the chances of nest failure (Richards and Packer, 1998), here defined as failure to produce any detected B1 offspring. I use a generalised linear model (GLM) with binomial and normal errors to investigate whether both nest failure rates and the number of workers produced differ between years, and whether co-founded nests were less likely to fail.

Finally, to place *L. calceatum* sociality in a broader context it is useful to compare my results with published data from other closely related species. With additional data from more recently published work and the present study I follow Bourke (1999) and use Spearman's rank correlation coefficient to test for a relationship between worker brood size and caste-size dimorphism within the *Lasioglossum* subgenus *Evylaeus* (see Table C.1 in Appendix C for data and sources).

2.3 Results

2.3.1 Nest founding and nesting success

In Sussex, March was considerably warmer in 2012 than in 2013, leading to an extended period of foundress emergence in 2012. In contrast, the spring of 2013 started later, resulting in a shorter foundress emergence period (Figs. 2.3, 2.4). After the early start in 2012, the weather deteriorated and was very wet for much of the remaining spring and summer (Fig. 2.3a). After foundress emergence in 2013, however, the weather was much drier with extended periods of sunshine and a summer heat wave (Fig 2.3a, b). A significantly greater proportion of nests failed to produce any detected B1 offspring in 2012 than in 2013 ($X^2_{1,125}$=19.578, p<0.001; 2012: 84%, n=84 failed, n=16 successful, 2013: 37%, n=10 failed, n=17 successful). A small proportion of nests were co-founded (5% (5) in 2012, 16% (4) in 2013) with up to three provisioning co-foundresses in a nest, and these were significantly less likely to fail than singly founded nests ($X^2_{1,125}$=4.719, p=0.030).

2.3.2 Social phenotypes detected

Bees at Sussex were found to nest socially in both years, with workers provisioning a second brood of reproductives in all but 2-4 nests in 2012. The life cycle is summarised in Figure 2.4.
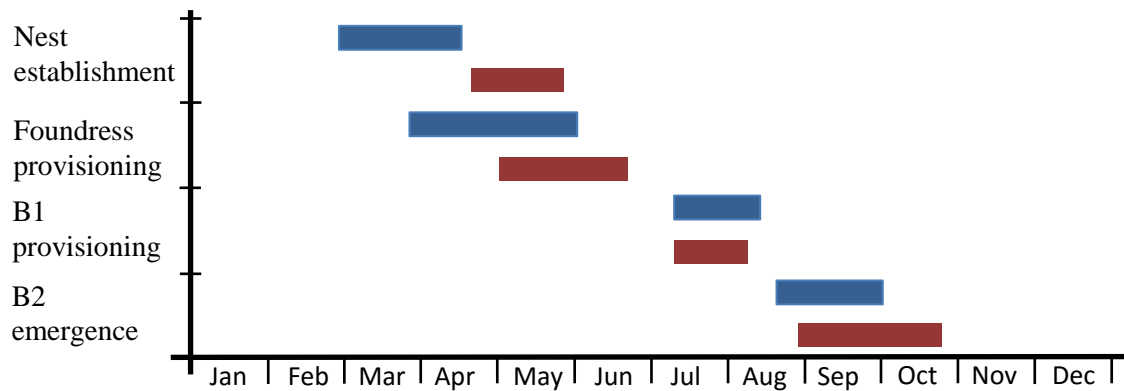
Figure 2.4
Nesting cycle of *Lasioglossum calceatum* on the University of Sussex campus in 2012
(blue bars) and 2013 (red bars). Temporal overlap between stages within each year
represents periods when not all bees were at the same stage.

Two nests at Sussex in 2012 at which B1 offspring were observed entering and leaving, but never provisioning, were deemed to be solitary. Two further nests in the same year were possibly solitary, although it was less clear because the foundresses were unmarked. All nests in 2013 with detected B1 offspring were social. Preliminary analysis of microsatellite data from B2 offspring excavated at Sussex in a subsequent year (see Chapter three) suggests that foundresses generally monopolised reproduction within nests (Davison and Field, in prep; see Appendix B for brief methodology). Bees at both Hexham and Inverness were solitary, B1 offspring observed entering nests but never provisioning. Some nests at Inverness and Sussex (see below) were co-founded, but observations to test for this were not made at Hexham. The sampling method employed at Hexham and Inverness is unlikely to have overlooked social nests, as there were no days at Sussex on which multiple nests were active where B1 provisioning was not observed at any nest.

A single individual marked as a worker in 2012 appeared during the nest-founding phase of 2013, but did not successfully initiate a nest, suggesting that some B1 offspring entered directly into hibernation. Most offspring entering directly into hibernation would not have been marked if they did not start provisioning: in both years at Sussex unmarked bees were observed leaving and entering nests after all workers there had been marked, and flying around the aggregation as foundresses do in spring when searching for a nesting site.

2.3.3 Geographic size variation

There was a significant effect of site on foundress size ($F_{4, 111}=9.372$, $p<0.001$). Tukey's HSD test revealed that foundresses from both Dartmoor and Inverness were significantly smaller than
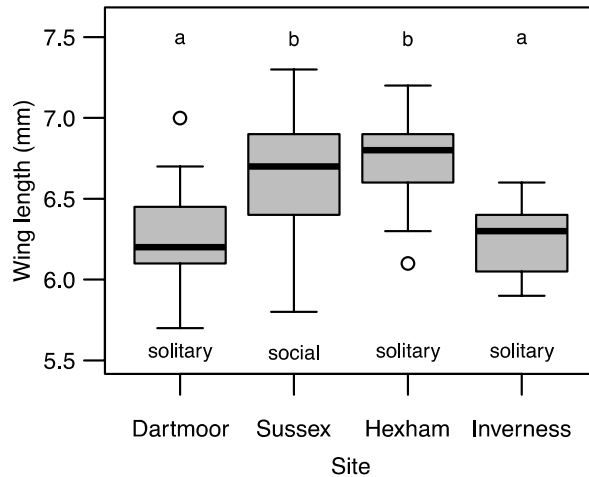
Figure 2.5
Boxplot showing the wing lengths of *Lasioglossum calceatum* foundresses from sites with different social phenotype. Dartmoor n=15, Sussex n=73, Hexham n=17, Inverness n=11. For each box and whiskers, the thick horizontal line shows the median, the box the interquartile range and the whiskers the maximum/minimum value where outliers are not depicted. Separate circles depict outliers, which are data points more than 1.5 times the interquartile range below or above the 25th or 75th percentile respectively. Letters above the boxes denote significant differences (see text for statistics).
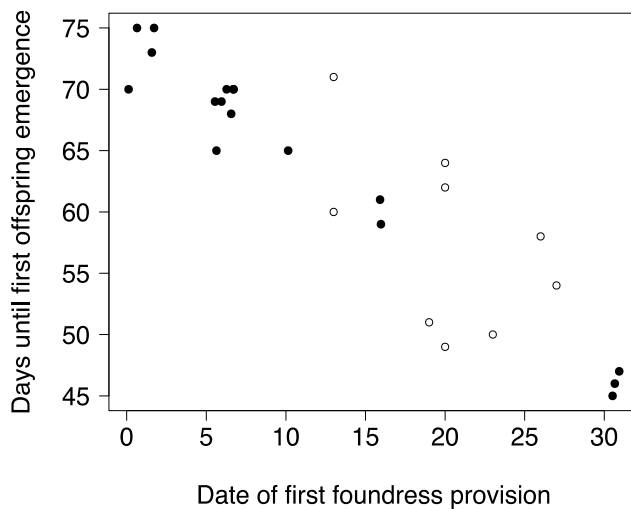
Figure 2.6
Relationship between the date on which a *Lasioglossum calceatum* foundress was first recorded provisioning and the number of days until that foundress' first offspring emerged. Data are shown for both years; open circles are from 2012 and filled circles from 2013. The later a foundress began provisioning, the shorter the time before her first offspring emerged (see text for statistics). Day 0 is April 30. Individual points are horizontally jittered to show overlapping data.

those from Sussex and Hexham (Fig 2.5; see Table C.2 in Appendix C for pairwise comparisons).

2.3.4 Foundress provisioning and B1 emergence

The time between the date of a foundress' first recorded provisioning trip and the date of her first B1 offspring emerging decreased linearly with date of first provision ($F_{1,25}$=115.49, p=0.001; Fig. 2.6). This pattern meant that earlier-provisioning foundresses did not produce
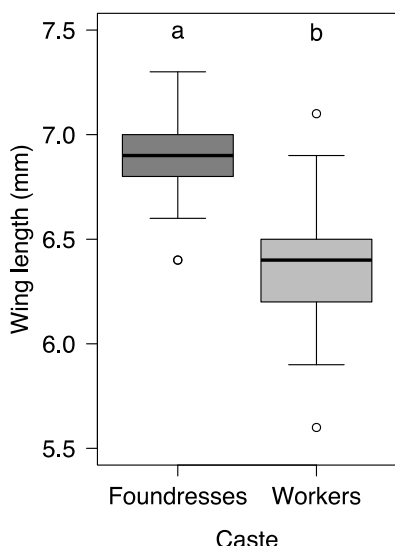
Figure 2.7
Boxplot showing the wing lengths of *Lasioglossum calceatum* foundresses and the workers they produced from both years at Sussex. Letters above the boxes denote a significant difference (see text for statistics). Foundresses n=22, workers n=89. For each box and whiskers, the thick horizontal line shows the median, the box the interquartile range and the whiskers the maximum/minimum value where outliers are not depicted. Separate circles depict outliers, which are data points more than 1.5 times the interquartile range below or above the 25th or 75th percentile respectively.

offspring that emerged earlier ($F_{1,25}$=2.704, p=0.113). Foundresses that began provisioning earlier did not produce more workers ($F_{1,25}$=2.704, p=0.335).

2.3.5 Bee size and number of workers

Foundresses produced workers significantly smaller than themselves (Fig. 2.7; n=18 nests, X^2_1=51.655, p<0.001), with a mean within nest caste-size dimorphism of 6.6% (foundresses=6.88mm±0.06, workers=6.39±0.03). There was no effect of year (X^2_1=0.011, p=0.918) such that foundresses that successfully produced workers were the same size in both years, and produced workers of the same size. Among nests caste-size dimorphism ranged from 0-13%, with four nests containing one or more workers that were the same size as the foundress, and there was large size overlap between castes (Fig. 2.7). Larger foundresses did not produce larger workers (X^2_1= 2.443, p= 0.295).

Foundresses produced a mean of 2±0.36 workers in 2012 (range 1-5) but were significantly more productive in 2013, producing 3.5±0.42 in 2013 (range 1-4) (n=17 nests in both years, W=70, p=0.009). Similarly, larger foundresses also did not produce more workers (X^2_1=0.451, p=0.502). Co-founded nests, however, did produce significantly more workers than singly-founded nests (X^2_1=8.217, p=0.004, mean number of offspring produced: singly-founded=2.35±0.30, co-founded=4±1.09, 2012 n=5/100, 2013 n=4/27).

2.3.6 Natural enemies

The halictid cuckoo parasite *Sphecodes* was continuously present in small numbers at Sussex in 2012, and a single parasitic fly (species unknown) was observed following a foundress to her nest and subsequently entering. Bee flies (*Bombylius*), known to parasitize *Lasioglossum* (Wyman and Richards, 2003; Boesi et al., 2009), were also present at the aggregation during spring. Only a single *Sphecodes* female was observed in the spring of 2013. This was caught and later identified as *S. monilicornis*, known to be a cuckoo of *L. calceatum* (Bogusch et al., 2006 and references therein). Ants (*Lasius* sp.) attacked nests during the foundress-provisioning phase, preventing foundresses from entering their nests with pollen, and also raided nests during the B1 worker phase.
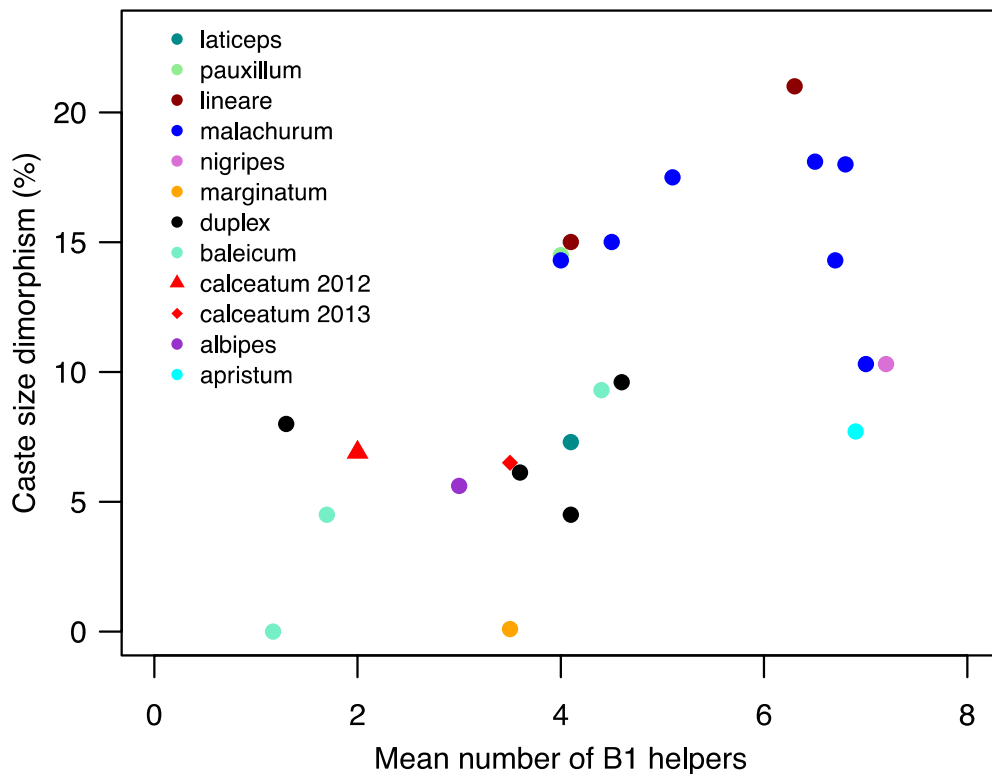
Figure 2.8
Scatterplot showing the significant positive correlation between the number of first brood workers (colony size) and caste-size dimorphism in eleven species from the *Lasioglossum* subgenus *Evylaeus* (see Table C.2 for data sources), including the new data from *L. calceatum* reported in this chapter. Species names are given in the legend. Species with more workers tend to have greater caste size dimorphism (see *Social level in Evylaeus*). More socially specialized species tend towards the upper right of the plot.

2.3.7 Social level in Evylaeus

Across studied populations of *Evylaeus* there was a significant positive correlation between the number of first brood workers and caste-size dimorphism (r=0.651, p=0.001, n=24). The less socially specialised species tend towards the lower left of Figure 2.8, and the more specialised the upper right. Results from Sussex place *L. calceatum* in the lower left portion of Figure 2.8, indicating that it is relatively less socially specialised than other members of the subgenus.

2.4 Discussion

Socially polymorphic sweat bees are of particular interest for elucidating the behavioural and genetic changes associated with the origins of eusociality. Nevertheless, few studies have focussed on temperate-zone socially polymorphic species. In this chapter I determined the social phenotype of *L. calceatum* at different latitudes in the UK and investigated its life cycle across two years at the southernmost site (Fig. 2.1). The results presented in this chapter confirm that *L. calceatum* is socially polymorphic within the UK. Nests in the northern UK (Hexham and Inverness) were solitary, while those in the south at Sussex were social. Sociality at Sussex was characterised by on average 2-3.5 workers per nest and a small mean caste-size dimorphism of 6.6%. The number of functional workers produced and rate of nest failure differed significantly between years, highlighting the effect of inter-year environmental fluctuations on soil-nesting Hymenoptera. I now discuss social polymorphism and sociality in *L. calceatum*.

2.4.1 Social phenotype in the UK

Most nests at Sussex, in the south of the UK, were social with a first brood of provisioning workers and a second brood of reproductives. In contrast nests at Hexham in the north of the UK (Fig 2.1) were solitary, and foundresses produced only a single brood of non-provisioning reproductives. These results indicate that solitary nests reported by Field (1996) from Dartmoor, an area of southern upland in the UK, are consistent with the altitude-based polymorphism originally reported in Japan by Sakagami and Munakata (1972). Foundresses at Dartmoor and Inverness were smaller than those at Sussex and Hexham, consistent with other sweat bees in which those persisting at higher latitudes or altitudes are smaller (Kirkton, 1966; Soucy, 2002; Field et al., 2012, see Chapter 4).

It is likely that sociality in northern and upland areas is precluded because the season is too short for more than one brood (Soucy, 2002, Kocher et al., 2014; but see Miyanaga et al., 1999). Indeed, in both 2012 and 2013 foundress provisioning occurred later at Hexham and Inverness

respectively than at Sussex. Similarly, Field et al. (2012) reported that northern *H. rubicundus* foundresses began provisioning considerably later than those in the south, and that their offspring took longer to develop. In both cases B1 offspring in the north probably emerge too late to successfully rear a second brood because nests are initiated later and cooler temperatures lengthen development time (Table 2.1; Weissel et al., 2006; Hirata and Higashi, 2008). Such constraints probably also limit body size and lead to the significantly smaller size of foundresses from Dartmoor and Inverness relative to those from Sussex (Fig. 2.5). In light of this it is interesting that foundresses from Hexham and Sussex did not differ in size. One possible explanation is that adult body size follows a saw-tooth cline (Roff, 1980; Field et al., 2012). Foundresses just to the north of the transition between social and solitary nesting might be relatively less time-stressed than those just to the south, because they must rear only a single brood per year instead of the two required for sociality (Field et al. 2012). In consequence, offspring may be able to attain a larger size because they can spend longer developing, if foundresses can provide them with extra food, which is likely to confer benefits such as increased tolerance of cooler temperatures and survival through hibernation (Stone, 1994; Potts, 1995; Brand and Chapuisat, 2012).

At least two nests at Sussex in 2012 were confirmed to be solitary, demonstrating that *L. calceatum* can sympatrically express both phenotypes as in other polymorphic halictids (Packer, 1990; Soucy, 2002). One explanation for this observation is that B1 offspring emerging later in the season are less likely to remain as workers because there is not enough time remaining to successfully raise a second brood (Hirata and Higashi, 2008; Field et al., 2010). However, offspring from the solitary nests at Sussex in 2012 were among the first and last to emerge, although the sample size was very small (n=9). Factors other than time of year may be important in determining social phenotype. Packer (1990) suggested that foundress absence could induce B1 offspring to enter hibernation. This is unlikely here because foundresses in the two solitary nests were still alive at the time of offspring emergence, demonstrating that foundress presence does not always result in sociality (Hirata and Higashi, 2008). Yanega (1989, 1993) proposed that mating soon after eclosion could induce offspring to hibernate directly. This is impossible to test, but seems unlikely because offspring emerging simultaneously at other nests became workers and they would have had the same access to males. Moreover, although theoretically plausible under certain conditions (Field and Lucas, 2013), this mechanism of caste determination has been shown not to occur in *L. albipes*, the sister species to *L. calceatum* (Plateaux-Quénu and Packer, 1998). Caste-biasing mechanisms are still poorly understood in halictids, and pre-emergence mechanisms mediated via nutrition provided by the foundress cannot be ruled out (Richards and Packer, 1994; Brand and Chapuisat, 2012).

The B1 female from 2012 seen again in 2013 demonstrates that B1 offspring can successfully overwinter, even though this female did not successfully found a nest in 2013. Observations of B1 offspring searching the nesting area and investigating nests in both years further strongly suggest that brood divalency occurs in *L. calceatum*, as is well known in *H. rubicundus* (Yanega, 1989).

2.4.2 Foundress provisioning and B1 offspring

Contrary to the pattern found by Field et al. (2010) in *H. rubicundus*, there was no evidence that the B1 offspring of earlier-provisioning foundresses emerged earlier. Instead, development time of B1 offspring decreased linearly throughout spring (Fig. 2.6). Strikingly, in 2013 three former co-foundresses began provisioning at least three weeks after most other foundresses, but their offspring emerged at a similar time. This suggests that foundresses could not use time of provisioning to anticipate time of offspring emergence, and hence social phenotype. Temperature is known to affect larval development time (Weissel et al., 2006), and Field et al. (2012) attributed a similar pattern in another population of *H. rubicundus* to increasing growth rates caused by rising temperatures throughout spring. It is also possible that earlier-provisioned offspring suffer higher mortality because larval mortality rates are likely to be greater when development is longer (Roff, 1980). Nevertheless, unless offspring that are provisioned earlier in the season suffer extremely high mortality across all nests, larval mortality alone could not explain the linear decline in development time show in Figure 2.6. It is likely that the start of provisioning closely corresponds with the date of egg-laying, as sweat bees are thought to provision enough for one egg per day (Richards, 2004). Another possibility is that the time between foundress provisioning and offspring emergence does not represent development time (Yanega, 1997). Weissel et al. (2006) showed that a 5°C difference in temperature (around the difference in temperatures experienced by early and late-provisioned bees, see Figures 2.3b, 2.4, 2.6) resulted in a difference in development time of approximately 15 days. The difference in observed development time between the first and last provisioned B1 offspring in Figure 2.6 is around 30 days. Therefore, the possibility that early-provisioned B1 females completed development but delayed eclosion cannot be discounted (e.g. Wcislo et al., 1993). Excavating nests prior to B1 emergence could help resolve this issue.

The number of functional workers produced was related to neither foundress size nor the date of first provision. A positive relationship might be expected because (i) earlier-starting foundresses would have a longer period during which to provision, and/or (ii) larger foundresses should be able to carry more pollen and provision for longer. Larger bees could provision for longer because they are more tolerant of lower temperatures (Stone, 1994; Potts, 1995), although Field

et al. (2012) found no effect of size on foraging parameters. However, when provisioning their first brood, foundresses must take other factors into account such as increasing risk of mortality or parasitism through foraging, and potential future reproductive conflict within the nest. (Cant and Field, 2001; Strohm and Bordon-Hauser, 2003; Zobel and Paxton, 2007). Foundresses may therefore derive significant genetic benefits from provisioning sufficient offspring to raise a second brood, while remaining alive both to care directly for their own developing B1 offspring (Knerer, 1969; Plateaux-Quénu, 2008) and to lay B2 eggs (Field et al., 2010). Consequently, foundresses probably cease B1 provisioning at a point that optimises their fitness given these factors.

One possible benefit of large size not assessed here might be better reproductive dominance within nests (e.g. Breed and Gamboa, 1977). Worker size was independent of foundress size so that caste-size dimorphism was largest in nests with the largest foundresses. In nests of *H. ligatus*, foundresses that were the largest relative to their workers appeared to gain a greater share of reproduction (Richards et al., 1995; Richards and Packer, 1996). This may not be the case in all species, however: reproduction was successfully monopolised in nests of *H. rubicundus* where workers and egg-layers did not differ in size (Field et al., 2010). The future application of genetic markers to B2 offspring will help to resolve this issue in *L. calceatum* (Davison and Field, in prep).

Results from this study provide further evidence that caste-size dimorphism in *L. calceatum* is geographically variable. In France foundresses are on average 8.3-13% larger than workers (Plateaux-Quénu, 1992), whereas in Japan, Sakagami and Munakata (1972) reported 3.5-5.5%, and in the present study mean caste-size dimorphism was 6.6%. The reason for such differences remains unclear, but it could be that caste-size dimorphism is greater in areas where bees experience warmer temperatures (Sakagami and Munakata, 1972; Soucy, 2002), a pattern generated experimentally by Plateaux-Quénu and Plateaux (1980). This could be because the longer growing season further south allows the production of larger reproductives. If there is little advantage in also producing larger workers (Strohm and Liebig, 2008), worker size may remain constant or at least increase at a slower rate. Therefore, caste-size dimorphism would be larger at lower latitudes and could explain the discrepancy in measurements reported between the present study and that of Plateaux-Quénu (1992).

Previous studies on *Halictus ligatus* have emphasised how inter-year variation in environmental conditions can affect key characteristics such as caste-size dimorphism (Richards and Packer, 1996). Nest failure was significantly greater and the mean number of workers produced significantly fewer in 2012 than 2013. Development of B1 offspring occurred mostly during

June and early July (Fig. 2.3), which in 2012 were much wetter than in 2013 (Fig. 2.1). In 2012, 84% of nests failed to produce any detected offspring, whereas in 2013 the nest failure rate was only 38%. It seems possible that the higher nest failure rate and smaller brood sizes in 2012 compared with 2013 resulted from differential brood mortality between years due to intense summer rainfall in 2012. Poor weather is unlikely to have affected foundress provisioning in 2012, because most foundresses provisioned during the warm and sunny weeks of May (Fig. 2.4). High rates of nest failure are common in halictids (Ulrich et al., 2009), particularly during the foundress phase when nests are most vulnerable (Sakagami and Fukuda, 1989). Failure can be accentuated by cool and wet weather, when brood become mouldy (Richards and Packer, 1995; Soucy, 2002), and the results here highlight how strongly weather conditions can influence reproductive success of ground-nesting Hymenoptera.

2.4.3 Social level in *L. calceatum*

Across both years at Sussex, mean within-nest caste-size dimorphism was 6.6%, and foundresses produced a mean of two and 3.5 workers in 2012 and 2013 respectively. Both figures are small in comparison with more specialised obligate social species in the *Lasioglossum* subgenus *Evylaeus* (Packer and Knerer, 1985; Wyman and Richards, 2003; Fig. 2.8). Although foundresses were significantly larger than workers there was still considerable overlap in size (Fig. 2.6a). Obligate primitively eusocial species such as *L. malachurum* not only produce more workers but also show distinct and almost non-overlapping bimodality in caste sizes (Knerer, 1980; Wyman and Richards, 2003), reflecting an increased specialisation for eusocial nesting. The significant cross-species correlation between group size and caste-size dimorphism may reflect an adaptation to reduce kin conflict if foundresses more easily behaviourally dominate a larger number of smaller workers (Kukuk and May, 1991; Bourke and Franks, 1995).

The ability to nest solitarily is not necessarily lost in obligate eusocial nesters (Rehan et al., 2013), but polymorphism probably limits the degree to which social behaviour can become specialised. Nevertheless, *L. calceatum* may be more socially specialised than other polymorphic species. For example, *L. calceatum* nests socially in areas where its polymorphic sister species *L. albipes* is solitary (Plateaux-Quénu et al., 2000), and B1 females at Sussex chose to become workers later in the season than B1 offspring of *H. rubicundus* (Field et al., 2010). Together, this suggests that sociality in *L. calceatum* may occur over a wider range of conditions than in other polymorphic species; perhaps suggesting that social phenotype may be less plastic. Field transplantation experiments (Field et al., 2010, 2012) will be required to

ascertain the existence or extent of social plasticity in *L. calceatum* (see Chapter 3). Interestingly, *L. albipes* is thought to not be plastic (Plateaux-Quénu et al., 2000).

Chapter three

Loss of plasticity in the socially polymorphic sweat bee *Lasioglossum calceatum*

Abstract

Eusociality is characterised by reproductive division of labour within social groups, where some individuals forgo direct reproduction to instead help raise kin. Socially polymorphic sweat bees are ideal models for addressing the mechanisms underlying the transition from solitary living to eusociality, because individuals in the same species can express either social or solitary behaviour. A key question is whether alternative social phenotypes represent environmentally induced plasticity or fixed genetic differences between populations. In this chapter I focus on *Lasioglossum calceatum*, in which northern or high altitude UK populations are solitary, whereas more southern or low altitude populations are typically eusocial. To test whether social phenotype responds to local environmental cues, I transplanted adult females from a solitary, northern population, to a southern site where native bees are typically eusocial. Most native southern nests were social, with foundresses producing small first brood (B1) females that became workers. In contrast, nine out of ten nests initiated by transplanted bees were solitary, in which female offspring were the same size as transplanted foundresses and entered directly into hibernation. Only one of these ten nests became social. Social phenotype was unlikely to be related to cues such as conditions experienced by nest foundresses when provisioning B1 offspring, or B1 emergence time, both previously implicated in social plasticity in two other socially polymorphic sweat bees. Results presented in this chapter suggest that social phenotype in *L. calceatum* is predominantly determined by fixed genetic differences between populations, and that plasticity is in the process of being lost by bees from northern populations.

3.1 Introduction

Eusociality is characterised by some individuals within a social group forgoing direct reproduction to assist in the production of collateral kin (Wilson, 1971), and its origin is considered to be a major transition in evolution (Maynard Smith and Szathmáry, 1995; Bourke, 2011a). Once regarded as a challenge to evolutionary theory, functional questions about how eusociality first evolved have largely been answered within the framework of inclusive fitness theory (Hamilton, 1964; Bourke, 2011a, b). However, comparatively less attention has been paid to determining the mechanisms by which individual offspring become workers or reproductives (e.g. Yanega, 1997; Kapheim et al., 2012, 2015a; Field et al. 2012). Interrogating these behavioural, physiological and genetic processes requires taxa that straddle the divide between solitary and social nesting, and where it is possible to observe individuals making the choice to either remain at the nest as a worker, or become a reproductive (Field et al., 2010; Rehan and Toth, 2015).

Sweat bees (Hymenoptera: Halictidae) are ideal models for this purpose because they lack fixed castes and exhibit a broad range of social behaviour, from solitary nesting to primitive eusociality (Schwarz et al., 2007). From as few as two independent origins within the subfamily Halictinae, sociality has been repeatedly lost (Danforth, 2002; Danforth et al., 2003; Gibbs et al., 2012a). In some sweat bee lineages sociality has been lost completely through the evolution of oligolecty (floral specialisation), which can preclude sociality because flowers are available for only a small portion of the year (Danforth, 2002), or parasitism. Others have reverted to solitary nesting only in parts of their range, and are therefore termed socially polymorphic (Danforth, 2002; Soucy and Danforth, 2002; Kocher et al., 2014). Socially polymorphic sweat bees therefore offer unparalleled opportunities for investigating the roles of environmental and genetic factors in the transition from solitary nesting to eusociality (Chapuisat, 2010).

Social and solitary life cycles in polymorphic sweat bees are characterised by the number of broods raised: sociality requires at least two consecutive broods of offspring, whereas solitary nesting requires only one. In both cases mated females (foundresses) emerge in spring from hibernation and initiate subterranean nests. They then mass provision a first brood (B1), providing each offspring with a ball of pollen and nectar in separate, sealed brood cells. In social populations, at least some females remain at the nest as workers to provision a second brood (B2) of reproductive offspring. In solitary populations all female offspring mate then enter hibernation. Workers may also switch castes and become replacement queens within their nest if the original nest foundress dies before the end of the season (e.g. Mueller, 1991; Field et al., 2010). In contrast with social nests, in solitary nests of socially polymorphic species, all B1

females mate and directly enter hibernation to found their own nests in the following year. Season length has long been implicated as a key proximate constraint on the distribution of alternative social phenotypes, because sociality can occur only where the season is long enough to allow the bees to rear two broods (Sakagami and Munakata, 1972; Hirata and Higashi, 2008; Kocher et al., 2014; Davison and Field, in press, Chapter 2).

The early stages of eusociality are thought to have involved initial plasticity and its subsequent loss (West-Eberhard, 1996, 2003; Rehan and Toth, 2015). However, comparatively little is known about the extent to which alternative phenotypes in polymorphic sweat bees result from environmentally mediated plasticity, or represent genetically distinct strategies. Some insight has been gained from work on *Halictus rubicundus* Christ in the United Kingdom (UK) and North America. Reciprocal field transplants of foundresses between social and solitary nesting sites in the UK have revealed that social phenotype is plastic (Field et al., 2010, 2012). Bees from a southern, social site all nested solitarily when transplanted to a northern site where social nesting by native bees had never been observed. Likewise, the majority of bees transplanted to the south from the north nested socially, despite social behaviour having never previously been observed in the northern population (Field et al., 2010). First brood offspring in nests that became social emerged earlier in the season than offspring from nests that were solitary, suggesting that time remaining in the season may be an important cue influencing whether offspring remain at the nest as workers (Hirata and Higashi, 2008; Field et al., 2010). Population genetic work strongly suggested there are no barriers to gene flow among populations expressing social and solitary behaviour (Soro et al., 2010).

In contrast, Soucy and Danforth (2002) uncovered considerable mitochondrial differentiation between social and solitary populations of North American *H. rubicundus*, indirectly suggesting that, in North America, social phenotype might have a fixed genetic component. Non-plasticity has been demonstrated experimentally in only a laboratory common garden study of *Lasioglossum albipes* Fabricius (Plateaux-Quénu et al., 2000). Foundresses from different populations where native bees express alternative social phenotypes could not be induced to switch phenotypes in the laboratory, after exposure to conditions designed to mimic those experienced by populations expressing the alternative phenotype. These results suggest that there are probably some fixed genetic differences between bees from social and solitary populations (Plateaux-Quénu et al., 2000). However, without the realism of a natural field setting it is not possible to fully account for the effects of unmeasured environmental variables, and field transplants are required to test comprehensively for social plasticity (Yanega, 1997; Field et al., 2012).

Lasioglossum calceatum Scopoli is a common and widespread socially polymorphic sweat bee of the Palearctic (Sakagami and Munakata, 1972; Pesenko et al., 2000; Davison and Field, in press, Chapter 2). Although the existence of social polymorphism and social behaviour is now well established in *L. calceatum* (Davison and Field, in press, Chapter 2), almost nothing is known about the mechanisms underlying the expression of alternative phenotypes. Therefore *L. calceatum* is an ideal candidate for conducting field transplants to test the extent to which social phenotype is plastic. In this chapter I test for social plasticity by transplanting foundresses from a northern UK site where only solitary behaviour is observed, to a southern UK site where native bees express primitive eusociality (Davison and Field, in press, Chapter 2).

In this chapter I focus on three characteristics of B1 females linked to social phenotype: pollen collection, body size and emergence time. First, B1 females that become workers typically begin bringing pollen back to the nest within one or two days of emergence (Davison, pers. obs). Second, sweat bee workers tend to be smaller than their mothers (Packer and Knerer, 1985; Schwarz et al., 2007). Indeed, foundresses native to the southern UK site produce workers that are significantly smaller than themselves (Davison and Field, in press, Chapter 2), while offspring in solitary populations are the same size as their mothers (Sakagami and Munakata, 1972, Soucy, 2002). Third, the decision of an offspring to stay at the nest as a worker may depend on reaching adulthood sufficiently early in the season, and later-emerging offspring might therefore be more likely to directly enter hibernation (Hirata and Higashi, 2008; Field et al., 2010). However, although pollen collection is intrinsically linked with sociality, small size is not synonymous with social behaviour: workers in some sweat bee populations may be the same size as foundresses (Field et al., 2010), and smaller 'worker-sized' offspring may emerge too late to be workers (Hirata and Higashi, 2008).

If social phenotype is plastic, bees transplanted from solitary to social sites should respond adaptively to the new environmental conditions, and express sociality or solitary bivoltinism at the new site (Field et al., 2010, 2012). If, however, there are fixed genetic differences for social phenotype between social and solitary populations, offspring of transplanted foundresses should enter directly into hibernation without working. While most native nests were social, most transplanted foundresses produced offspring that were the same size as themselves and which entered directly into hibernation instead of becoming workers. This result suggests that environmental factors may have played a less significant role in determining social phenotype than fixed genetic differences between the two populations.
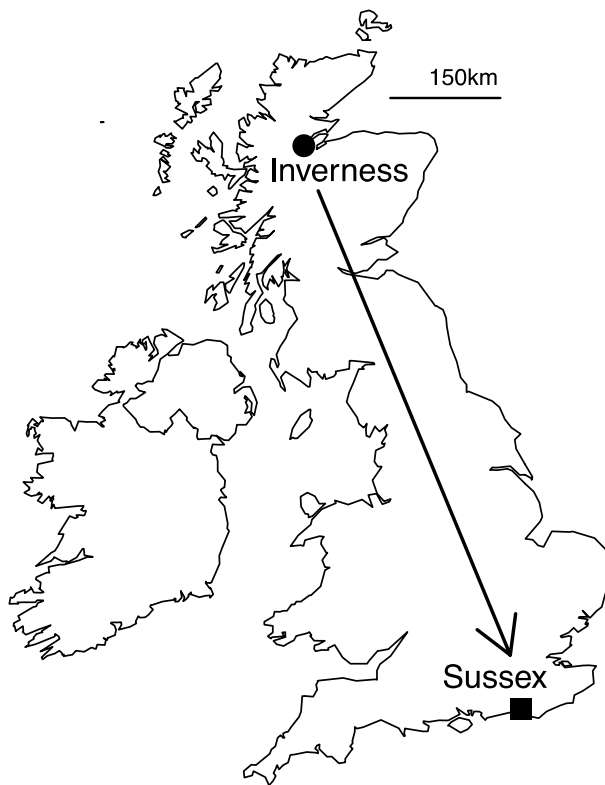
Figure 3.1
Map of the UK and Ireland showing where bees were collected (Inverness) and transplanted to (Sussex).

150km

Inverness

Sussex

3.2 Methods

3.2.1 Transplant

Foundresses were transplanted from Dunglass Island near Inverness in the north of the UK, to a nesting aggregation at the University of Sussex campus in the south of the UK (Table 3.1; Fig. 3.1; see also Chapter 2 for details of the study sites). Social nests of *L. calceatum* contain at least some offspring that remain as workers but social nesting has never been observed at Inverness (Davison and Field, in press, Chapter 2). Transplantation was carried out in two distinct periods; August 2014 (autumn transplant) and May 2015 (spring transplant). Autumn-transplanted bees were freshly emerged reproductive females, captured as they returned to their nests from feeding/mating flights at Inverness, and approximately 70 were caught during two trips to Inverness on 15-16 August 2014 and 27 August 2014. Spring-transplanted bees were nest foundresses, captured on return from feeding or provisioning flights on 16 May 2014. Most of the 202 foundresses captured in spring were not carrying pollen, and therefore were likely to have been caught prior to or just after initiating their own nests. In both cases bees were caught at the aggregation with an insect net, and marked on the clypeus and thorax with a single spot of enamel paint (Revell® and Humbrol™ enamel model paints) with a pin. Marks denoted the

time of transplant (autumn or spring) and additionally, for the spring-transplanted bees whether or not the bee was provisioning when caught, as determined by the presence of pollen loads on the tibial scopae. Immediately after capture, and during travel, bees were kept in separate plastic tubes in a cold box containing ice packs. At all other times and prior to release bees were kept in a fridge.

In the evening of their arrival at Sussex after both collecting trips, autumn-transplanted bees were released into 14L plastic buckets filled with soil and embedded into the ground away from the nesting aggregation at Sussex. Artificial nest holes (15-20cm) were created in each bucket by pushing a metal rod into the soil, and buckets were covered with netting to prevent bees escaping from them. Early the following morning the netting was removed, and bees were thus released into an insect-proof cage that covered the buckets. This was filled with flowers so that bees could continue to feed before overwintering. The cage prevented any mating between transplanted females and native males, and therefore any offspring produced contained genes exclusively from the source population. Before the start of spring 2015, buckets containing the autumn-transplanted bees were embedded directly at the native aggregation. Spring-transplanted bees were released directly into artificial holes among nests initiated by native bees at Sussex over a three-day period, starting from the day of their arrival at Sussex (17-19 May 2015). Bees were released each evening by placing them in a short section of plastic tubing, which was held vertically over the entrance to an artificial nest hole, so allowing the bee to walk down the tube and into the hole. Due to the restricted number of bees at Sussex, it was not possible to conduct a control transplant to test for the effect of transplantation on social phenotype. However, previous transplant studies of *H. rubicundus* showed that being transplanted did not affect social phenotype (Field et al., 2010, 2012). Furthermore, behaviour of *L. malachurum* also appeared to be unaffected by transplantation (see Chapter 5), and the ready nesting of sweat bees in cages shows they are robust against removal from their natal environment (Plateaux-Quénu et al., 2000). Therefore, it is unlikely that the behaviour of transplanted *L. calceatum* was affected by transplantation per se.

Table 3.1 Details of the sites used in the study

Location	Latitude/ Longitude/	Temperature $^{o}C^{a}$	Season length[b] (months)	Altitude (masl)	Native social phenotype
Sussex	50.864 N/-0.084 W	17.4	6.1	82	Social
Inverness	57.554 N/-4.456 W	13.4	4.8	5	Solitary

[a]Mean land surface temperature 1981-2006 (Hay et al., 2006).
[b]Calculated as the mean number of days in the season during which the LST is greater than 16°C (see Chapter 4 for details).

All native and transplant-initiated nests where the foundress was marked and that produced B1 female offspring were included in the final analysis. Therefore, sample sizes simply reflect nests that did not fail prior to the emergence of B1 female offspring.

3.2.2 Spring phenology of native and spring-transplanted foundresses

This experiment was designed so that foundresses would be transplanted from Inverness to Sussex in both the autumn of 2014 and the spring of 2015. All transplanted and native bees were then observed during the 2015 season to determine whether of not B1 females became workers. Native foundresses were first observed provisioning on 20-April 2015; however, because the season started later in Inverness than at Sussex it was not possible to transplant foundresses until mid-May (Fig. 3.2). In consequence, the first spring-transplanted foundresses did not begin provisioning until 20-May 2015, four weeks after the first native foundresses. Nevertheless, not all native foundresses had finished provisioning before spring-transplanted foundresses started, and there was overlap in the timing of provisioning between native and transplanted foundresses (Fig. 3.2). Two native foundresses began provisioning *after* spring-transplanted foundresses (Fig. 3.5a).
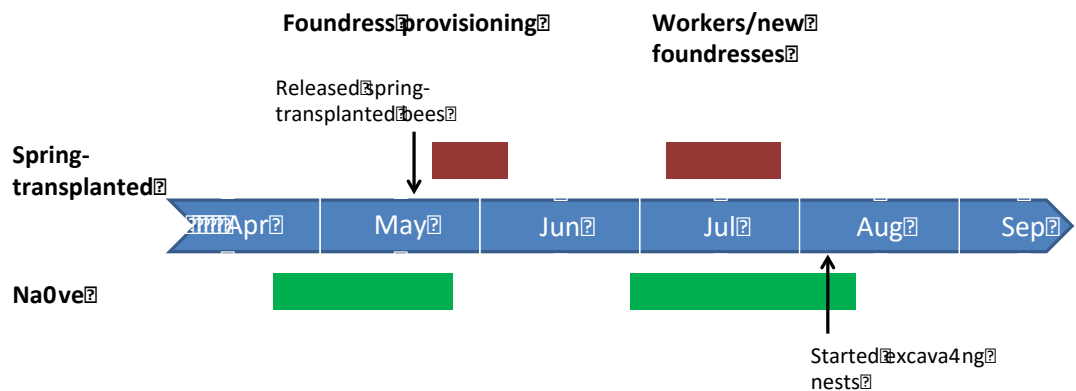
Figure 3.2
The timing and duration of key events for spring-transplanted (red) and native (green) nest foundresses. Solid bars show the periods during which activity was observed, and represent all bees in that cohort. Not all bees within each cohort, represented by a coloured bar, began or finished individual stages on the same day. Bars therefore represent the first and last days on which different individual bees within a cohort were observed. Blank space between bars shows periods of bee inactivity

3.2.3 Foundress demography and body size

The timing of foundress provisioning and offspring emergence has been implicated as a potentially key factor influencing social phenotype (Hirata and Higashi, 2008; Field et al. 2010). Accordingly, the first date of foundress provisioning, the date of first B1 female offspring emergence and offspring development time (calculated as the time between provisioning and emergence) were recorded at all native and transplanted nests. A nest was considered to have been successfully initiated when the foundresses began provisioning; foundresses were then caught with an insect net as they emerged from their nest after a provisioning event had been observed. This ensured that foundresses had learned the locations of their nests before capture, and would be able to return when released. Each foundress was given a unique combination of two enamel paint spots (Revell® and Humbrol™ enamel model paints) applied to the thorax with a pin. Wing length was measured to the nearest 0.1mm with digital callipers, as the distance between the outer edge of the tegula and the end of the forewing. Each nest was marked by an individually numbered nail. During the foundress provisioning phase the nesting aggregation was divided into two sections, and these were observed on alternate days. Nests were continually observed alongside the marking and measuring of foundresses on every day of suitable weather (n=29 observation days). In addition to the first date on which a foundress was observed provisioning, each subsequent day on which foundresses provisioned was also recorded.

3.2.4 Determining social phenotype and offspring size

Social phenotype of native and transplanted bees was determined by observing whether any B1 offspring at a nest became workers. Workers tend to begin provisioning within one or two days of emergence, whereas directly hibernating offspring are recorded entering the nest for several days but never with pollen (Davison, pers. obs.). Offspring deemed to be workers were caught on emergence from their nest after they were observed provisioning. They were then measured and marked with a single paint spot on the thorax, with different workers in each nest being given a different colour. The wing lengths of offspring that did not become workers and hibernated beneath their natal nests were recorded after nests were excavated at the end of the season (Fig. 3.2). With the help of an additional observer, nests were continually observed during the summer (n=26 observation days), and all B1 activity was recorded.

3.2.5 Nest excavations

All nests were excavated towards the end of the B2 active phase. Nests were excavated to (i) assess productivity and second brood parentage in social nests (results to be reported elsewhere, but see Chapter 2), and, pertinent to the present study, (ii) to uncover any B1 offspring that entered into hibernation beneath their natal nests so they could be measured. Excavations took place after activity had ceased in spring-transplanted nests, but before activity had ceased in all native nests (Fig. 3.2). This was to ensure that B2 offspring did not have time to eclose as adults and leave the nest. Buckets containing nests were removed from the aggregation and carefully tipped upside down on a table. The bucket was then gently removed and the freestanding mass of soil turned the right way up. Soil was gradually scraped away with a knife until all nests in the bucket had been identified and their contents collected and recorded. Nests located outside of buckets at the aggregation were excavated by digging a vertical trench adjacent to the nests, and then excavating laterally towards the nest entrances. At all nests, all brood and adult bees (workers and foundresses) were removed with tweezers, recorded and stored in ethanol. Brood cells in *L. calceatum* are arranged in clusters, and therefore it was easily possible to be certain that all brood have been collected. Excavations were continued well below the level of brood cell clusters in order to detect hibernating B1 offspring.

3.2.6 Confirming offspring population of origin

Live foundresses were not found in nests initiated by transplanted foundresses. I therefore used the program Structure (Pritchard et al., 2000) to confirm that unmarked adults excavated in or beneath nests initiated by transplanted foundresses were Scottish in origin (i.e. they had been produced by a transplanted foundress). Individuals were genotyped at 10 loci originally developed for the closely related species *L. malachurum* (Parsons and Field, in prep; see appendix A for primers and details of the PCR protocol). All adults, larvae and pupae were included (n=171 individuals) for which at least six loci had been successfully genotyped. Three replicates were run using a burn-in period of 100,000 reps with admixture assumed and uncorrelated allele frequencies, each with K=1-3. I then implemented the Evanno method (Evanno et al., 2005) within the program Structure Harvester (Earl and Vonholdt, 2012) to determine the best fitting value of K. Individuals of unknown origin were then checked to see whether they were assigned to the Inverness or Sussex population.

3.2.7 Data analyses

To determine whether transplanted bees exhibited plasticity I first examined three characteristics associated with social nesting: worker behaviour, offspring size and development time.

First, I tested whether the observed pattern of social phenotype exhibited by native and transplanted bees represented (i) environmentally mediated plasticity or (ii) fixed genetic differences between the two populations. The timing of B1 offspring emergence is thought to be a key factor mediating the decision of B1 offspring to become a worker. Therefore, a significant effect of 'source' would indicate fixed genetic differences between populations, whereas a significant effect of 'emergence date' would be indicative of plasticity. However, foundresses were transplanted from Inverness to Sussex and initiated provisioning after most native foundresses had started provisioning. In consequence, differences in social phenotype between native and transplanted bees could have been due to disparities in environmental conditions experienced by provisioning foundresses. In order to control for this effect, I also considered temperature throughout the period during which each foundress provisioned. This was calculated as the mean of daily average temperature for each day between a foundress' first and last observed provisioning event, yielding 'provisioning temperature'. I used temperature across each foundress' entire provisioning period, rather than *just* the days on which a foundress provisioned, because conditions experienced between days suitable for provisioning may also inform a foundress' provisioning strategy. I analysed the effect of 'source', 'emergence date' and 'provisioning temperature' on 'phenotype' using a generalised linear model (GLM) with binomial errors. Given that later-provisioned offspring also emerged later (Fig. 3.5a), I checked for collinearity among explanatory variables (Dormann et al., 2013) by examining variance inflation factor (VIF) scores, using the function 'vif' in the R-Package 'car' (Fox and Weisberg, 2011). I employ a conservative threshold of VIF≥2.5 to identify collinearity (Allison, 2012). For all variables VIF scores were low (<1.3), indicating that there was no significant collinearity.

Second, because sociality in *L. calceatum* is associated with workers that are smaller than their mother (caste-size dimorphism), I examined whether 'source' affected the size of B1 female offspring produced by native and transplanted foundresses. As there were multiple offspring per nest, I use a generalised linear mixed model (GLMM) to test for effects of 'caste' and 'source' on B1 'wing length' with 'nest' included as a random factor. I initially included a caste/source interaction to test whether foundresses from different sources produced offspring of different sizes relative to themselves.

Third, I tested for differences in development time of B1 offspring between native and transplanted bees (the time between a foundress' first observed provisioning event and her first female offspring emergence). Development time was considerably left skewed, and so I used the function *powerTransform* in the package 'car' (Fox and Weisberg, 2011) to transform the data. Offspring of transplanted and native foundresses were different in size, and I therefore include 'size' to control for this difference. As a measure of size for the first-emerged offspring in social nests I use wing length of the first-marked worker. For B1 offspring in solitary nests it was not possible to know which offspring was the first to emerge, and therefore I use the mean wing length of offspring excavated at the end of the season.

Additionally I wanted to compare B2 productivity between native and transplanted social nests. However, because only a single nest initiated by a transplanted foundress became social it was not possible to test for an effect of 'source' on B2 productivity. I therefore analyse overall B2 productivity using a GLM with negative binomial errors, with 'number of workers' as the only explanatory variable, and show productivity of the single social transplant-initiated nest in comparison with native nests (see Fig. 3.6).

I use a chi-squared test with Yates' correction to compare the frequency of successful spring nest initiation between autumn and spring-transplanted Scottish bees, and a Fisher's exact test to compare the frequency of subsequent successful offspring production. Foundresses were considered to have successfully initiated nests after they had started provisioning. Successful offspring production was determined by the presence or absence of B1 offspring. Finally, I use a chi-squared test to compare the frequency of nest failure between nests initiated by native or transplanted foundresses. As above, a nest was considered to have failed if no B1 offspring emerged.

For all models I report significance values when removing terms from the minimal adequate model, after stepwise reduction from the maximal model (Crawly, 2013). All analyses were conducted in the *R* environment (R Development Core Team, 2013). Results are presented ±1 standard error.

3.3 Results

3.3.1 Nesting success of transplanted bees

Of the 70 foundresses transplanted from Inverness to Sussex in the autumn of 2014, only four (5.7%) successfully initiated nests in spring 2015, of which two (2.8% of total) subsequently
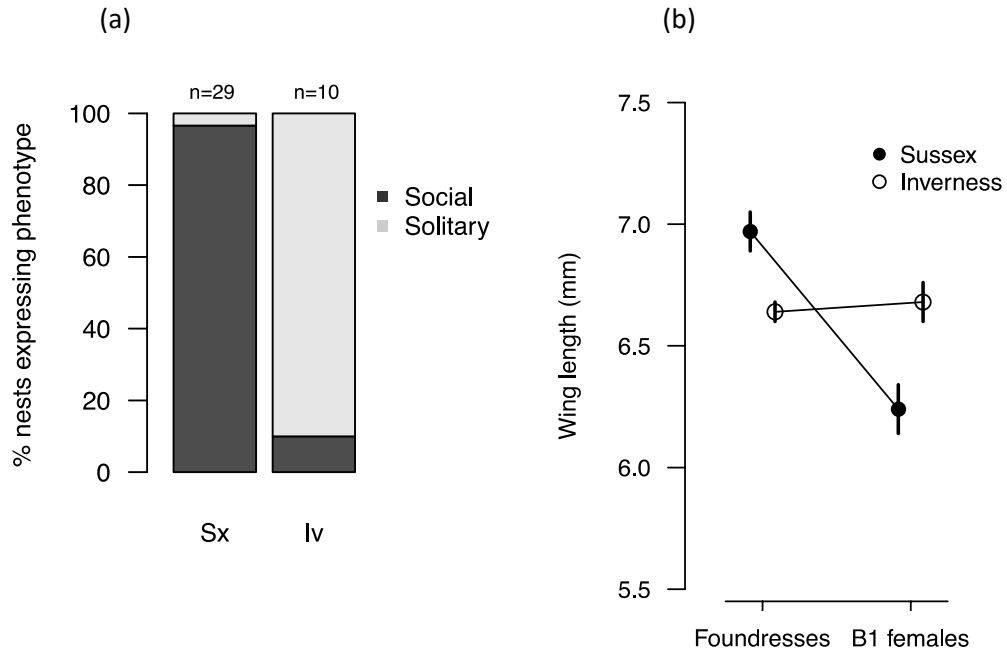
Figure 3.3
(a) Proportion of native nests at Sussex (Sx) and nests initiated by spring-transplanted foundresses from Inverness (Iv) that expressed social or solitary behaviour.
(b) Mean wing lengths (millimetres) of native (Sussex) and transplanted (Inverness) foundresses and their B1 female offspring (±1SE). Foundresses: n=18 from Sussex, n=5 from Inverness. B1 females: n=51 from Sussex, n=13 from Inverness. Significant caste/source interaction X^2_1=20.302, p<0.001.

produced B1 offspring. Of the 202 foundresses transplanted to Sussex from Inverness in spring 2015, 21 (10.4%) successfully initiated nests in spring, of which ten (4.9% of total) produced B1 offspring. Spring and autumn-transplanted foundresses were equally likely to initiate nests and start provisioning in the spring of 2015 (X^2=0.862, p=0.353), and to produce B1 offspring (Fisher's exact test: p=0.737). Once nests had been initiated, the rate of failure to produce at least one detected B1 offspring did not differ between nests initiated by native and transplanted foundresses (X^2=0.019, p=0.819, native=56.6% failed, transplanted=52.4% failed).

3.3.2 Social phenotype

Social phenotype was recorded at 29 native and ten spring-transplanted nests. It was not possible to unequivocally determine social phenotype at the two nests initiated by autumn-transplanted foundresses; in one two males only were detected in the B1 generation, and the other nest produced a single B1 female that was observed to provision once but then disappeared. At the nest that produced only two males it was not possible to distinguish between the foundress adopting a deliberate strategy to produce males only (e.g. Kapheim et al. 2015a)
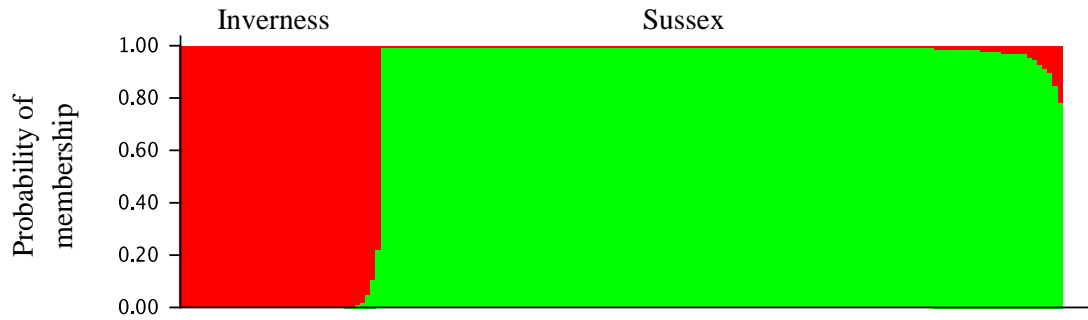
Fig. 3.4

Clustering of *Lasioglossum calceatum* foundresses from Inverness (red) and Sussex (green), and the offspring they produced. Individuals clustered strongly into two populations (K=2 determined by the Evanno method, delta K=1637.99), n=171 individuals. All individuals know to be from Inverness or Sussex were correctly assigned to each population. All putative offspring of transplanted Inverness foundresses excavated from beneath their nests were confirmed to belong to the Inverness population. Three B1 females that initiated independent summer nests were confirmed to be from Sussex.

and non-matedness. In either case it is not possible to know whether any female offspring would have become workers. At the nest where the single B1 female died after provisioning once it was not possible to assign her as a worker, because B1 offspring may provision a small number of times and then enter hibernation (e.g. Yanega, 1989).

All but one of the native nests produced female offspring that became workers (Fig. 3.3a), with a mean of 3.1±0.33 functional workers per nest (n=28). By contrast, nine of the ten spring-transplanted Scottish nests that successfully produced female B1 offspring did not become social (Fig. 3.3a). In these nests and the single solitary native nest, B1 females were repeatedly observed entering the nest but never with pollen. The single transplanted nest that became social produced three female offspring, all of which became workers. Worker behaviour was clear-cut at this nest because each female was observed provisioning on at least ten occasions over three or more separate days (Yanega, 1989). The analysis of bees using the software Structure further confirmed that all offspring excavated from beneath nests initiated by transplanted foundresses were of Scottish origin. The Evanno method (Evanno et al., 2005) suggested K=2 best explained the data (delta K=1637.99), in line with bees originating from two separate populations. On visualisation of the output (Fig 3.4 it was found that all individuals of known origin were correctly assigned between each cluster, and the putative Scottish offspring were assigned to the cluster containing only other Scottish individuals.

3.3.3 Bee size

As expected, native foundresses produced B1 females smaller than themselves. In contrast, transplanted foundresses produced offspring the same size as themselves, and which were larger than native workers (caste/source interaction X^2_1=20.302, p<0.001; Fig. 3.3b). Two of the three offspring produced in the single social Scottish nest were the same size as native workers, while the third was slightly larger than then foundress (Fig. 3.3b). The B1 female offspring excavated from beneath the single solitary native nest was the same size as other bees that became workers (Fig. 3.3b).

3.3.4 Offspring emergence date, development time and productivity

Development time of the first female offspring did not differ between nests initiated by native and transplanted foundresses, and decreased linearly as the date when a foundress first started provisioning became later ($F_{1,30}$=292.58, p<0.001). The first female offspring of transplanted foundresses still emerged later overall than those from native nests (Fig. 3.5a; Wilcoxon signed rank test: W=27.5, p<0.001). Note that, in contrast to Figure 2.6 in the previous chapter, data in Figure 3.4 are presented showing date of first B1 female offspring emergence on the y-axis, as opposed to the number of days between a foundress' first date of provisioning and first B1 female offspring emergence. However, I suggest that transplanted bees were not solitary simply because their B1 offspring emerged later in the season, or because transplanted foundresses provisioned later in the spring. Nests initiated by native foundresses were significantly more likely to become social than nests initiated by transplanted foundresses after controlling for the effects of foundress provisioning temperature and offspring emergence date (provisioning temperature $X^2_{1,34}$=0.739, p=0.390; emergence date $X^2_{1,34}$=1.613, p=0.204; bee source $X^2_{1,34}$=5.565, p=0.021). Furthermore, in the present study first brood females of transplanted foundresses still emerged about a week earlier than the earliest-emerging native offspring in two previous years (Fig. 3.5b; Davison and Field, in press, Chapter 2). Therefore although they emerged later in the present study, B1 offspring of transplanted foundresses emerged relatively early in comparison with B1 offspring from previous years that still became workers.

Excavations of nests prior to the emergence of second brood offspring revealed that overall, productivity among native nests significantly increased with the number of workers in a social nest ($X^2_{1,15}$=4.944, p=0.0.027; Fig. 3.6). The single social nest initiated by a transplanted foundress produced only a single B2 offspring despite having three workers, whereas the three native nests that also had three workers produced three, five and ten B2 offspring respectively (Fig. 3.6). I also noted a disparity in foundress behaviour between the social and solitary
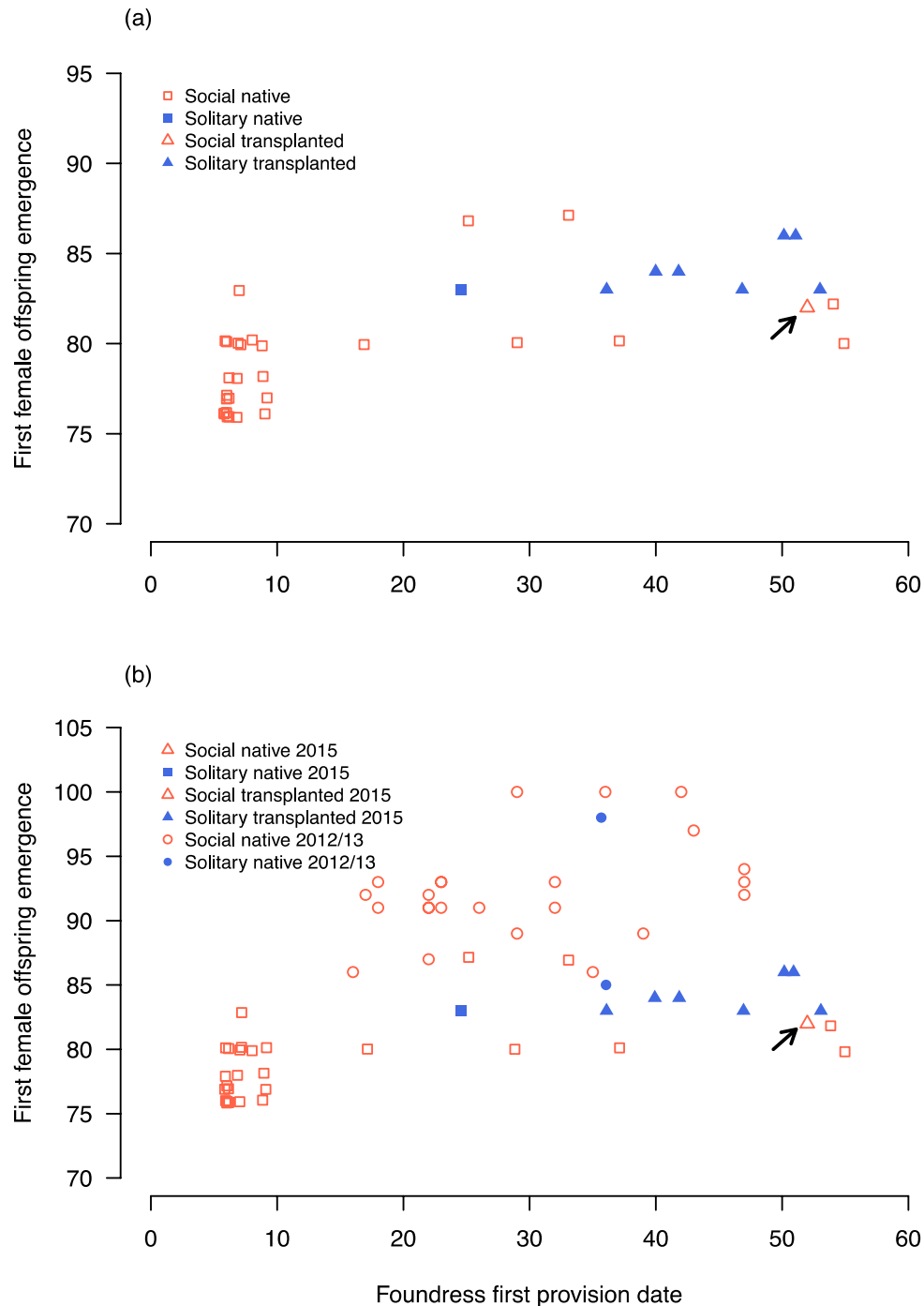
Figure 3.5

Relationship between the date of a foundress' first spring provisioning event and the emergence date of the first female offspring. Note the y-axis is different to Figure 2.6. Data shown are from (a) the present study only and (b) additionally with data from native bees collected in 2012/2013 (note: this is the same data as shown in Figure 2.6). Red symbols show nests where at least some offspring became workers, and blue symbols where all female B1 offspring did not become workers. The arrow shows the single social nest initiated by a transplanted female. Points are jittered to reveal multiple overlapping observations. Day zero is 14-April and day 70 is 23-June, standardised across years.
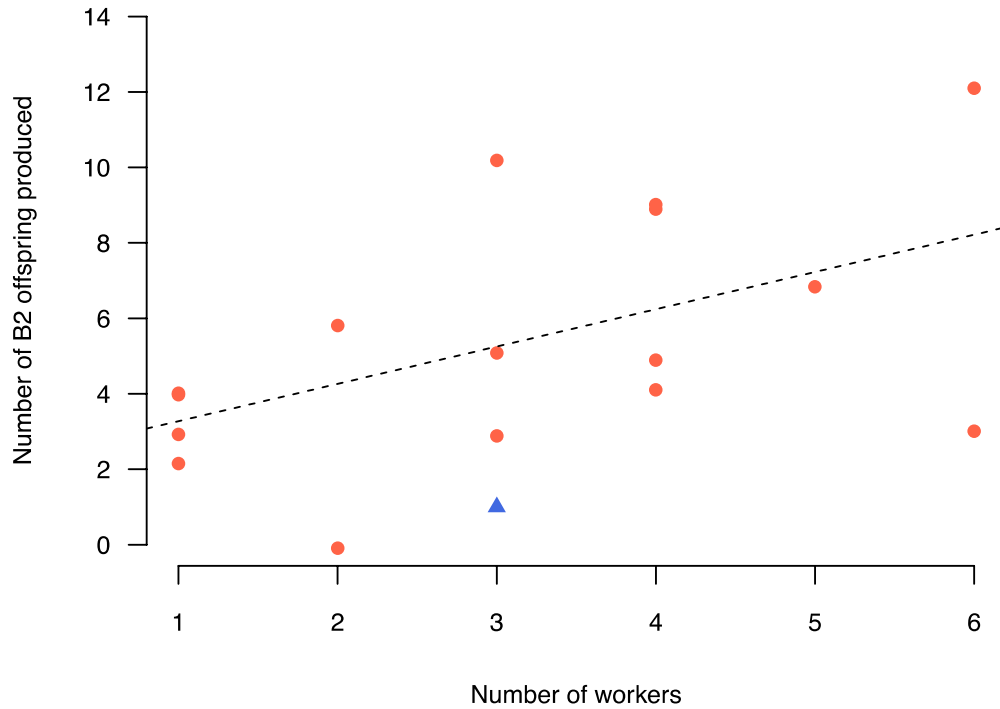
Figure 3.6
Relationship between the number of provisioning workers in a nest and the number of B2 male and female offspring produced. Orange circles represent native nests, and the filled blue triangle the single social transplanted nest. Points are jittered vertically to reveal multiple overlapping observations. The dashed line shows least-squares regression for native nests.

transplanted nests. The foundress in the in single transplant social nest did not leave the nest once her offspring had emerged, despite being observed daily in the nest entrance. In contrast, foundresses in the solitary nests were frequently observed leaving the nest on nectaring trips alongside their offspring.

3.3.5 Summer nests

Three new nests were independently initiated and provisioned by single individuals after B1 offspring emergence in the summer of 2015. B1 females began emerging on 29 June and B1 activity was observed at most nests by 7 July, before the three summer nests were first observed: two were first recorded on 16 July and the third on 17 July. Independent summer nest founding by offspring of transplanted bees would also represent plasticity because this behaviour has not been recorded at Inverness. In the Structure analysis, all three individuals were placed within the Sussex population, revealing that these individuals were highly likely to be native to Sussex. Independent summer nest founding has not previously been observed at Sussex (Davison and Field, in press, Chapter 2), and therefore represents the discovery of a new behaviour by *L. calceatum*.

3.4 Discussion

Socially polymorphic sweat bees exhibit both social and solitary behaviour within the same species, and are of particular interest for elucidating the behavioural and genetic mechanisms underlying the origins of eusociality (Chapuisat, 2010). Nevertheless, few studies have sought to address this experimentally by transplanting bees between different environments (see Plateaux-Quénu et al. 2000 (common garden lab test); Field et al., 2010, 2012 (common garden field test)). In this chapter I transplanted nest foundresses within the UK, from a northern population where only solitary behaviour is expressed to a southern population where most native bees nest socially (Davison and Field, in press, Chapter 2; Fig. 3.1). The two sites were approximately 800km apart, and represent areas of contrasting temperatures and season length (Table 3.1). The principal finding from this chapter is that offspring at the majority of nests initiated by transplanted foundresses did not become social (Fig. 3.3a), and the results suggest that this could be independent of environmental effects. Native and transplanted foundresses also produced offspring of different sizes. Offspring of native bees were significantly smaller than their mothers, but offspring of transplanted bees were the same size as their mothers and significantly larger than native offspring (Fig. 3.3b). Together these results tentatively suggest that social phenotype has a fixed genetic component in *L. calceatum*. Fixed genetic differences between social and solitary populations of sweat bees have previously been inferred for *H. rubicundus* in North America (Soucy and Danforth, 2002), and suggested in the laboratory for *L. albipes* (Plateaux-Quénu et al. 2000). However, these studies either lack direct evidence (Soucy and Danforth, 2002) or the realism of a natural field setting (Plateaux-Quénu et al. 2000). My results therefore provide the first experimental evidence for fixed genetic differences between bees from social and solitary populations in a socially polymorphic sweat bee. I now discuss the evidence for the significant role of fixed genetic differences in determining social phenotype in *L. calceatum*, and the wider implications of losing plasticity.

3.4.1 Evidence for genetic control of sociality

The results presented in this chapter present two lines of evidence suggesting that observed social phenotype in *L. calceatum* was largely determined by fixed genetic differences between bees at Sussex and Inverness. First, transplanted foundresses produced B1 offspring significantly larger than native B1 offspring, and which did not differ in size from themselves (Fig. 3.3b); in contrast native foundresses produced offspring significantly smaller than themselves. Second, after controlling for environmental cues that could potentially influence whether offspring become workers, social phenotype was significantly predicted by source site while environmental cues remained non-significant. Conditions experienced by provisioning

foundresses or by offspring upon emergence are predicted to influence social phenotype if the decision to become a worker is environmentally mediated (Figs. 3.3a, 3.5; Hirata and Higashi, 2008; Field et al., 2010, 2012).

Decisions about social phenotype can be made during two distinct periods of the life cycle; when foundresses are provisioning first brood offspring, and when offspring subsequently emerge as adults. Workers are typically smaller than reproductives in sweat bees (Fig. 3.3b; Schwarz et al., 2007; Davison and Field, in press, Chapter 2). Offspring size is largely determined by the amount of food provided in the cell (Roulston and Cane, 2002), and therefore a foundress' provisioning strategy should depend on whether offspring are likely to become workers. For foundresses, matching offspring size to the anticipated future behaviour of offspring is likely to confer considerable benefits. Provisioning is costly (Cant and Field, 2001; Strohm et al., 2002; Weissel et al., 2012), and when first brood offspring are likely to become workers investment of resources into the first brood represents a trade off against future survival to produce second brood offspring (Cant and Jonstone, 1999; Field et al., 2010). In consequence, costs incurred through the production of large offspring that nonetheless end up as workers could result in premature foundress death (e.g. Field et al., 2010). Conversely, small offspring that enter hibernation may make inferior nest foundresses (Strohm et al., 2002; Beekman et al., 2003; Brand and Chapuisat; but see Weissel et al., 2012). Therefore, maternal investment in first brood offspring should be strongly influenced by whether those offspring are likely to become workers. The large offspring produced by transplanted foundresses indicates that unlike socially plastic sweat bees (Field et al., 2012) foundresses from Inverness did not alter their provisioning strategy.

Nevertheless, offspring must also emerge sufficiently early in the season if they are to become workers and successfully rear reproductive offspring (Field et al., 2010), and these two decision points are not necessarily mutually dependent. In a study of *L. baleicum* Cockerell, foundresses provisioned small, worker-sized offspring that nonetheless emerged too late in the season and entered hibernation (Hirata and Higashi, 2008). Conversely, Field et al. (2010) showed that *H. rubicundus* foundresses produced large offspring that nonetheless remained at nests as workers. In the present chapter most transplanted foundresses produced large offspring that entered hibernation directly regardless of emergence time, suggesting that foundresses did not anticipate offspring becoming workers. However, as shown by Field et al. (2010), offspring emerging sufficiently early can still become social if the appropriate behavioural switches are in place.

Despite emerging slightly later than native offspring, offspring of transplanted foundresses still emerged earlier than almost all native offspring in two previous years (Fig. 3.5b, Davison and

Field, in press, Chapter 2). This result is potentially important because it suggests there would have been sufficient time remaining in the season to rear a second brood, and hints that the date of offspring emergence per se might not be a critical factor influencing the social phenotype of *L. calceatum*. Although other factors such as temperature are likely to play a role in environmentally mediating behaviour (Forrest et al., 2010), photoperiod is probably a critical cue because it is robust against inter-annual variation in weather conditions (Bradshaw and Holzapfel, 2007). However, bees transplanted from Inverness are likely to have been adapted to the photoperiod regime experienced at that latitude (Bradshaw et al., 2004; Lehmann et al., 2015). As a consequence offspring of transplanted foundress may interpret photoperiodic cues differently to offspring of native bees. In particular, if transplanted and native bees perceive the same time of year, each might interpret it differently because any given date is likely to be closer to the end of the active season at Inverness than Sussex, where the season is longer (Table 3.1). Any such effects are impossible to dissect, however, in the absence of a reciprocal transplant or controlled laboratory experiment (Bradshaw et al., 2004).

These results suggest two possible conclusions. First, environmental cues received after transplantation to Sussex did not cause foundresses to reduce maternal investment in first brood offspring. Second, emerging earlier in the season did not induce offspring of transplanted foundresses to remain as workers, and environmental cues played a limited role in determining social phenotype of transplanted bees. I confirmed that offspring of transplanted foundresses did not simply enter hibernation because their mother had died (e.g. Packer, 1990), as transplanted foundresses were still alive in 7 of 10 nests at the time of offspring emergence. This result agrees with previous observations of solitary behaviour by native offspring in the presence of the foundress (Davison and Field, in press, Chapter 2).

It was not possible to assess social phenotype at either of the two nests initiated by autumn-transplanted females. One foundress produced only two males, and therefore may not have been mated when caught at the Inverness source site. The second produced only a single female that was recorded provisioning once before disappearing, which could be the result of either mortality or dispersal. This meant that it was only possible to assess the behaviour of offspring produced by transplanted foundresses that were collected in spring, and which had already experienced temperature and day length cues associated with overwintering and spring emergence at the source site. An alternative explanation to non-plasticity in foundress behaviour therefore could be that any plastic response to environmental cues occurred prior to transplantation, and consequently would not have been expressed at Sussex (Thibert-Plante and Hendry, 2011). This possibility cannot be ruled out, and the future completion of a successful autumn transplant would shed light on whether this effect might have occurred.

3.4.2 Plasticity and its loss in Scottish bees

The interpretation that fixed genetic differences between bees from social and solitary populations influences social phenotype agrees with a common garden laboratory study of the closely related polymorphic species *L. albipes* (Plateaux-Quénu et al., 2000). However, both the results presented in this chapter and those of Plateaux-Quénu et al. (2000) also show that the ability of offspring from solitary populations to express sociality has not been lost completely (Fig. 3.3a). In the present chapter all three offspring at one nest initiated by a transplanted foundress became workers and provisioned the nest. The reasons why all offspring in only this nest became workers are unclear.

Behavioural changes associated with sociality occurred in both offspring and the foundress. First, each offspring was observed provisioning on at least ten occasions. This meets Yanega's (1989) definition of a worker as a bee that provisions on at least six occasions, or enough to theoretically provision a single cell. Second, the behaviour of the foundress also changed in line with her offspring. In common with native foundresses in social nests, the social Scottish foundress was never observed to leave the nest after the second day on which her offspring had become active. In contrast, transplanted foundresses at nests that did not become social were regularly recorded leaving their nests alongside newly emerged offspring, presumably to feed on nectar. Third, although circumstantial, it is worth noting that two of the three offspring that became workers were among the smallest produced by transplanted foundresses (data not shown). It is possible that this represents an evolved provisioning strategy in relation to size (e.g. Field et al., 2012), but the limited data precludes further investigation. A general conclusion, however, is that the role of the environment in actively shaping social phenotype in *L. calceatum* and *L. albipes* is considerably reduced in comparison with plastic species such as UK *H. rubicundus* (Field et al. 2010, 2012).

Phenotypic plasticity can be lost once environmental conditions become predictable (Masel et al., 2007; Pfennig et al, 2010). In these circumstances, loss of plasticity may occur because its maintenance incurs costs. In reality, however, costs of plasticity appear to be largely negligible or absent (Snell-Rood et al., 2010; Murren et al., 2015). Alternatively, plasticity may be lost via genetic drift and subsequent genetic assimilation (Masel et al., 2007; Pfennig et al., 2010), when circumstances in which both phenotypes are routinely expressed no longer arise (Smith et al., 2015; Cini et al., 2015). One phenotype only is expressed at the solitary Inverness population, because worker behaviour has never been observed in three years of observations (Davison and Field, in press, Chapter 2). Other traits such as patterns of foundress provisioning may also be expected to diverge between populations, both due to expected future reproduction (i.e. the

production of a second brood) and adaptation to local weather patterns (e.g. Field, 1996). However, some behaviours characteristic of solitary nesting such as nest founding and hibernation of some first brood offspring, are common to both population types (Davison and Field, in press, Chapter 2).

In order for differentiation to occur, this scenario would also require mechanisms leading to minimal gene flow between social and solitary populations (Lenomand, 2002). This appears to be the case in North American *H. rubicundus*, where proximately situated populations exhibiting social or solitary behaviour show more mitochondrial differentiation than to more distant populations of the same social phenotype (Soucy and Danforth, 2002). In the absence of physical barriers to gene flow, one possible mechanism could be phenological differences in the production of offspring (Soucy and Danforth, 2002; Quintero et al., 2014; Weis, 2015). In sympatry, social nests produce reproductive offspring later than solitary nests; however, assortative mating may be difficult to achieve because the first brood in social nests contains males (Plateaux-Quénu, 1992; Davison, pers. obs.), as well as females that mate and enter hibernation (see Chapter two; Davison and Field, in press). Controlled mating experiments and detailed field studies of bees from adjacent social and solitary populations may prove illuminating (Soucy and Danforth, 2002).

The early stages of sociality likely involved flexibility in the timing of gene expression, where some offspring behave as workers and others as reproductives (West-Eberhard, 2003; Rehan and Toth, 2015). Social plasticity could therefore most easily be lost in solitary populations if emerging offspring only ever received cues associated with entering diapause, such as late times of emergence (Field et al., 2010) or rapid mating (Yanega, 1989, 1992, 1997; Lucas and Field, 2013). Selection at loci regulating the timing of provisioning behaviour during the social life cycle could then be relaxed, because provisioning would occur only at one time during the year: by foundresses provisioning B1 offspring in spring, instead of by foundresses *and* by workers provisioning B2 offspring later in the year. This could then lead to changes in the response threshold at which worker behaviour is expressed, and its eventual loss from the solitary population (Abouheif and Wray, 2002; Suzuki and Nijhout, 2006). A possible change in the response threshold suggests an alternative explanation for the apparent general lack of plasticity exhibited by transplanted bees: environmental cues might not have been sufficiently strong to induce social nesting (e.g. Sikkink et al., 2014). For example, within the UK primitively eusocial sweat bees are restricted to the south (Falk and Lewington, 2015). Therefore, it is possible that *L. calceatum* would express sociality if transplanted further south, or offspring may need to emerge earlier than native bees for plasticity to be induced. Nevertheless, it remains the case that bees from Inverness appeared to respond differently to environmental cues

than did bees from Sussex. Currently it has not been possible to directly compare the behaviour of native and transplanted *H. rubicundus* B1 female offspring (Field et al., 2010, 2012). Future transplants may reveal that although *H. rubicundus* is socially plastic, the threshold at which bees from solitary populations will express sociality is higher.

The limited data hint that the plastic sociality expressed by transplanted bees was ineffective; despite having three workers (mean for native nests=3.1±0.33), productivity at this nest was lower than at any native nest that produced second brood offspring (Fig. 3.6). Such a result would not be surprising given that bees at Inverness never express sociality, and parallels the inept behaviour of slave-making ants when deprived of their slaves (Wilson, 1975; Stuart and Alloway, 1985).

The behavioural repertoire of first brood offspring at Sussex is, in contrast, considerably broader. First brood offspring may either become workers or enter hibernation (Fig. 3.3a; Davison and Field, in press, Chapter 2), and in the present chapter I observed three first brood offspring initiating their own summer nests. It is therefore possible that B1 females at Sussex retain a greater degree of plasticity than at Inverness (Field et al., 2010), although in practice this may depend on the extent to which cues associated with B1 diapause at Sussex and Inverness are the same. First brood offspring in other polymorphic species exhibit a similar range of reproductive options (Yanega, 1989; Yagi and Hasegawa, 2012), but early diapause and independent nest founding by B1 females are also recorded from some apparently obligate social sweat bees (Rehan et al., 2013; Richards et al., 2015) and so are probably not synonymous with the ability to adaptively adopt a completely solitary phenotype. I did not perform the reverse transplant from Sussex to Inverness, and this could be the subject of future work.

3.4.3 Future considerations

The result that social polymorphism in UK *L. calceatum* is probably based on fixed genetic differences suggests some interesting questions for future research additional to conducting a reverse transplant. First, the existence of fixed differences between populations in which individuals express social or solitary behaviour will of particular interest for studies investigating the genetic basis of sociality (e.g. Kocher et al., 2013). Second, few studies have examined the extent to which social polymorphism has promoted population differentiation (e.g. see Soucy and Danforth, 2002; Zayed and Packer, 2002; Soro et al., 2010), or considered whether polymorphism could facilitate ecological speciation (Rundle and Nosil, 2005; Thibert-Plante and Hendry, 2011). It would prove particularly fruitful to locate the geographical region

where the transition from social to solitary nesting occurs, where conditions in different years could favour either social or solitary nesting. Moreover, it would be interesting to transplant bees from a solitary population much further south than in the present chapter, where selection for plasticity may have persisted and bees may express more flexibility than in the present chapter. In general, the cornucopia of social variation exhibited by sweat bees demands that species are studied in detail throughout their geographic range, and in a variety of environmental contexts (Wcislo and Danforth, 1997; Wcislo, 1997). Finally, it would be interesting to address an obvious question; why does *L. calceatum* appear to largely have lost plasticity while *H. rubicundus* has not? Arguments based on separate phylogeographic histories (e.g. Field et al., 2010) might not apply because in Europe both species occupy a broadly similar area (Pesenko et al., 2000).

Chapter four

Season length, body size and social polymorphism in sweat bees: a novel test of the saw-tooth model

Abstract

Annual insects are predicted to grow larger at lower latitudes because the growing season is longer. Transitions from one to two generations per year can occur when the season becomes sufficiently long, which can cause sharp decreases in body size because time available to develop is halved. The resulting saw-tooth clines have been investigated only in solitary taxa with free-living larvae. We investigated size clines in socially polymorphic sweat bees: a unique system in which latitudinal transitions between solitary and social nesting occur within the same species, characterised by the absence or presence of workers, and offspring are individually mass provisioned by adults. We aimed to examine how the body size of nest foundresses changed with season length, and determine whether transitions in social phenotype generate saw-tooth size clines. We measured *Lasioglossum calceatum* and *Halictus rubicundus* nest foundresses previously collected across more than 1000 km of latitude encompassing the transition between social and solitary nesting. Using satellite-collected temperature data to estimate season length, we show that both species were largest where the season was longest. Body size increased linearly with season length in *L. calceatum* and non-linearly in *H. rubicundus*, but the existence of saw-tooth clines was not supported in either species. Our results suggest that because the amount of food consumed by offspring during development is entirely determined by adults, environmental and social influences on the provisioning

strategies of adult bees may be more important factors than available feeding time in determining offspring body size in socially polymorphic sweat bees.

4.1 Introduction

Intraspecific geographic variation in life history traits is ubiquitous across insect taxa (Roff, 1992; Stearns, 1992). Clinal variation occurs when a trait changes continuously across an environmental or ecological gradient (Ridley, 2004). Geographic variation in body size has received considerable research attention for more than 150 years (Blanckenhorn and Demont, 2004), and in insects can play a key role in traits such as potential fecundity and overwintering success (Honěk, 1993; Smith, 2002). Within species, body size frequently varies either positively or negatively with latitude or altitude (Chown and Gaston, 2010): positive clines occur where body size increases with increasing latitude or altitude, frequently cited as examples of Bergmann's rule (Bergmann, 1847; Ray, 1960), here termed Bergmann clines (BCs), and negative clines occur where body size decreases with latitude or altitude, and are termed converse-Bergmann clines (CBCs) (Blanckenhorn and Demont, 2004). In a recent review of size clines in insects, Shelomi (2011) showed that approximately equal numbers of studies reported latitudinal BCs, CBCs or no cline at all, and concluded that the taxonomic distribution of size clines among insects was "highly idiosyncratic" (Shelomi, 2011 pg. 511).

In temperate zones, a portion of the year is unfavourable for growth or reproduction (Bradshaw and Holzapfel, 2007). The remaining time represents the growing season; a window during which the immature form present in most insects (typically a larva or nymph) must feed and grow (Gullan and Cranston, 2010). Assuming that development time and body size are positively correlated, theory predicts that CBCs should most frequently be observed in species where generation time is long relative to the length of the growing season (Chown and Gaston, 1999; Blanckenhorn and Demont, 2004). In taxa such as moths and crickets, where development time takes up a considerable proportion of the growing season, immatures must either reach an advanced stage of development or fully develop and eclose as an adult before the onset of winter (Gullan and Cranston, 2010). In many cases the growing season therefore places a limit on the amount of time the free-living pre-adult form can spend feeding and developing, and adult body size correspondingly is often found to follow a CBC (Nylin and Svärd, 1991; Mousseau, 1997). By contrast, species with short generation times relative to season length are capable of growing larger at higher latitudes because season length does not constrain their size (Blanckenhorn and Demont, 2004). The resulting BC then arises either because it is adaptive, or a consequence of temperature-mediated physiological processes (see Blanckenhorn and Demont, 2004 and references therein).

Offspring in taxa exhibiting CBCs attain a larger body size where the season is longer because they can spend longer developing, and fitness tends to be maximised when the whole season can be utilised for growth and reproduction (Masaki, 1972). However, as development time increases so too the likelihood of successfully reaching adulthood decreases, for example due to predation or susceptibility to extrinsic environmental factors (Johansson and Stocks, 2005). Roff (1980) therefore predicted that there comes a point along a CBC, from high to low latitude, when fitness gains derived from attaining ever-greater size are outweighed by the increased risk of mortality resulting from ever-longer development. Roff (1980) therefore suggested that south of this point, selection should favour a bivoltine life cycle and, because development time must therefore be halved, that there should be a concomitant sharp drop in body size. At lower latitudes, as season length increases further, body size in bivoltine populations could increase again and thus the population would exhibit a saw-tooth size cline (Roff, 1980; Fig. 4.1). As predicted, saw-tooth size clines are observed in a variety of groups where there are latitudinal changes from univoltine to bivoltine life cycles, including Orthoptera (Masaki, 1972 [6]; Mousseau and Roff, 1989) and Lepidoptera (Nygren et al., 2008; Välimäki et al., 2013).
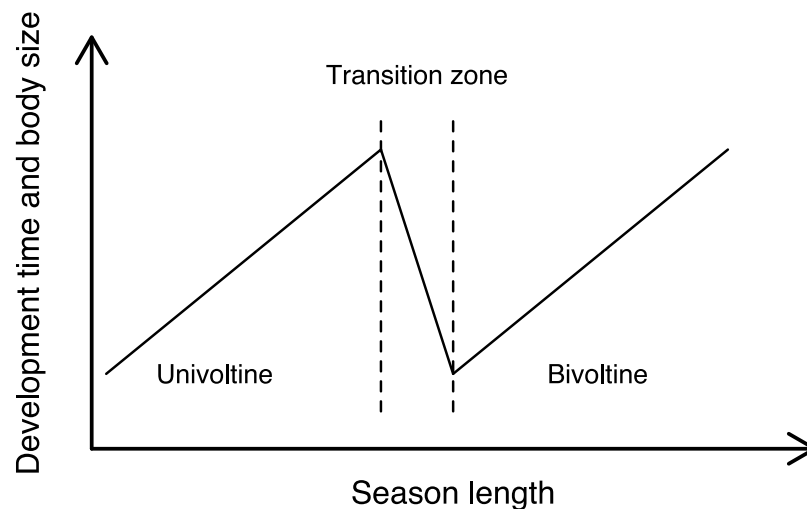
Figure 4.1
A hypothetical saw-tooth cline. After Roff (1980) and Nygren et al. (2008). In socially polymorphic sweat bees, univoltine populations are solitary while bivoltine populations are social (see text).

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[6] Roff (1980) states that he developed his model 'without any knowledge of the saw-tooth patterns observed by the [*sic*] Masaki and others'

Further, size clines are frequently found to have a genetic basis when individuals are reared in common gardens (e.g. Masaki, 1967; Blanckenhorn and Fairbairn, 1995; Telfer and Hassall, 1999). However, saw-tooth size clines are not always found and the relationships between body size and season length may be complex (Kivelä et al., 2011; Välimäki et al., 2013). One reason for more complex relationships between body size and season length is countergradient variation in growth rate (Conover and Shultz, 1995; Blanckenhorn and Demont, 2004). Countergradient variation occurs where a higher growth rate evolves to counter the effect of shorter available development time, and this can over, under, or perfectly compensate for clinal variation in development time (Conover and Shultz, 1995). Consequently, size clines can become partially mitigated, nullified or even overcorrected (Blanckenhorn and Demont, 2004).

One group in which the presence of saw-tooth clines might have more far-reaching implications are socially polymorphic sweat bees (Hymenoptera: Halictidae). Social polymorphism occurs when the same species can exhibit both solitary and social behaviour, characterised by the presence of either one or two broods of offspring per year respectively (Soucy and Danforth, 2002). In both the solitary and social life cycle, mated females (foundresses) emerge from hibernation in spring and independently initiate subterranean nests. Foundresses then mass provision separate brood cells, each with its own ball of pollen and nectar, which the developing offspring will consume entirely before pupating and eclosing as an adult. Body size is strongly correlated with the amount of food consumed during development (Plateaux-Quénu, 1983; Richards and Packer, 1994). Importantly, because each mass provisioned cell contains only a single offspring, the amount of food consumed during development is entirely dependent on how much pollen and nectar the foundress collects and provisions the cell with. In social nests this represents the first brood (B1) from which some or all female offspring remain at the nest as workers and help their mother to produce a second brood (B2) of reproductive offspring. As with other social insects, workers in sweat bees are typically smaller than the foundress (Schwartz et al., 2007).

In solitary nests, however, the worker brood is omitted and all first brood female offspring mate and enter hibernation to become foundresses the following year. Social behaviour is typically expressed from only lower latitudes or altitudes, probably because in northern or high altitude areas there is not enough time in the season to successfully rear two broods (Soucy and Danforth, 2002; Hirata and Higashi, 2008; Field et al., 2010; Kocher et al., 2014; Davison and Field, in prep, Chapter 5). In consequence, northern or high altitude populations exhibit only solitary nesting, and the geographic distribution of social and solitary phenotypes is analogous to the bivoltine and univoltine populations of other taxa such as butterflies, moths and crickets. Field et al. (2012) therefore predicted that the transition between social and solitary phenotypes

might generate a saw-tooth size cline: single-brooded, solitary-nesting bees just to the north of the transition zone would be released from some of the time-stresses acting on double-brooded, socially-nesting bees just to the south (Fig. 4.1).

In this chapter I investigate whether two socially polymorphic sweat bees exhibit saw-tooth size clines along a gradient of increasing season length, from the north of the UK to France. *Lasioglossum calceatum* Scopoli and *Halictus rubicundus* Christ are widely both distributed throughout the Palaearctic and Holarctic respectively (Pesenko et al., 2000; Pesenko, 2005), and are socially polymorphic throughout their ranges (Soucy and Danforth, 2002; Sakagami and Munakata, 1972; Davison and Field, in press, Chapter 2). In the UK both are known to nest socially in the south, but solitarily in the north or at high altitude in the south (Field, 1996; Soro et al., 2010; Field et al., 2012; Davison and Field, in press, Chapter 2). Foundresses from social populations tend to be larger (Field et al., 2012; Davison and Field, in press, Chapter 2); however, measurements have been limited to single locations or a few widely scattered sites, whereas it is necessary to consider body size continuously across a broad latitudinal range to determine whether saw-tooth clines occur. If a saw-tooth cline is generated by the transition between social and solitary behaviour as predicted by Roff (1980), it should be signified by a concurrent sharp change in size. If, however, the change in social phenotype has no effect, a linear cline should result. The results presented in this chapter generally support the existence of CBCs in sweat bees, but not the existence of saw-tooth clines.

4.2 Methods

4.2.1 Sampling range

Although *L. calceatum* is known to nest socially in France (Plateaux-Quénu, 1992), no studies of *H. rubicundus* from there have been published. However, it is highly likely that *H. rubicundus* nests socially in the regions of France sampled in this study because it is social in southern Britain, and elsewhere in its range the expression of social phenotype is closely linked to latitude and altitude (Soro et al., 2010; Field et al., 2012). Therefore, the range of latitudes sampled for both species should encompass the transition zone between social and solitary behaviour although at present it is not known exactly where the transition occurs.

4.2.2 Specimens

Specimens were sourced from museum, private and university collections spanning the years 1895-2014. In total 487 *L. calceatum* and 356 *H. rubicundus* specimens from Britain and France

(a)   (b)
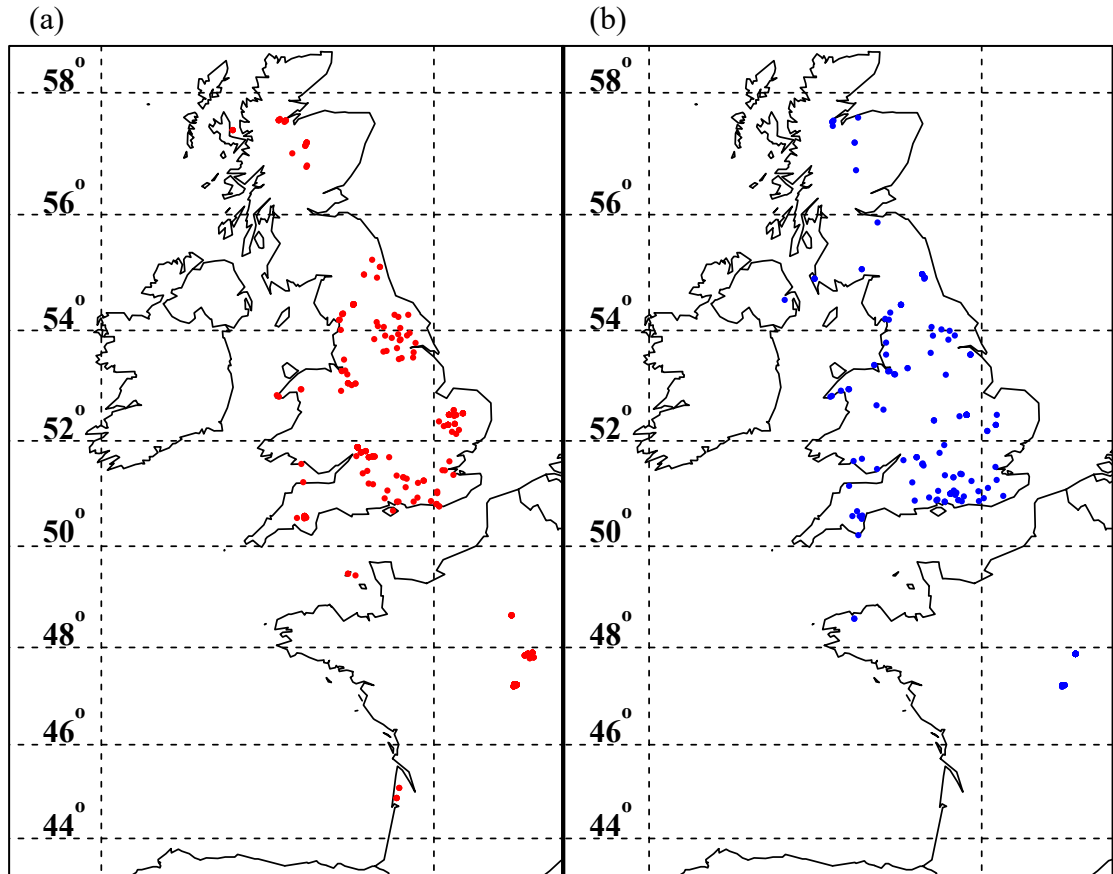
Figure 4.2

Maps showing the location of specimen sampling locations within the United Kingdom and France for specimens of (a) *Lasioglossum calceatum* and (b) *Halictus rubicundus* that were measured and entered into the size cline analysis. Note that the number of specimens sampled from each location is not indicated. Horizontal dashed lines are drawn at intervals of two degrees of latitude. In each panel, the right hand vertical dashed line shows zero degrees longitude (Greenwich Meridian), and the left had vertical line -10 degrees longitude.

covering 45-58 and 47-58 degrees of latitude respectively were measured (Fig. 4.2). Bee size was recorded as foundress head width, which is a widely used measure of body size in sweat bees (e.g. Soucy, 2002; Brand & Chapuisat, 2012), correlates strongly with mass and is easily measured from pinned specimens (Stubblefield and Seger, 1994). Head width was measured as the widest point across the head in full-face view including the compound eyes. Insect exoskeletons are robust to changes in shape or size over time (Daly, 1985). Therefore, despite some specimens being over one hundred years old their size as measured was the same as the year in which the individual was caught. *L. calceatum* and *H. rubicundus* workers tend to be slightly smaller than foundresses (Field et al., 2012; Davison and Field, in press, Chapter 2; but see Field et al. 2010). In order to ensure the smaller size of workers did not confound the analysis, bees that were caught after 15 June were excluded. This cut off is justified because in the south of England (where season length is longest in the UK) *L. calceatum* workers have never been observed before July (Davison and Field, in press, Chapter 2), and the earliest *H.*

rubicundus workers have rarely been observed only at the very end of June (Field et al., 2010, 2012). Further south in France *L. calceatum* workers are typically observed earlier; however, all French specimens of both species were captured in either March or April, well before the period of worker emergence (Plateaux-Quénu et al., 2000).

For all specimens, the location and date of capture were recorded. Data on specimen labels varied considerably in detail from precise coordinates and date of capture to vague or indecipherable place names without a date. Specimens without at least a place name and full date (i.e. including at least the month of capture), or where the place name was illegible or impossible to verify, were disregarded because it was not possible to determine with sufficient accuracy where or when the specimen was caught. If the only location data was a verifiable place name, this was considered sufficiently accurate and coordinates for that place were used as the given location for the specimen. In a few cases the name of only a near-shore island was provided, and because these islands were very small the centre of the island was used as the specimen location. All such coordinates were obtained from Google Maps©. Digital coordinates were used in the final data set. Many specimen labels provided an Ordnance Survey (OS) grid reference, a system used to map Great Britain and which is not compatible with digital coordinates. OS coordinates were converted to digital using Grab a Grid Reference Tool provided by the Bedfordshire Natural History Society[7]. The tool shows a satellite image map and a square overlaying the area referred to by the given OS coordinate, which varied from two to three figures in accuracy. In each case the centroid of the square was taken as the specimen location and its coordinates obtained from Google Maps©. Depending on the accuracy of the OS coordinates given, squares were either 100x100 m or 1x1 km.

Head width measurements of most specimens were made at the University of Sussex using a light microscope with an eyepiece graticule. Specimens kindly made available by the Oxford University Museum of Natural History were measured digitally on site.

4.2.3 Estimating season length

I estimated season length at all sampling locations as a measure of the time available in the year for growth and reproduction. Following Kocher et al. (2014), I use the number of days in the year on which land surface temperature (LST) exceeds 16°C to estimate the likely length of the bee active season. To estimate season length I used temporal Fourier processed LST data from the National Oceanographic and Atmospheric Administration's (NOAA) Advanced Very High

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[7](available at http://www.bnhs.co.uk/focuson/grabagridref/html/)

Resolution Radiometer (AVHRR) polar-orbiting satellites (Hay et al., 2006). Temporal Fourier analysis (TFA) is a noise reduction technique that describes variation in naturally occurring cycles such as temperature as a series of summed sine curves of different amplitude and phase (Rogers, 2000; Scharlemann et al., 2008). Data are based on 14 daily images at a spatial resolution of 8x8km, spanning a 20-year time series from August 1981 to September 2001 (inclusive). For present purposes I utilised the annual, bi-annual and tri-annual cycles, describing over 90% of variation from the original data (Hay et al., 2006), to reconstruct average annual LST profiles for each sampling location.

TFA data were imported into the software ArcGIS (Version 9.3), where the amplitude, phase and mean LST for each 8x8 km pixel containing sampling locations were extracted using the 'sample' function. Averaged annual LST profiles for each pixel (*td*) were reconstructed by summing (Eq. 1) the annual (Eq. 2), bi-annual (Eq. 3) and tri-annual (Eq. 4) sine curves and adding the mean LST

$$td = \sum_i t_i + a_o \qquad \text{(Eq. 4.1)}$$

where *i*=1-3

$t_1 = a_1 * \sin(((d+365/4-p_1) * 2 *\pi) / 365)$ (Eq. 4.2)

$t_2 = a_2 * \sin(((d+182.5/4-p_2) * 2 * \pi) / 182.5)$ (Eq. 4.3)

$t_3 = a_3 * \sin(((d+121.66667/4-p_3) * 2 * \pi) / 121.66667)$ (Eq. 4.4)

where t_i is the given temperature profile, a_i is the amplitude and p_i the phase of the annual, bi-annual and tri-annual cycles receptively, *d* is days (1-365) in the year and a_0 the mean LST. Season length for all sampling locations within each pixel was then the number of days from the averaged annual LST profile (*td*) on which the LST was greater than 16°C (Kocher et al., 2014).

4.2.4 Data analysis

For the data to support the saw-tooth hypothesis body size should follow the non-linear pattern shown in Fig. 4.1. It is unknown precisely where the transition between social and solitary behaviour occurs, therefore I follow Kivelä et al. (2011) and use polynomial regression to objectively test whether latitudinal size variation in *L. calceatum* and *H. rubicundus* supports the saw-tooth hypothesis. Specimens from the same 8x8 km pixel returned the same value for season length, and specimens were caught from different years. For each species I therefore use a generalised linear mixed model (GLMM) to analyse geographic variation in head width with

'pixel' and 'year' as random effects. Explanatory variables included were season length, the square of season length and the cube of season length (Kivelä et al., 2011). Support for the saw-tooth hypothesis would be indicated if the model generated a significant positive cubic term for season length. Maximal models were checked for normality and heteroskedasticity of residuals before proceeding with stepwise model reduction (Crawley, 2013). In the analysis of *H. rubicundus,* residuals were highly non-normal. Head width was therefore transformed using the function *powerTransform* in the package 'car' (Fox and Weisberg, 2011). The square and cube of season length were divided by 100 and 10,000 to avoid scaling issues between the three explanatory variables. Analyses were conducted in the *R* environment (R Development Core Team, 2013), using the lme4 package (Bates, 2015) for generalised linear mixed models (GLMMs).

4.3 Results

Head width was positively associated with season length in both *H. rubicundus* and *L. calceatum*, supporting previous work suggesting that sweat bees follow CBCs. However, the precise relationship between head width and season length differed between the two species (Table 4.1; Fig. 4.3a, b). In *L. calceatum*, head width increased linearly with season length, and there was no evidence of the predicted saw-tooth cline (Fig. 3b). In contrast head width in *H. rubicundus* generated a significant positive cubic term for season length, indicating that the relationship was non-linear. However, the pattern of change in head width with decreasing season length does not conform to predictions outlined by Roff

Table 4.1
Parameter estimates for linear mixed effects models in the two bee species studied. P-values were obtained by removing each variable from the final model.

Species	Variable	Estimate	SE	t	P
H. rubicundus	Intercept	39.716	9.542	4.162	
	Season length	10.421	6.829	1.526	0.129
	Season length^2	-0.255	0.094	-2.726	0.007**
	Season length^3	0.107	0.037	2.988	0.003**
L. calceatum	Intercept	2.115	0.095	22.468	
	Season length	0.001	<0.001	2.287	0.024*
	Season length^2	<0.001	<0.001	-0.73	0.464
	Season length^3	0.004	0.004	1.131	0.311

Figure 4.3
Head width in relation to season length in (a) *Lasioglossum calceatum* and (b) *Halictus rubicundus*. Note the difference in the length of the x-axes. Lines shown are for (a) linear regression line and (b) the Loess regression line. Green dots represent specimens from France, red dots are specimens from the United Kingdom. See Table 4.1 for statistics.

(1980) (Fig. 4.1) because there is no sudden decrease in size that could signal a transition from solitary to social nesting (Fig. 4.3b).

4.4 Discussion

Insects with long generations times relative to the length of the season are expected to show CBCs because further north the shorter season limits the time available for growth (Blanckenhorn and Demont, 2004). Moreover, species that transition from bivoltine to univoltine life cycles can often show saw-tooth clines in body size (e.g. Mousseau and Roff, 1989). Saw-tooth clines are predicted to occur because time stresses experienced by developing offspring in the bivoltine population just to the south of the transition zone are temporarily relaxed for univoltine offspring just to the north (Roff, 1980; Fig. 4.1). Previous studies have focussed exclusively on solitary taxa such as crickets and moths that have free-living larvae and lack parental care (e.g. Mousseau and Roff, 1989; Kivelä et al., 2011). Socially polymorphic sweat bees present an interesting alternative test of the saw-tooth model, because offspring are mass provisioned and alternative phenotypes are characterised by the presence or absence of a worker generation prior to the production of reproductives (Field et al., 2010). In this chapter I tested for saw-tooth size clines in two socially polymorphic sweat bees, *Lasioglossum calceatum* and *Halictus rubicundus*, across a gradient of increasing season length from the north of the UK to France. In both *L. calceatum* and *H. rubicundus*, head width was largest where the season length was longest, but I found no evidence for saw-tooth clines. I now discuss these results in the context of social behaviour, social polymorphism and behavioural plasticity.

4.4.1 Clinal variation in body size

The largest foundresses of both *L. calceatum* and *H. rubicundus* were from areas with the longest season length. However, neither *L. calceatum* nor *H. rubicundus* showed clear evidence of following a saw-tooth size cline in head width (Fig. 4.3a, b), as proposed by Field et al. (2012) and Davison and Field (under review, Chapter 2). Head width in *L. calceatum* increased linearly with season length across both the UK and France. By contrast, head width in *H. rubicundus* from the UK showed almost no cline at all whereas the larger specimens from France showed a slight CBC. The positive linear cline in *L. calceatum* is consistent with previous findings that northern foundresses are smaller than those in the south (Davison and Field, in press, Chapter 2). Similar previous findings in *H. rubicundus* (Soucy, 2002; Field et al., 2012) were only partially supported, however, because although specimens from areas with the longest season lengths were the largest, within the UK head width showed almost no relationship with season length. The smaller size of northern UK *H. rubicundus* may have been

underemphasized due to the comparatively small sample size and high degree of scatter on the data, but the general pattern within the UK is clearly different from the one found in *L. calceatum* (Fig. 4.3a, b). Despite returning a significant positive cubic term for season length (Table 4.1), the non-linear cline exhibited by *H. rubicundus* does not fit with Roff's (1980) prediction of an increase in body size at the transition zone. Instead, head width seems to respond to season length differently in France and the UK. It is unlikely that this discontinuity is directly related to polymorphism per se, because the known distribution of alternative phenotypes of both species strongly suggests the transition to solitary nesting occurs within the UK (Soro et al., 2010; Davison and Field, in press, Chapter 2). As such the transition from social to solitary nesting was not reflected as a sudden change in head width in either *L. calceatum* or *H. rubicundus*.

The general explanation that northern bees experience a shorter growing season leading to reduced body size (Chown and Gaston, 1999; Blanckenhorn and Demont, 2004) clearly cannot account fully for either observed pattern. It is also unlikely that countergradient variation in growth rates can explain the lack of saw-tooth clines; reciprocal transplants of *H. rubicundus* (Field et al., 2012) and a recent transplant of northern UK *L. calceatum* to a southern site (Davison and Field, in prep, Chapter 3) provide no evidence for genetic countergradient variation in growth rate (Blanckenhorn and Demont, 2004; Kivelä et al., 2011). Instead, growth rates appear to be heavily influenced by temperature (Field et al., 2012; Davison and Field, in press, Chapter 2; but see Field et al., 2010). It is likely that other factors linked with life history and sociality can better explain the observed patterns.

4.4.2 Social polymorphism as a distinct system

In contrast to other taxa in which larvae or nymphs are free-living, sweat bees mass provision their offspring and body size is highly correlated with the amount of food an offspring is provided with in its brood cell at the start of development (Plateaux-Quénu, 1983; Richards and Packer, 1994). Therefore constraints on provisioning may contribute to the general pattern in both species of larger size in areas with longer seasons, in addition to season length per se. Specifically, bees nesting further north may experience environments in which resources are more scarce or available for a shorter period of time, or in which there are fewer opportunities to provision (Field, 1996; Richards, 2004; Field et al., 2012). Together, these effects could lead foundresses to allocate each individual offspring with less food (Field et al., 2012).

In both *L. calceatum* and *H. rubicundus*, foundresses were largest where the season is longest. Within the region where bees nest socially there might also be a north-south cline in the number

of workers produced by foundresses. This could occur because further south, foundresses emerge earlier and therefore have more time or more plentiful resources to provision extra workers (Plateaux-Quénu, 1992; Davison and Field et al., under review, Chapter 2 but see Richards et al., 2015). Because halictid eggs are large relative to their body size (Iwata and Sakagami, 1966), the rate at which workers in more populous nests collect provisions could outstrip the ability of the foundress to lay eggs. One possible outcome is that workers use excess provisions to resource their own eggs (Wyman and Richards, 2003; Richards et al., 2015). Alternatively, cells destined to contain female offspring could instead receive more resources if foundresses can effectively suppress worker oviposition, which could then increase both the size and future fitness of second brood females (Franks and Crespi, 1989; Boomsma and Eickwort, 1993). There is almost no data available with regard to number of workers in social nests of either *L. calceatum* or *H. rubicundus* at different latitudes, but other sweat bees show trends for producing more workers in years of better weather, or have larger colonies at lower latitudes (Richards and Packer, 1996; Wyman and Richards, 2003). In contrast colonies of *Polistes* do not appear to show any significant size variation with season length, perhaps because their open nests are more vulnerable to whole nest predation (Reeve, 1991).

Differences in provisioning between social and solitary nests are also likely to have contributed to the lack of detected saw-tooth clines. Multiple workers provision the reproductive second brood offspring of social nests, but a lone foundress provisions all offspring in solitary nests (in *L. calceatum* foundresses occasionally co-found, see Chapter 2). Therefore, because workers in social nests together collect resources more rapidly and can strategically provision bigger cells with more food (Kamm, 1974; Richards, 2004), the sizes of reproductive females from social and solitary nests are probably not directly comparable. Consequently, although body size overall increases with season length in *L. calceatum*, a saw-tooth cline itself may not occur because any additional advantage gained by increased development time just north of the transition zone is partially negated by provisioning constraints on solitary bees. Sweat bees provide extended parental care (Plateaux-Quénu, 2008), and brood mortality is decreased where adults remain in the nest until offspring eclosion (Soucy, 2002). Since foraging is risky (Cant and Field, 2001), patterns of provisioning by workers and solitary foundresses just to the south and north of the transition zone respectively are likely to be very different (e.g. Field 1996). Solitary foundresses just to the north of the transition zone therefore may not be able to capitalise on the temporary increase in available development time because they are unable to provision female offspring with sufficient extra food.

Additionally, because social phenotype is plastic in *H. rubicundus* but appears to be less so in *L. calceatum* (Field et al., 2010, 2012; Davison and Field, in prep, Chapter 3), the transition zone

between alternative phenotypes may be broader in *H. rubicundus*. If foundresses in solitary nests are not able to provision offspring as large as those provisioned by workers from social nests, then a broad region where both phenotypes occur in sympatry could act to smooth out any existing cline in B2 offspring. Moreover, in social nests of *H. rubicundus* a proportion of B1 females do not become workers and instead directly enter hibernation, emerging the following year as smaller nest foundresses (Yanega, 1988, 1989). This behaviour has been demonstrated in *L. calceatum* (Davison and Field, in press, Chapter 2), and could add to the smoothing effect described above.

One other explanation for why saw-tooth clines were not detected could be that only the B1 offspring in social nests experience constraints set by voltinism, whilst B2 offspring experience constraints set by season length (Kivelä et al., 2011). This scenario appears to occur in some solitary taxa where development time is longer in the second generation than the first (Masaki, 1972), and where the saw-tooth cline is more pronounced when only the directly developing generation from the bivoltine population is considered (Nygren et al., 2008). A saw-tooth cline may therefore become apparent if only the worker generation from social regions is included instead of foundresses, because smaller B1 offspring enable more time for B2 offspring to attain a larger size, which probably increases overwintering success (Beekman et al., 2003; Brand and Chapuisat, 2012; but see Weissel et al., 2012) because diapause is costly (Sakagami et al., 1984; Weissel et al., 2012). An obvious confounding factor, however, is that the size difference between queens and workers (caste-size dimorphism) is intrinsically linked with sociality in sweat bees (Schwartz et al., 2007; Field et al., 2012; Davison and Field, in press, Chapter 2; but see Field et al., 2010). Moreover, the production of small workers is likely to result from a complex suite of selective pressures, such as reducing kin conflict within the nest and prolonging foundress longevity to lay B2 eggs (Breed and Gamboa, 1977; Field et al., 2010).

Caste-size dimorphism is generally assumed to have originated via maternal manipulation (Richards and Packer, 1994; Brand and Chapuisat, 2012), but the above scenario suggests an intriguing alternative. Intergenerational size differences similar to the caste-size dimorphism reported in *L. calceatum* have been recorded in the solitary bivoltine sweat bee *L. villosulum* (Plateaux-Quénu et al., 1989). This pattern possibly occurs at least in part because larger second-generation females must endure hibernation (Beekman et al., 2003; Brand and Chapuisat, 2012; but see Richards and Packer, 1996; Weissel et al., 2012), or because resources are more plentiful in the summer (Kim and Thorpe, 2001; Richards et al., 2015). This suggest that patterns paralleling caste-size dimorphism could have originated prior to sociality, and later been co-opted as a mechanism to reduce kin conflict between queens and workers in social taxa (Lin and Michener, 1972; Michener, 1990). Such an explanation challenges the idea that

maternal manipulation played a key role on the origin of workers in social sweat bees, and deserves further investigation. Investigating size clines in a solitary bivoltine sweat bee could help to separate the relative importance of sociality and voltinism in determining how size responds to changes in season length. However, apart from the work of Plateaux-Quénu et al. (1989), solitary bivoltine sweat bees remain almost entirely unstudied, and provide potentially rich ground for future research.

4.4.3 Implications for sociality

A key implication for sociality that arises from constraints set directly or indirectly by season length is the possibility of clinal variation in caste-size dimorphism. This could be important because queens may more easily dominate workers in nests where caste-size dimorphism is greater (Kukuk and May, 1991; Richards and Packer, 1996, but see Field et al., 2010). Field et al. (2012) proposed that one outcome of maximising the size of B2 offspring in social populations could be a gradient of decreasing caste-size dimorphism with increasing season length. This scenario predicts that the size of nest foundresses and workers should be most divergent closest to the transition zone, yet the available data for *L. calceatum* and *H. rubicundus* imply the opposite pattern. Caste-size dimorphism in *L. calceatum* studied in the south of the UK is around 6.6% (Chapter 2; Davison and Field, in press, Chapter 2); however, in more southern French populations dimorphism is greater at 8.3% and 13% (Plateaux-Quénu, 1992), and the same pattern is found in Japan (Sakagami and Munakata, 1972). Similarly, data from North American *H. rubicundus* and other species also suggest that caste-size dimorphism is greatest in areas where the growing season is longer (Yanega, 1989; Packer et al., 1989; Soucy, 2002), but it is not clear how the size of workers varies in relation to foundresses to generate these patterns. One possibility is that worker size does not follow any size cline, because there may be little advantage in producing larger workers (Strohm and Liebig, 2008; see Chapter 6 and a longer discussion in Chapter 7; but see also Richards and Packer 1996). Instead of investing in larger workers, larger southern foundresses might instead produce more of them (Robin, 1988). This could act to increase caste-size dimorphism still further if nests with more workers provision each B2 female offspring with more food (Frank and Crespi, 1989). Further work simultaneously examining size clines in workers and queens could prove particularly fruitful, as well as detailed studies of wild social nests to determine whether nests situated further south contain a greater number of workers.

Chapter five

Severe environmental barriers to sociality: a field transplant of the obligate primitively eusocial sweat bee *Lasioglossum malachurum*

Abstract

Complex social behaviour such as eusociality is thought to buffer against the negative effects of harsh or unpredictable environmental conditions. Nonetheless, before the evolution of traits such as perenniality and nest thermoregulation, these factors may in fact inhibit the evolution of eusociality. I utilise the obligate primitively eusocial sweat bee *Lasioglossum malachurum* to investigate environmental constraints on the early stages in the evolution of eusociality, and examine the ability of bees to respond adaptively to novel environmental conditions. Short season length is thought to be a key factor precluding the expression of sociality, because there is insufficient time to produce both a first brood (B1) of workers and a second brood (B2) of reproductives. In this chapter, I test whether shorter seasons preclude sociality by transplanting newly-founded nests of *L. malachurum* from the south to the north of the United Kingdom (UK), far beyond its natural range. I predicted this would result in delayed nesting phenology, foundresses would provision fewer B1 offspring, foundresses would produce more males and that the delayed phenology would preclude successful production of B2. In one year when bees were transplanted after provisioning in the south, B1 females emerged and provisioned B2. In another year when nests were transplanted before provisioning began in the south, nesting phenology was delayed by approximately seven weeks in the north relative to the south, but foundresses in the north provisioned as many cells as foundresses at Sussex. However, it is

probable that no B1 offspring in the north completed development due to intense rainfall. In both years it is highly likely that the delayed phenology would have precluded successful provisioning and emergence of second brood offspring. I conclude that shorter seasons do actively preclude sociality in sweat bees, and that *L. malachurum* appears to exhibit only very limited behavioural plasticity.

5.1 Introduction

The distribution and expression of social behaviour among arthropods is strongly influenced by environmental conditions (Purcell, 2011). Determining how extrinsic environmental factors affect the formation of social groups is critical to understanding the origin of complex social behaviours, such as eusociality (Korb and Heinz, 2008). Among bees, eusociality has arisen at least four times (Bourke, 2011a; Cardinal and Danforth, 2011; Gibbs et al., 2012a), and is characterised by largely non-reproductive daughters remaining at the natal nest as workers to help their mother rear more offspring (Wilson, 1971). Analyses conducted across taxa show that harsher or more variable environmental conditions might actually favour eusociality because the presence of multiple individuals can act as a buffer against the environment (Hoiss et al., 2012; Kocher et al., 2014). However, an important consequence of raising workers prior to reproductive offspring in annual temperate taxa is that the colony cycle is necessarily lengthened relative to the length of the active season. Eusociality may therefore be favoured in harsher environments only after the evolution of characteristics associated with more socially advanced groups, namely rapid offspring development, nest thermoregulation or a perennial life cycle (Kaspari and Vargo, 1995; Hoiss et al., 2012; Kocher et al., 2014). By contrast, season length and localized geographic and temporal environmental variation are thought to play a particularly significant role in shaping intra- and interspecific variation in social organization of primitively eusocial species (Richards and Packer, 1996; Fucini et al., 2009; Kocher et al., 2014).

Primitively eusocial sweat bees (Hymenoptera: Halictidae) are one group in which there is considerable variation in social behaviour both within and between species, and environmental factors are thought to play a key organizational role (Wcislo, 1997; Schwarz et al., 2007). Nesting behaviour ranges from solitary to obligate primitive eusociality, with intermediate socially polymorphic species that exhibit both social and solitary behaviour (Wcislo, 1997; Schwartz et al., 2007). Sweat bees therefore represent an ideal group with which to examine the role of the environment in shaping the origin and early evolution of eusociality. Primitive eusociality in sweat bees is characterised by the presence of at least two generations; a first brood (B1) of typically smaller female workers (and a varying proportion of males) provisioned

by a solitary, overwintered nest foundress, and a second brood (B2) of larger reproductives provisioned by the B1 workers. Social polymorphism occurs in species where southern or low altitude populations produce workers, but northern or high altitude populations do not (Soucy and Danforth, 2002; Cronin and Hirata, 2003; Davison and Field, in press, Chapter 2). Obligate social species are found only in southern or low altitude areas, suggesting that social behaviour is temporally precluded where the season is too short for bees to rear consecutive broods (Kocher et al., 2014). In at least one socially polymorphic species the expression of sociality is plastic and may be associated with the amount of time remaining in the season after the emergence of B1 offspring (Field et al., 2010, see also Hirata and Higashi, 2008).

The potential for the environment to stochastically affect social organization is revealed in marginal environments, where inter-year variation in local weather conditions can dramatically affect phenology, demography and colony social organisation (Packer et al., 1989; Richards and Packer, 1996). The length of the colony cycle is directly affected by season length, and in areas with a longer season nests are initiated earlier (Field et al., 2012; Richards et al., 2015). The timing of nest initiation is thought to be a key constraint in the expression of sociality, and in at least two socially polymorphic species the date of offspring emergence has been directly implicated in whether offspring remain at the nest as workers (Hirata and Higashi, 2008; Field et al., 2010).

First brood offspring emerging later in the season may not have enough time to provision a second brood, or there may not be time for B2 offspring to successfully develop (Hirata and Higashi, 2008; Field et al. 2010). Foundresses that initiate nesting earlier in the year can also experience higher rates of mortality later in the season, which can then increase the reproductive options for any worker that becomes a replacement queen (Richards, 2000; Richards et al., 2015). Furthermore, inter-year environmental variation in weather conditions can affect the number and size of offspring produced, probably mediated via limitations on provisioning opportunities and resources available to provisioning foundresses and workers (Richards and Packer, 1996; Richards, 2004; Richards et al., 2015). In turn this can result in contingent variation in caste-size dimorphism, which can directly affect the outcome of dominance interactions and queen-worker conflict over reproduction within the nest (Breed and Gamboa, 1977; Richards et al., 1995; Richards and Packer, 1996).

The proportion of males in the worker brood can also vary within and between species. In solitary nests, approximately equal numbers of male and female offspring are produced; however, in social nests worker broods are female-biased (Soucy, 2002; Hirata and Higashi, 2008). The degree of female-bias is greatest in the most socially specialised species, in which

worker broods can comprise less than 2% males (Packer and Knerer, 1985; Wyman and Richards, 2003). In the socially polymorphic North American *Halictus rubicundus*, social nests situated further north produce the greatest proportion of males in the worker brood (Yanega, 1993), possibly as a bet-hedging strategy in marginal environments that enables more B1females to enter hibernation (Seger and Brockman, 1987; Yanega, 1989). Lengthening photoperiod is thought to influence the laying of male eggs (Yanega, 1989, 1997), but it is unknown to what extent obligate social sweat bees might be able to adjust the sex ratio in response to environmental cues. The B1 sex ratio can vary between years in obligate social species (Richards and Packer, 1996), although it is not clear whether this represents an adaptive response by foundresses.

The extent to which mobile taxa (i.e. non-sessile) can persist outside of their range has received little experimental attention (Sexton et al., 2009). Moreover, those that have, have specifically addressed social polymorphism within the range of the species (Plateaux-Quénu et al. 2000 (lab test); Field et al., 2010, 2012, Davison and Field, in prep, Chapter 3; and see Cronin, 2001 in an allodapine bee (Apidae: Xylocopinae)). Field transplants of polymorphic species show that bees either do not express sociality in northern environments (Field et al., 2010), or that sociality itself is in the process of being lost (Davison and Field, in prep, Chapter 3). However, it has yet to be demonstrated that season length is the critical factor, and to date no study has transplanted an obligate primitively eusocial bee into a novel environment. There is currently therefore a lack of experimental evidence supporting either the prediction that a social life cycle is temporally precluded at shorter season lengths (Kocher et al., 2014), or examining the true extent of behavioural plasticity, in obligate social sweat bees. Some latent behaviours are expressed only when bees are manipulated or subjected to unusual conditions, causing the expression of ancestral or otherwise previously unknown behavioural traits (e.g. Rehan et al., 2013; Quiñones and Wcislo, 2015). Moreover, if sociality is not precluded in northern environments, or apparently obligate social species are capable of non-social nesting, it would be interesting to ask why they do not currently persist further north.

In this chapter I transplant the obligate eusocial sweat bee *Lasioglossum malachurum* Kirby (Fig. 5.1) from the southern United Kingdom (UK) where it is known to nest socially (Packer and Knerer, 1985; Davison, unpubl.) to the northern UK, several hundred miles further north than its recorded distribution (Falk and Lewington, 2015; Fig. 5.2). Previous studies that have transplanted organisms to outside of their natural range have focussed on plants (e.g. Gebert and Eckhart, 2005; Angert et al., 2008), and therefore this represents a novel experimental approach for a mobile taxon.

Figure 5.1
Female *Lasioglossum malachurum* next to her nest entrance (behind her) in spring before transplantation from the source site at Denton.

Sociality in *L. malachurum* is obligate so far as is known, with a life cycle in Britain typical of many primitively eusocial halictids. In Britain *L. malachurum* is confined to southern England (Falk and Lewington, 2015; Fig. 5.2), and the following summary of the life cycle is based on nests from this region (Packer and Knerer, 1985; Davison, pers. obs.). Mated females emerge from hibernation and initiate subterranean nests in spring. Each female alone mass provisions a first brood (B1) of ≈7 sealed brood cells, each containing a single offspring and all the food required for development. B1 females emerge in summer and remain at the nest as helpers, provisioning a second brood (B2) of reproductives. Almost all B1 offspring are female and on average around 15% smaller than queens.

I made three predictions for how transplantation of *L. malachurum* to the north of the UK would affect social behaviour. First, I predicted that the shorter season and cooler temperatures would delay nesting phenology relative to control bees in the south, and that it would therefore not be possible for transplanted bees to successfully rear B2 offspring. Second, I predicted that due to less favourable weather conditions, foundresses would experience greater rates of both total nest failure (no B1 cells provisioned) and brood failure. Third, I predicted that to the extent that *L. malachurum* can be socially plastic, transplanted foundresses might actively respond to increased day length by producing a more male-biased first brood. By transplanting an obligate social species, this study represents the first experimental test of the general prediction that sociality is temporally precluded at higher latitudes, and of the extent of behavioural plasticity,
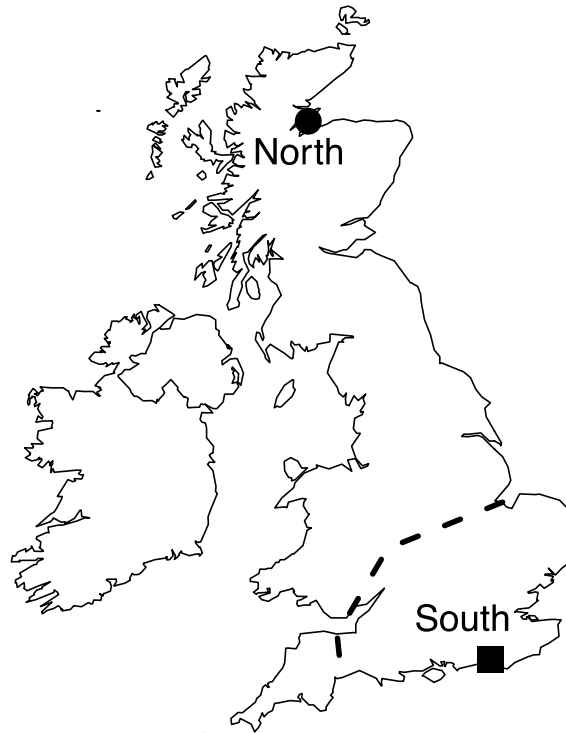
Figure 5.2
Map of the UK and Ireland showing the study sites. South represents both the source site for bees at Denton Downs and the University of Sussex to where control nests were transplanted. These are situated too close to one another to be shown independently. North represents Cromarty to where bees were transplanted, almost 500 miles north of the South. The dashed line shows the limit of the known distribution of *L. malachurum* in the UK (Falk and Lewington, 2015).

in an obligate social sweat bee.

5.2 Methods

I tested for behavioural plasticity and environmental constraints on the expression of eusociality in *Lasioglossum malachurum* by transplanting foundresses from the south to the north of the UK (Fig. 5.2). This is far beyond the natural range of obligate social sweat bees in the UK, and where only solitary behaviour is expressed in socially polymorphic species (Falk and Lewington, 2015; Field et al., 2012; Davison and Field, in press, Chapter 2). Foundresses were sourced from a substantial aggregation (>>1000 nests) located along a grassy footpath in the South Downs National Park at Denton, approximately eight miles from the University of

Figure 5.3
Field site at Cromarty to where buckets from Sussex were transplanted. The arrow indicates the precise location of buckets.

Sussex. Foundresses were then transplanted to the University of Aberdeen's Lighthouse Field Station at Cromarty in northern Scotland (Figs. 5.2, 5.3).

Transplants were made in two separate years, 2013 and 2015. In both years, bees removed from the source site had initiated nests in black 14L plastic buckets with drainage holes cut into the base. Buckets were filled with compressed soil from the nest site, and embedded adjacent to the nesting aggregation. These were first embedded during the winter of 2012/2013, with further buckets added during the winter of 2014/2015. Buckets were kept clear of vegetation and therefore offered bare areas of soil, which foundresses naturally colonised upon emergence from hibernation in the spring of 2013 and 2015 respectively. For clarity, I now describe the methods for the transplants carried out in 2013 and 2015 separately. Figure 5.4 details the chronology of events for the experiment conducted in 2015. Details for 2013 are not shown since both control and transplanted foundresses probably provisioned all of their offspring prior to transplantation, and therefore it was not possible to test phenological hypotheses.

5.2.1 2013 fieldwork

2013 transplant

In 2013 two buckets containing nesting foundresses and their developing B1 offspring (see below) were removed on 31 May and placed in refrigerated conditions (5°C) overnight. Over the following two days they were driven to Cromarty and embedded in the ground adjacent to a west-facing wall (Fig. 5.3). On 11-June a further two buckets were removed from Denton, refrigerated overnight and driven around for the two following days as a control to mimic conditions experienced by transplanted bees. These were then embedded at the University of
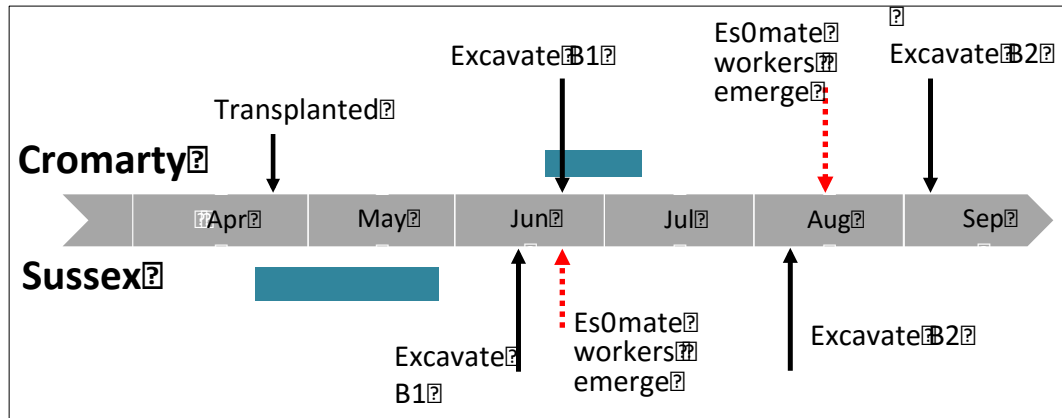
Figure 5.4
Timeline showing the key events in 2015 at Cromarty and Sussex from transplanting to the excavation of second brood. Blue bars show estimated periods of foundress provisioning.

Sussex (Fig. 5.2). During transportation, buckets were kept individually in a dark, cool environment to discourage any activity. Each was wrapped in two black plastic bin bags and placed in a sealed black plastic container with ice packs. Duvets were used to cushion the buckets from shocks experienced from transportation.

Focal nests were not observed prior to transplantation in 2013, but observations carried out by another PhD student at nearby nests indicated that foundresses had provisioned all of their B1 offspring before buckets were removed (L. Holt, pers. comm.). It is therefore unlikely that foundresses transplanted to Cromarty provisioned any B1 offspring subsequent to transplantation. Moreover, no further provisioning was observed at Denton after the removal of buckets used in the control transplant (J. Field, pers. obs.). Consequently all B1 offspring were almost certainly provisioned at Denton, and therefore we focus only on whether transplantation increased rates of brood failure and the behaviour of offspring upon emergence.

Nest excavations in 2013
To examine the failure rate of brood within nests, I excavated nests from one bucket each at Cromarty and Sussex, prior to first brood emergence, on 04 July 2013 and 12 July 2013 respectively. In both cases foundresses had ceased provisioning at the time of excavation, as indicated by the lack of newly provisioned brood cells. To examine whether offspring at Cromarty had successfully emerged and provisioned a second brood the remaining bucket was excavated on 13 August 2013, prior to the emergence of any B2 offspring. B2 offspring were not excavated at Sussex. Some nests contained alive workers and small larvae, therefore it is possible the provisioning had not stopped. The solid mass of soil was extracted from each bucket by tipping it upside down, and then gently removing the bucket. The soil mass was then

righted and excavated by carefully scraping with a knife, until individual nests were exposed. All pupae and foundresses present were recorded and stored in ethanol. I also noted whether offspring were alive or dead: dead larvae often appear misshaped or squashed, and alive larvae typically wriggle upon contact. In 2013 all offspring were pupae, and therefore the sex of offspring was easily determined by counting the number of antennal segments (12 in females and 13 in males). After transplantation, nests were not observed at either Sussex or Cromarty. Social behaviour was diagnosed by the presence of developing B2 offspring, which must have been provisioned by B1 workers. In *L. malachurum* each brood forms a separate cluster of cells. The B2 cell cluster is located beneath the B1 cell cluster, and therefore it is easy to distinguish between B1 and B2 brood.

5.2.2 2015 fieldwork

2015 transplant

Buckets were transplanted seven weeks earlier in 2015 than in 2013. I carried out observations at focal buckets on sunny days prior to transplantation, and individually marked newly initiated nests with numbered nails. Observations confirmed that foundresses did not begin provisioning before either control buckets or those transplanted to Cromarty were removed. All provisioning by foundresses was therefore undertaken subsequent to transplantation, and so for the 2015 transplant I focus on data collected from all stages of the life cycle. Four buckets were removed from Denton on 10 April 2015 and treated as controls. A further four buckets were removed on 16 April 2015, and embedded in the ground at Cromarty on 18 April 2015 in exactly the same location as buckets transplanted in 2013 (Fig. 5.3). During transit, buckets were treated the same as those in 2013. As in 2013 nests were not observed at either Sussex or Cromarty after transplantation, and social behaviour was determined by the presence of B2 brood (see previous section).

Nest excavations in 2015

Due to transplantation occurring much earlier in 2015, I additionally examined total nest failure, productivity and phenology at Sussex and Cromarty. Two buckets were excavated prior to first brood emergence on 10-12-June and 24-25-June at Sussex and Cromarty respectively. At the time of excavation it was not clear that foundresses at Cromarty had finished provisioning, as all nests contained very young larvae on large pollen balls. Nests were excavated for second brood offspring on 06 August and 08 September and Sussex and Cromarty respectively. Nests were excavated using the same method as in 2013, and all larvae, pupae and adults were recorded and stored in ethanol. As a measure of foundress provisioning effort, I noted the number of cells that *had been provisioned* (i.e. contained pollen) regardless of whether they contained developing

(a)

(b)
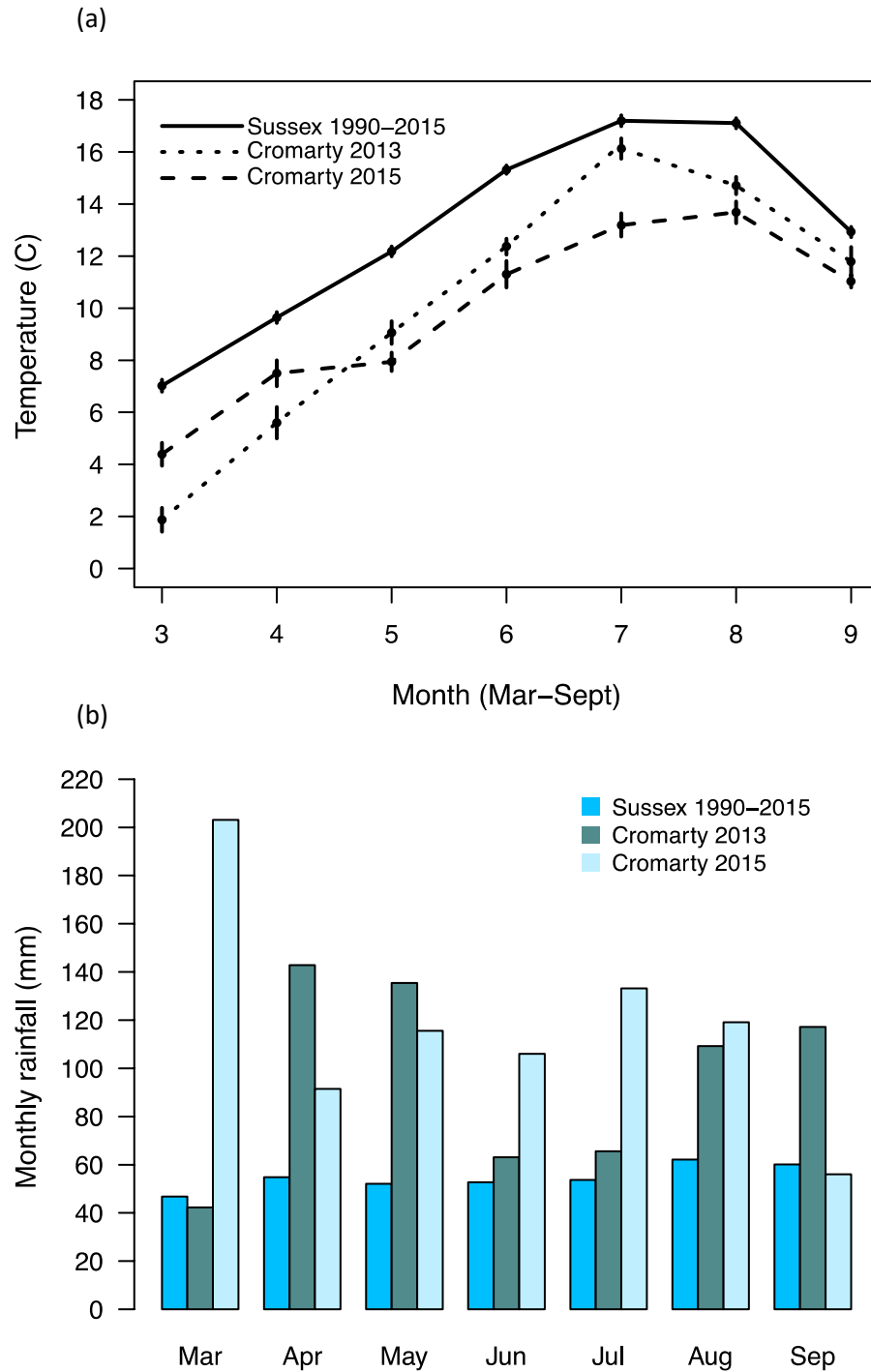
Figure 5.5
(a) Mean monthly temperature for Sussex (solid line, long term 1990-2015 average for SE England), and Cromarty in 2013 (short dashes) and 2015 (long dashes).
(b) Total monthly rainfall for Sussex (1990-2015 average) and Cromarty in 2013 and 2015, see legend for colours.

offspring. All B1 offspring excavated at Cromarty were larvae, and I therefore assigned sex by genotyping individuals using microsatellite markers (developed by P. Parsons and J. Field unpubl.). Offspring were genotype at twelve loci, and designated as haploid males when only a single allele was detected across all loci (see appendix A).

5.2.3 Climate and weather data

To provide a baseline for conditions typically experienced by *L. malachurum* at the source site study species I constructed 25-year time series of mean monthly temperature and rainfall for the southeast of England (Fig. 5.5a, b). Data covering 1990-2015 were downloaded from the UK Meteorological Office website[8]. To examine how conditions experienced by bees transplanted to Cromarty in both 2013 and 2015 deviated from those typically experienced at Sussex, temperature data were downloaded from a nearby web-based weather station located at Inverness Airport[9]. Localised rainfall data were not available; therefore I use regional monthly rainfall values for northern Scotland from 2013 and 2015 respectively as indicative of conditions at Cromarty in both years.

5.2.4 Data analysis

In 2015, buckets were transplanted to Cromarty early in the season, and before foundresses had begun provisioning at the source site. I therefore hypothesised that a greater proportion of foundresses transplanted to Cromarty would fail to produce any B1 offspring at all, experience higher brood mortality and that successful foundresses would overall provision fewer cells than control foundresses transplanted to Sussex. I used generalised linear models (GLMs) with binomial, negative binomial or normal errors as appropriate to compare the rate of nest failure and the number of cells provisioned (including and excluding completely failed nests) between nests transplanted to Cromarty and controls. B1 offspring excavated at Cromarty were significantly younger than those at Sussex (see below). To avoid artificially inflating the failure rate at Sussex (brood will have had more time in which to fail) I used the number of cells that *had been provisioned* (i.e. inclusive of those that had failed) to compare the number of potential B1 offspring provisioned by foundresses between sites. Analyses of B1 size and nest failure rates in 2015 thus focus on foundress provisioning opportunities. In 2013, nests most likely already contained brood prior to transplantation. I used a GLM with binomial errors to test whether transplantation to Cromarty resulted in a greater proportion of brood failing than at Sussex

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[8] http://www.metoffice.gov.uk/climate/uk/summaries/2015/october/regional-values
[9] http://www.wunderground.com

I further hypothesised that the phenology of bees transplanted to Cromarty in 2015 would be delayed relative to bees at Sussex. To examine this I scored the age of brood excavated from nests as follows: pollen ball= 0, very small larva= 1, small larva= 2, medium larva= 3, large larva= 4, white pupa (wp)= 5, wp brown eyes= 6, wp black eyes= 7, pigmented pupa= 8. Within nests there was very little variation in the age of brood and so the individual scores for brood in each nest were averaged. I then used the Wilcoxon signed rank test to examine differences in the age of brood between nests at Cromarty and Sussex. Although brood did not spend their whole development at Cromarty, I also scored the age of brood from 2013 to test whether being transplanted to Cromarty significantly slowed their development.

All analyses were conducted in the *R* environment (R Development Core Team, 2013), and the MASS package (Venables and Ripley, 2002) was used for performing GLMs with negative binomial errors. Results are presented ±1 standard error.

5.3 Results

5.3.1 Prediction 1: Phenology will be delayed at Cromarty relative to Sussex, and preclude the successful rearing of B2 brood

In 2015, first brood offspring excavated at Cromarty were predominantly very small larvae (see inset Fig. 5.6), whereas B1 offspring excavated at Sussex were almost all significantly older pupae (Fig. 5.6; W=32, p=0.006, $\bar{x}$ B1 age at Cromarty=1.59±0.36 Sussex=5.9±0.42). To estimate the difference in phenology between the two sites, I conservatively assumed that control foundresses began provisioning two weeks after being transplanted to Sussex. This is justified because by this time (23 April) provisioning had been observed both by bees at the source site and at a nearby aggregation of another social sweat bee (C. Couchoux, pers. comm.; Davison, pers. obs.). The pupal stage typically comprises about one third of total development time (see Table 14-2 in Yanega, 1997), and I estimated that B1 offspring excavated at Sussex had approximately another week of development left. Thus I take 17-June as their eclosion date, giving an estimated development time of 50 days, or about seven weeks. This is in line with development times previously reported for *L. malachurum* (Weissel et al., 2006). Most offspring excavated at Cromarty were only very small larvae, and cannot have been more than a week old. I therefore conservatively assume that most offspring at Cromarty were provisioned approximately one week prior to excavation (18 June), about the same time as B1 emergence at Sussex. Thus, the life cycle at Cromarty was roughly seven weeks delayed relative to Sussex.

Furthermore, temperatures throughout June, July and August at Cromarty in 2015 never greatly exceeded May temperatures at Sussex in the same year (Fig. 5.5a). Because higher temperatures later in the season would not have speeded up development at Cromarty, it is possible to conservatively assume a similar development time of 53 days to B1 offspring at Sussex. Therefore, if B1 offspring at Cromarty had survived to eclosion, I estimate they would not have emerged until around 9-August, again approximately seven weeks later than B1 offspring at Sussex.

There was no evidence of social nesting at Cromarty in 2015. Of 14 nests initiated in buckets transplanted to Cromarty, none contained any B2, and therefore failure to produce B2 offspring at Cromarty was 100%. Nest excavations aimed at locating B2 offspring at Cromarty revealed the presence of first brood chambers and some pollen in the soil, but no attempt
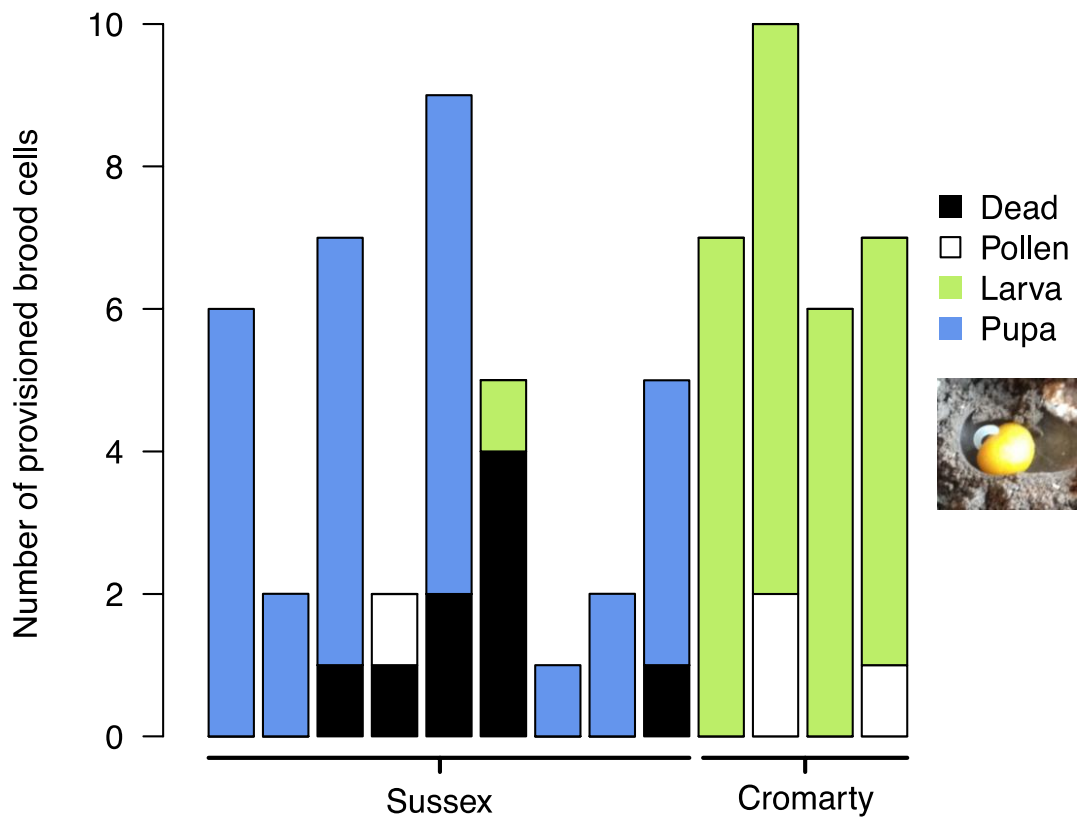
Figure 5.6
The number of B1 brood cells provisioned by foundresses at Sussex and Cromarty and the age of brood within each nest in 2015 (see legend). Note that each column represents a different nest. Inset shows a small larva feeding on a provision mass inside an opened cell. Note that there are no dead brood at Cromarty because offspring are considerably younger than at Sussex. Foundresses at Sussex and Cromarty provisioned equal numbers of B1 brood cells ($F_{13}=2.029$, $p=0.180$), mean number of cells provisioned: Sussex= 4.5 ± 1.58, Cromarty=6.2 ± 0.96.

to construct B2 cells. This suggests that foundresses *had* provisioned first brood offspring, but that these offspring failed to eclose. Moreover, the only evidence of adult bees at Cromarty was a single foundress-sized head buried in the soil. In contrast, at Sussex twelve nests were excavated before B2 emergence, and six (50%) contained provisioned B2 offspring (8.33± 2.30, range 3-19). The similarity of this failure rate to that of nests failing to produce any B1 offspring suggests that few nests failed completely once workers had emerged.

In 2013 first brood offspring were excavated from nine nests at Cromarty and 20 nests at Sussex. There was no difference in the age of offspring excavated from Cromarty or Sussex in 2013 (W=91.5, p=0.233; $\bar{x}$ B1 age at Sussex=6.14±0.09, $\bar{x}$ B1 age at Cromarty=5.86±0.14). It was therefore possible to directly compare the proportion of brood within nests that failed after being provisioned by the foundress. Second brood offspring were excavated from 11 nests at Cromarty in 2013 ($\bar{x}$=3.82±0.84 B2 offspring per nest), demonstrating that in these nests B1 females emerged and behaved as workers. Excavations were not conducted for second brood at Sussex in 2013. However, it is highly likely B1 females would have behaved as workers, as occurred in 2015.

5.3.2 Prediction 2: Foundresses transplanted to Cromarty will suffer increased failure to provision any B1 cells, successful foundresses will provision fewer B1 offspring, and a greater proportion of their brood will fail

In 2015 first brood offspring were excavated from five nests at Cromarty and nine nests at Sussex. There was no difference between Cromarty and Sussex in the proportion of foundresses that failed to provision any B1 offspring (X^2_1=0.158, p=0.691, Cromarty=14/19 (74%) failed, Sussex=11/19 (57%) failed). At nests where foundresses provisioned B1 cells, there was no difference between Cromarty and Sussex in the number of cells provisioned by foundresses (Fig. 5.6; F_{13}=2.029, p=0.180, Cromarty=6.2±0.96, Sussex=4.5±1.58). There was also no difference in the number of B1 cells provisioned when nests containing no cells (i.e. completely failed) were included (X^2_1=0.081, p=0.776, Cromarty=1.63±0.68, Sussex=2±0.65). In 2013 brood at Sussex and Cromarty were the same age, and it was therefore possible to test whether transplantation to Cromarty directly affected rates of brood failure. There was no difference in the proportion of dead brood in nests excavated at Cromarty or Sussex (X^2_{13}=0.70, p=0.403). Foundresses were equally likely to be found alive in their nests during excavations for B1 offspring in 2013 (Fisher's exact test: p= 0.205; foundresses detected at Cromarty=4/9, Sussex=15/20) and in 2015 (Fisher's exact test: p= 1; foundresses detected at Cromarty=2/5, Sussex=5/9).

5.3.3 Prediction 3: Foundresses transplanted to Cromarty will lay a greater proportion of B1 males

Only sex ratios from 2015 were considered, since foundresses provisioned offspring prior to transplantation in 2013. At Sussex, no males were detected in the nine nests that were excavated and contained B1 offspring, and therefore each foundress produced a 100% female first brood. The sex of offspring could be determined at only four of the five nests excavated at Cromarty; two produced 100% females and two produced a single male each (80% and 89% female-biased in each case).

5.4 Discussion

Environmental factors play a key role in the distribution and organisation of social behaviour among social insects (Purcell, 2011). In primitively eusocial species, social behaviour can be lost completely at high latitudes or altitudes (Soucy and Danforth, 2002; Fucini et al., 2009; Davison and Field, in press, Chapter 2), and is thought to be restricted to areas where the season is long enough for the production of workers prior to reproductives (Soucy and Danforth, 2002; Kocher et al., 2014). Climatic and environmental variation can also significantly affect nesting phenology and the expression of sociality (Richards and Packer, 1996; Rehan et al., 2013). However, the idea that season length directly precludes the expression of primitive eusociality has never been demonstrated experimentally, and the extent of latent behavioural plasticity present in obligate primitively eusocial species is unknown. I experimentally investigated both behavioural plasticity and environmental barriers to sociality in the obligate primitively eusocial sweat bee *Lasioglossum malachurum* (Fig. 5.1). I transplanted newly initiated nests containing foundresses from the south of the UK (Sussex) to northern Scotland (Cromarty), well beyond the natural range of *L. malachurum* (Fig. 5.2).

The principal findings of this chapter are that in 2013 B1 offspring emerged and behaved as workers by provisioning a second brood. However, in 2015 when foundresses were transplanted considerably earlier in the year, the number of first brood cells provisioned by foundresses did not differ between nests at Sussex or Cromarty (Fig. 5.6), but the phenology of the colony cycle was considerably delayed at Cromarty relative to Sussex (Fig. 5.4). Moreover first brood offspring provisioned at Cromarty in 2015 failed to eclose, leading to complete nest failure. There was also some limited evidence that foundresses at Cromarty in 2015 responded to environmental cues by producing more B1 male offspring, none of which were recorded at Sussex. These results demonstrate that *L. malachurum* will still express sociality in a northern

environment, but that harsher environmental conditions and the shorter season may preclude its long-term persistence.

5.4.1 Nesting phenology

The timing of nest initiation is thought to be a major constraint on sociality in temperate zones for species with an annual life cycle (Schwarz et al., 2007). I predicted that the nesting phenology of bees transplanted to Cromarty would be considerably delayed relative to control bees at Sussex. This prediction was supported, as.in 2015 when bees were transplanted to Cromarty early in the year, foundresses provisioned B1 offspring approximately seven weeks later at Cromarty than at Sussex. This meant that the period of B1 provisioning was almost non-overlapping between the two sites (Fig. 5.4), and that when adult offspring were eclosing at Sussex, most offspring at Cromarty had only just hatched as larvae (Fig. 5.6). Although B1 females failed to complete development at Cromarty, it is highly unlikely that had they emerged, they would have been able to rear a B2 to adulthood. This result agrees with previous work finding that sweat bees and polistine wasps that nest further north have nests that are initiated later in spring than those in the south (Reeve, 1991; Field et al., 2012; Richards et al., 2015), and that the timing of nest initiation is environmentally determined (Field et al., 2012). Moreover, it further supports a key prediction from a recent model examining the interaction between altitude and sociality in bees (Kocher et al., 2014): that obligate primitively eusocial species are absent from higher altitudes because the season is too short for the life cycle to be successfully completed.

Small sweat bees such as *L. malachurum* are particularly reliant on sufficiently warm temperatures to become active, because they are essentially thermo conformers (i.e. body temperature changes in response to the external environment) (Bishop and Armbruster, 1999). Foundress activity levels are positively associated with ambient temperature (Kamm, 1974; Field et al., 2012), which is therefore likely to be a key determining factor in the timing of nest initiation in spring (Potts, 1995). Temperatures at Cromarty lagged considerably behind the long-term average for the southeast UK, with May temperatures being matched at Cromarty only between June and July (Fig. 5.5a). Based on their age, most brood excavated in 2015 at Cromarty cannot have been provisioned before mid-June, which is consistent with the temperatures experienced by foundresses at Sussex when they provisioned their B1 offspring. Within a site, later nest initiation may be compensated by higher temperatures experience by developing offspring (Yanega, 1993; Field et al., 2012; Davison and Field, in press, Chapter 2), which can accelerate growth rates and appear to enable offspring to complete development at a similar time to those provisioned earlier in the season (Yanega, 1993; Weissel et al., 2006; Field

et al., 2012; Davison and Field, in press, Chapter 2, but see Field et al., 2010). Where nests are initiated later in the season because they are located relatively further north, developing offspring will never be able to achieve accelerated growth rates because temperature will always be cooler than further south (e.g. Fig. 5.5a), and consequently offspring will always emerge later (Field et al. 2012; Richards 2015).

The effects of temperature on the timing of nest initiation and development time could potentially be overcome by increasing developmental rate and tolerance of lower temperatures (Nylin and Gotthard, 1998; Gotthard et al., 2000). However, primitively eusocial sweat bees already tend to have shorter development times than solitary species (Kocher et al., 2014), and variation within species seems to be almost entirely determined by temperature (Weissel et al., 2006; Field et al., 2012; Davison and Field, in press, Chapter 2). Another way to truncate the life cycle would be to shorten development by becoming smaller (Richards and Packer, 1996, see Chapter 4), although it is unclear whether this alone could provide sufficient extra time. In contrast, bumblebees are able to effectively extend the season, because they are large and can raise their body temperature independently of the environment (Heinrich, 1979; Bishop & Armbruster, 1999).

In the present chapter, these factors meant that most offspring at Cromarty would be unlikely to have emerged until after the first week of August. This is later than workers in other social species studied in the far north of their range (Packer et al., 1989), and closely resembles the timing of offspring emergence at a nearby solitary population of *L. calceatum* (Davison, pers. obs.). Offspring in 2015 almost certainly emerged too late for a second brood to complete development before the end of the season. This result highlights an additional constraint on sociality in sweat bees: unlike most other bees, sweat bees must emerge, mate, feed and enter hibernation in the year they are born (Michener, 2007). Although this may sometimes provide a head start in spring (Matthews, 1991), it places more severe constraints on the timing of laying second brood eggs.

5.4.2 Foundress provisioning and brood failure

I predicted that foundresses transplanted to Cromarty would experience increased rates of nest and brood failure compared with control nests at Sussex, and that foundresses would provision fewer B1 brood cells. However, this prediction was partially supported: foundresses transplanted to Cromarty did not experience higher rates of nest or brood failure (in 2013 when a direct comparison was possible), and provisioned as many first brood cells as did foundresses at Sussex (Fig. 5.6). However, in 2015 it is probable that no B1 offspring completed

development and therefore brood failure was likely to have been 100%. It is not possible compare directly with Sussex, but this figure I clearly higher: six of 12 control nests at Sussex in 2015 contained B2 brood and therefore must have contained B1 female offspring that successfully emerged as adults. The number of first brood offspring provisioned by *L. malachurum* foundresses appears to be relatively consistent across populations throughout Europe (Packer and Knerer, 1985; Paxton et al., 2002; Strohm and Bordon-Hauser, 2003), and, as the results show, even when transplanted to a new environment. In contrast, Richards et al. (2015) found that *Halictus ligatus* foundresses initiating nests earlier in the season actually produced *fewer* workers than foundresses at another site further north, where nesting was initiated around a month later. Together this suggests that season length may have little impact on foundress provisioning behaviour to produce B1 offspring (Richards et al., 2015). Rather, foundresses may always provision some minimum number of workers required for the successful production of reproductive offspring (Strohm and Bordon-Hauser, 2003), or local availability of spring flowers or differences in weather conditions might limit opportunities for foundresses to provision (Richards et al., 2015). Like many social species, *L. malachurum* is well known to be highly polylectic (Westrich, 1989; Polidori et al., 2010). This may have allowed the foundresses I transplanted to Cromarty to more easily utilise available resources and provision the same number of cells as foundresses nesting at Sussex.

It was not possible to assess the quantity of pollen and nectar provided to each offspring, which may have revealed hidden provisioning constraints at Cromarty. Although foundresses were able to provision an equivalent number of first brood offspring at Cromarty and Sussex, it is possible that each was allocated with less food if fewer resources were available (Kim and Thorpe, 2001). For example, variation in resource availability and provisioning opportunities due to fluctuating environmental conditions is thought to directly impact the size of offspring in *H. ligatus* (Richards and Packer, 1996; Richards, 2004). The late spring and summer at Cromarty in 2015 were exceptionally wet (Fig. 5.5b; P. Thompson pers. comm.), and foundresses may therefore have had fewer or shorter windows of suitable weather during which to provision relative to those at Sussex. Whatever the case, however, it is difficult to generalise about constraints set by resource availability because the proximity and abundance of suitable floral resources is largely a stochastic property of the transplant locality (e.g. Field et al., 2012).

It is possible that another consequence of persisting in harsher conditions is increased failure to produce offspring (e.g. Packer et al., 1989). In 2015 there was no difference between Cromarty and Sussex in the rate of failure of foundresses to provision any B1 cells at all, indicating that there was no difference in foundress mortality early in the colony cycle. Yet, whereas at Sussex workers successfully provisioned a second brood, there was no evidence that any first brood

offspring emerged at Cromarty. When nests at Cromarty were excavated at the end of the summer there was no evidence that B1 offspring had emerged and attempted to provision a second brood, and no live adult bees were found either within or beneath nests. In fact the only evidence that foundresses had nested at all were areas of loose soil that had contained the first brood cells, and a single dead foundress-sized bee's head buried in the soil. It is therefore probable that any provisioned first brood offspring failed to complete development. In 2013 by contrast, when nests were transplanted after the first brood had been provisioned, developing offspring in nests transplanted to Cromarty did not experience elevated mortality compared with nests at Sussex, and workers successfully emerged to provision a second brood.

In both 2013 and 2015 brood were present in nests during June and July. It is likely that in 2015 the exceptional levels of rainfall in the northern UK during this period contributed to the total failure of nests prior to offspring emergence (Fig. 5.5b). By contrast, during the same period 2013 rainfall in the northern UK was much closer to the long-term average for southeast England (Fig. 5.5b). Ground-nesting Hymenoptera can be particularly vulnerable to heavy and persistent rainfall, because excessive rainfall can cause nests to waterlog and brood or provision masses to become mouldy (Richards and Packer, 1995; O'Neill, 2001; Soucy, 2002). However, unlike the species studied by Richards and Packer (1995) and Soucy (2002), first brood cells of *L. malachurum* are arranged in a cluster surrounded by a partial cavity (Sakagami and Michener, 1962; Packer and Knerer, 1986). This has probably evolved to improve drainage (Sakagami and Michener, 1962; Packer, 1991), and it is perhaps surprising that high levels of rainfall appear to have caused complete brood mortality. One reason could be that the buckets were largely kept free from vegetation (Packer and Knerer, 1986), but *L. malachurum* frequently nests in bare soil (Davison, pers. obs, see Fig. 1.8a in Chapter 1). Another possible reason why all B1 offspring at Cromarty appeared to die in 2015 is that *L. malachurum* is poorly adapted to such high levels of rainfall. Rainfall across the northern UK was approximately double the south-eastern UK average (Fig. 5.5b), and *L. malachurum* typically does not inhabit wet regions (Pesenko et al., 2000). Nevertheless, nests of obligate social species naturally persisting in very marginal environments can suffer exceedingly high failure rates when extreme weather is a regular occurrence, and in some cases whole nesting aggregations can fail completely (Packer et al., 1989).

5.4.3 Sociality and behavioural plasticity

My third prediction was that foundresses transplanted to Cromarty would respond to increases in season length by producing a greater proportion of male B1 offspring. This prediction was only partially supported: no B1 males were detected in control nests at Sussex in 2015, or in any

of the 2013 nests in which B1 offspring had been provisioned prior to transplantation. First brood males have been recorded only exceptionally rarely in ours and others' studies of *L. malachurum* in the southern UK (Packer and Knerer, 1985; L. Holt, pers. comm.; Davison, pers. obs.). However, single males were excavated from two out of the four nests excavated at Cromarty in 2015, yielding around 7.5% males across all brood cells, higher than the 0% for nests at Sussex and 2.3% reported by Packer and Knerer (1985).

In general, for annual species eusocial nesting is a high-risk strategy, particularly in marginal environments, because the payoff in boosted reproductive output is delayed until after worker emergence (Fu et al., 2015). One way around this problem is for some first brood offspring to mate and directly enter hibernation (Yanega, 1989), in which case it could prove adaptive to produce more B1 males. I found limited evidence in 2015 that foundresses transplanted to Cromarty increased the proportion of males in their first brood. It is possible that in the present study, foundresses responded to the longer photoperiod by laying some male eggs. However, it is not possible to tell from the data presented in this chapter whether this would have induced some B1 females to enter hibernation early, and it is difficult to conclude anything beyond the possibility that males were produced in response to transplantation. There is currently only limited evidence from other studies that *L. malachurum* exhibits early diapause of worker brood females, and not from the first brood (Wyman and Richards, 2003).

One way of mitigating the effects of environmental stochasticity is to express behavioural plasticity. Within its natural range, *L. malachurum* exhibits variation in the number of worker broods produced and the proportion of mated workers (Wyman and Richards, 2003; Richards et al., 2005); however, the extent to which aspects of behaviour in *L. malachurum* can be plastic in response to novel environmental conditions is unknown. Results presented in this chapter indicate that social plasticity in *L. malachurum* is likely to be limited; offspring that emerged in 2013 behaved as workers, and there was only limited evidence that foundresses in 2015 responded by producing a more male-biased first brood.

Species that are able to exhibit greater plasticity can cope better with variable environmental conditions, or invade new environments (Pfennig et al., 2010). For example, full social plasticity enables *H. rubicundus* to switch from social to solitary nesting when transplanted from the south to the north of the UK (Field et al., 2010, 2012). Obligate social sweat bees with the least flexible life cycles are least resilient if conditions are marginal for social nesting (Packer et al. 1989).

When offspring in the present study emerged as adults at Cromarty in 2013 they began provisioning second brood offspring instead of attempting to hibernate, as evidenced by the presence of developing second brood offspring. It is not possible to discount that a small number attempted to hibernate away from the natal nest, but since there are unlikely to have been any males present, early hibernation may not have been an option. However, the fact that offspring in 2013 did successfully provision a second brood at Cromarty, demonstrates that *L. malachurum* is not absent from more northern areas of the UK because conditions always preclude worker activity, or that they simply cannot survive. In this chapter I demonstrate that *L. malachurum* foundresses at Cromarty were capable of provisioning as many B1 offspring as foundresses at Sussex, and that offspring can successfully emerge and behave as workers to provision a second brood. In July 2013, temperature and rainfall data suggests that conditions experienced by B1 offspring were much closer to the long-term average in the southeast UK than in 2015 (Fig. 5.5a, b).

Consequently, the results presented in this chapter indicate that in some years it *may* be possible for *L. malachurum* to express sociality at Cromarty, provided that first brood offspring can emerge sufficiently early in the season and conditions in the summer are relatively benign. Such conditions probably occur only very rarely, if at all, and in reality the weather is likely to be highly variable between years (e.g. Richards and Packer, 1996; Fig. 5.5a, b). Moreover, had foundresses been transplanted to Cromarty earlier in 2013 it is highly likely that nesting phenology would have been significantly delayed as it was in 2015, because temperatures at Cromarty are consistently lower than at Sussex (Fig. 5.5a). Consequently, it is highly uncertain whether second brood offspring would have emerged sufficiently early in 2013 to successfully enter hibernation.

5.4.4 Conclusion

In this chapter I transplanted the obligate social sweat bee *L. malachurum* from the south (Sussex) to the north (Cromarty) of the UK in two separate years. I predicted this would (i) result in delayed nesting phenology that would preclude successful emergence of B2 offspring (ii) increased nest and brood failure and fewer first brood offspring and (iii) a greater proportion of males in the first brood. Prediction (i) was strongly supported: transplantation to Cromarty delayed nesting phenology by approximately seven weeks, and it is highly unlikely that B2 offspring could have been reared to adulthood. However, prediction (ii) was largely unsupported, because transplanted foundresses provisioned just as many B1 brood cells and experienced similar rates of brood failure as control nests at Sussex. Nevertheless, in 2015 all B1 offspring probably failed to complete development due to intense rainfall, suggesting that

conditions for developing offspring at Cromarty can be less predictable than those at Sussex. Prediction (iii) was partially supported as no B1 males were laid by control foundresses at Sussex, but two out of four foundresses transplanted to Cromarty each produced a single male. I conclude that shorter seasons do actively preclude sociality in sweat bees, and that *L. malachurum* may exhibit only very limited behavioural plasticity.

Variability both between years and between sites places limitations on short-term studies at single locations. Moreover, wild bees choose their own nesting environments, and therefore it is possible that Cromarty may not have been suitable for *L. malachurum*. One way around these limitations is to transplant bees to multiple sites, which would subject nesting bees to a range of microclimates and levels of resource availability.

Chapter six

Group size and individual quality in the primitively eusocial sweat bee *Lasioglossum malachurum*

Abstract

Studies of social groups in varied taxa, ranging from social insects to cooperatively breeding birds and mammals, frequently find that productivity increases with group size. However, results of correlative studies are potentially confounded by factors such as the quality of breeders or territories, which can only be addressed by experimentally manipulating group size. Such studies in birds frequently demonstrate that helpers really do contribute to group productivity, yet have rarely been conducted in social insects. In this chapter, I investigate the effect of foundress quality on group productivity (number of second brood reproductives produced) in the obligate primitively eusocial sweat bee *Lasioglossum malachurum*. Workers were removed from treatment nests, such that all possible new group sizes of between zero and five were created. I predicted that if higher quality foundresses produce more workers, nests manipulated to have fewer workers should still produce more reproductive offspring than unmanipulated nests containing the same post-treatment number of workers. Conversely, if foundresses incur costs through the production of more workers, originally larger nests might produce fewer offspring than unmanipulated nests. I found that, post manipulation, the number of workers in a nest was the most significant predictor of productivity. Nevertheless, there was also a significant negative effect of original group size on productivity, suggesting that producing more workers is costly. Examination of worker provisioning data indicated that

workers from originally larger groups tended to provision on fewer days, but did not provision more intensively. These results suggest that the negative effect of group size on productivity might be mediated via the quality of workers, and that the potentially lower quality of workers from larger groups represents a cost to producing more workers.

6.1 Introduction

Social organisms form groups, ranging in complexity from a single offspring remaining with its mother, to the elaborate societies of ants, honeybees and termites comprising thousands or even millions of individuals (Wilson, 1971). Such extreme group size variation among species has informed our understanding of the evolution of social complexity (Bourke, 2011a). However, group size also has important implications within species, such as for the survival of breeders and offspring, or the number of offspring produced (Michener, 1964; Emlen, 1997). Studies in various taxa have demonstrated that productivity increases with group size, including primitively eusocial bees (Smith et al., 2007; Thompson and Schwartz, 2006), foundress associations of vespoid wasps (Shreeves and Field, 2002; Tibbetts and Reeve, 2003) and helpers in some cooperatively breeding birds (Rabenold, 1984; Emlen and Wrege, 1991), mammals (see Russell, 2004) and fish (Brouwer et al., 2005). Benefits of group size vary among taxa. In bees the presence of helpers can increase productivity in two non-exclusive ways; by mitigating extrinsic causes of brood mortality such as parasitism (Rehan et al., 2011), and by increasing gross productivity (Brand and Chapuisat, 2013). In cooperatively breeding vertebrates both of these effects have been documented (Portelli et al., 2009), but also others such as increased survival of breeders to future breeding attempts[10] (e.g. in birds Cockburn et al., 2008).

A key question concerns the quality of individuals within groups and their contribution to productivity. In correlational studies suggesting a positive relationship between group size and productivity, results are confounded because group size may be correlated with other causal factors. For example, better quality breeders might be able to rear more helpers or workers, and ultimate productivity could be a causal correlate of breeder quality rather than the number of helpers (Cockburn, 1998). One powerful way to investigate the effects of individual quality and group size is to manipulate the number of individuals within groups (Emlen, 1997; Dickinson and Hatchwell, 2004). Experiments manipulating the number of helpers in cooperatively breeding birds, for example, have frequently found a positive effect of helpers on brood survival and productivity when controlling for confounding variables such as breeder and territory quality (e.g. Brown et al., 1982; Brouwer et al., 2012; Browning et al., 2012). Removal

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[10] Although this may apply to some bees in the tribe Xylocopini (family Apidae), which can live for two years (Gerling and Hermann, 1978).

experiments in social insects also reveal that helpers do often contribute towards group productivity (Field et al., 2000; Shreeves et al., 2003; Brand and Chapuisat, 2013). These studies demonstrate more conclusively that number of helpers is an important factor determining the quantity of offspring successfully reared, although the relationship is not always straightforward (e.g. Komdeur, 1994).

One group in which such manipulation experiments might prove particularly fruitful is the primitively eusocial sweat bees (Hymenoptera: Halictidae). Sweat bees have an annual life cycle and often nest in aggregations (Michener, 2007), therefore potentially presenting fewer confounding variables than cooperatively breeding vertebrates that occupy territories of varying quality and breed in multiple years (Cockburn, 1998). The following is a brief summary of the life cycle typical of a temperate zone primitively eusocial sweat bee (see Schwarz et al., 2007). Mated females (foundresses) emerge from hibernation in spring and initiate subterranean nests. Foundresses mass provision a first brood (B1) of offspring individually in sealed brood cells, each containing a single offspring and all the food required for development. B1 females emerge in summer and remain at the nest as workers to provision a second brood (B2) of reproductives. Reproductive offspring emerge at the end of the summer, mate, and females enter hibernation. Males die before winter and play no role in nesting.

Strohm and Borden-Hauser (2003) investigated the costs and benefits associated with increased group size in *Lasioglossum malachurum* Kirby, and found increased productivity of reproductive offspring to be a significant advantage. They suggested that producing extra workers entailed few costs to the foundress because no trade-off was evident between the numbers of workers and the number of reproductive offspring produced. However, together with data from previous studies (e.g. Boomsma and Eickwort, 1993; Smith et al., 2007), these results potentially remain confounded by the quality of individuals within the group. There is likely to be a trade-off between investment in current versus future reproduction (e.g. Gustafsson and Pärt, 1990; Ward, 2009), and better quality foundresses might be able to produce both more workers and more reproductives than lower quality foundresses (von Nordwijk and de Jong, 1986; Reznick et al., 2000). Strohm and Borden-Hauser (2003) did not report foundress quality, and it could be for example that better quality foundresses are larger and hence able to produce more or larger workers, although Zobel and Paxton (2007) reported no relationship between foundress size and number of B1 workers. A hidden cost to producing extra workers could become manifest in the quality of the workers themselves (Stearns, 1992; Kramer and Schaible, 2013). Strohm and Borden-Hauser (2003) found no relationship between the number and mass of workers produced, suggesting no such trade-off, yet without manipulation experiments it is difficult to demonstrate this conclusively. Another benefit of

producing more workers could be that they provision larger B2 female offspring (Frank and Crespi, 1989).

To my knowledge there are only two examples of group size manipulation experiments in sweat bees (Mueller, 1991; Brand and Chapuisat, 2013). Mueller (1991) experimentally investigated split sex ratios in *Augochlorella aurata*[11] by removing nest foundresses. Brand and Chapuisat (2013) therefore represents the only removal study to explicitly investigate colony productivity. By removing a single helper from approximately half of the nests studied, Brand and Chapuisat (2013) were able to estimate the contribution that individual workers made to productivity by comparing reproductive output with control nests where no workers were removed. Removal of a single helper significantly reduced B2 productivity when colonies were small, but the effect lessened with increasing colony size. Removing workers from nests containing four or more workers did not have a significant effect on reproductive output of the nest. However, this study was limited in scope because relative colony sizes remained the same. That is, removing a single worker ensured that larger colonies still remained large relative to smaller colonies, and therefore any effects of foundress or worker quality on productivity are impossible to infer. For example although it was possible to measure the contribution of a single worker to colony productivity, it was not possible to tell whether the smaller contribution of individual workers in larger groups was related to economic inefficiencies or to a lower quality of workers in larger groups.

In this chapter, I investigate the effect of foundress and worker quality on the number of B2 offspring produced in the primitively eusocial sweat bee *Lasioglossum malachurum*. For a given initial group size of *n* workers, I removed different numbers of workers at different nests to create all possible new group sizes of between zero and five. For example, groups originally comprising four workers were manipulated to contain zero, one, two, or three workers, or left unmanipulated as controls (see *Methods*). In this way it was possible to directly control for the quality of foundresses, because productivity in control groups containing *n* workers can be compared with productivity in originally larger groups which have been manipulated to contain *n* workers. This method explicitly takes into account problems of individual quality not accounted for in previous studies (e.g. Strohm and Borden-Hauser, 2003; Brand and Chapuisat, 2013).

It is possible to formulate three predictive hypotheses with respect to foundress quality and B2 productivity. H1: Foundresses vary in intrinsic quality, and higher-quality foundresses produce

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[11] *A. striata* is an earlier synonym (Coelho, 2004)

more B1 workers than lower-quality foundresses; this predicts that higher quality foundresses, which originally produced more workers, should still produce more B2 offspring when groups of varying original size are made of equivalent post-treatment size (Fig. 6.1a, i). H2: Foundresses vary in intrinsic quality, but higher quality foundresses do not produce more B1 workers, or the production of more workers equalises foundress quality; this predicts that for a given post-treatment group size there will be no relationship between original group size and B2 productivity (Fig. 6.1a, ii). H3: Foundresses that produce more workers incur costs; this predicts that for a given post-treatment group size there will be a negative relationship between original group size and productivity (Fig 6.1a, iii).

The above hypotheses assume that the quality of workers is equal across group sizes, and that the resulting predictions are due to variation in the quality of foundresses (H1 and H2), or costs incurred by the foundress (H3). However, workers should also vary in quality as predicted by life history theory (Stearns, 1992). Nevertheless, variation in worker quality is expected to augment the hypotheses outlined above. For example, if higher quality foundresses produce more workers that themselves are of higher quality, this can be considered to be an extension of initial high foundress quality (H1). Alternatively, if higher quality foundresses produce more workers, but which were individually of lower quality, this can be considered as a cost of worker production (H3). If, however, high foundress quality were offset by low worker quality, the prediction from H2 might be supported. It is nonetheless possible to assess the role of worker quality by observing provisioning behaviour (see Methods, section 6.2.4): higher quality workers should be recorded provisioning on more days.

Frank and Crespi (1989) predicted that nests containing more workers would produce larger female reproductive offspring. Hypothesis six (H4) therefore proposes that nests containing a greater post-treatment number of workers will produce larger B2 female offspring; this predicts a positive relationship between the post-treatment number of workers and the size of B2 female offspring.

Larger individuals are thought to possess more resources overall, which they can allocate towards multiple traits and are thought to mask negative correlations between traits (e.g. von Nordwijk and de Jong, 1986; Reznick et al., 2000). Therefore, I posit two additional hypothesis. H5: Larger foundresses have more resources overall to allocate towards B1 workers; this predicts a positive relationship between foundress size and both the number and size of workers, and a positive relationship between original group size and worker size. H6: Larger foundresses possess more resources and gain survival benefits; this predicts that larger
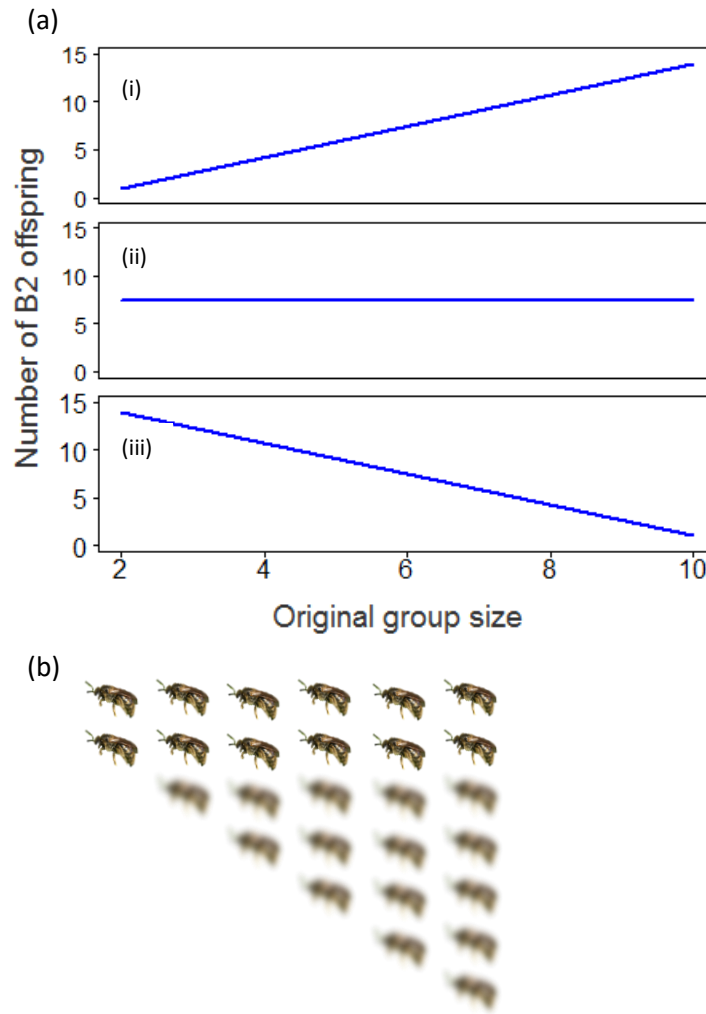
Figure 6.1

(a) Three possible predicted relationships between original group size and B2 productivity for nests manipulated to contain two workers. In each panel, workers in nests originally containing >2 workers have been removed so that each is left with a post-treatment number of only two workers. H1 (i): if higher quality foundresses produce more workers, foundresses that originally produced more workers should still produce more B2 offspring when the number of workers is equalised. H2 (ii): if the number of workers produced is independent of foundress quality, or the production of more workers equalises foundress quality, there should be no relationship between original group size and the number of B2 offspring produced. H3 (iii): if foundresses incur costs through producing more workers, a negative relationship between original group size and the number of B2 offspring produced should result. The same scenario applies to all post-treatment group size of between zero and five workers (see *Methods*).

(b) Manipulations required to create the scenario shown in (a), with original group size shown above each column. Original group sizes of between two and seven workers only are shown. Each bee represents a worker, and blurred bees represent workers that were removed to create the smaller post-treatment group size shown by the in-focus bees.

foundresses should be more likely to successfully produce offspring and survive through to the worker-provisioning phase.

6.1.1 Study organism

Lasioglossum malachurum is a widespread bee of the western Palaearctic, although in Europe its distribution is restricted to central and southern areas (Falk and Lewington, 2015). Sociality in *L. malachurum* is obligate so far as is known (Davison and Field, in prep, Chapter 5), with a life cycle in Britain typical of many primitively eusocial halictids. Mated females (foundresses) emerge from hibernation and initiate nests in spring. They then provision ≈6-7 brood cells that comprise the first brood (B1), almost all of which will become female workers. Workers are approximately 15% smaller than queens (Packer and Knerer, 1985; L. Holt unpubl.), and collectively provision a second brood (B2) of reproductives. During this period the foundress, now queen, remains in the nest, does not engage in foraging and probably more often than not remains alive to lay B2 eggs (Paxton et al., 2002). Instead of initiating nests in spring, some females attempt to take over those of other foundresses (Smith and Weller, 1989). These are termed 'floater' females (Zobel and Paxton, 2007), which remain at the nesting aggregation investigating nest entrances and do not engage in foraging.

6.2 Methods

6.2.1 Study sites

L. malachurum foundresses were sourced from a substantial aggregation (>>1000 nests) near to the University of Sussex in the South Downs National Park (50.811 N, 0.076 W). Black 14L plastic buckets with drainage holes drilled into the base were embedded adjacent to the footpath (Fig. 6.2a, b), and refilled with the excavated soil after large stones had been removed. Buckets were embedded so that rims were flush with the surrounding surface. To ensure soil did not fall out of the drainage holes, and to prevent bees from digging down into the surrounding soil, a layer of metal gauze was inserted before filling with soil. Soil was tamped firmly as each bucket was incrementally filled. Eighteen buckets were embedded in January and February 2013, and a further 20 were embedded in February 2014.

6.2.2 Foundress marking

Foundresses naturally began initiating nests in the buckets during the spring of 2014, and new nests were marked with individually numbered nails (Fig. 6.2c). The first foundresses were

observed provisioning their nests in mid-April 2014. After being observed provisioning their nests with pollen, foundresses were individually caught with a handheld net upon emergence. Foundresses were held by the legs between the first finger and thumb and marked with a single dot of white enamel paint (Humbrol^{TM} enamel model paint), applied to both the mesonotum and clypeus with a pin. Foundresses were not individually observed in the field in spring while they were provisioning B1 offspring. Foundresses were marked solely to distinguish them from workers, which were identified as unmarked bees emerging from nests later in the season (see section 6.2.4). In most cases right forewing length was measured, from the outer edge of the tegulum to the wing tip, with digital callipers to the nearest 0.1mm. Waiting until a foundress was observed provisioning before marking ensured both that the bee was not a 'floating female', and that it had learnt its nest location and would not become lost upon release. Floating females previously occupying a foundress' burrow were identified as bees emerging without pollen very soon after the provisioning foundress entered.

6.2.3 Bucket removal

Before treatment manipulations took place, buckets were transplanted to the University of Sussex campus (Fig. 6.2d). Buckets were removed for transplantation when provisioning had finished that day and all active nests were closed. After each bucket had been removed it was covered with two black plastic bin bags taped at the top to create a dark environment, and new buckets were used to replace those that were removed. Removed buckets were driven with extreme care along farm tracks back to the road and then to the Sussex campus where they were re-embedded in a flat, sheltered grassy area adjacent to a meadow (Fig. 6.2d). Five buckets were transplanted on 21-May 2014. At this time some foundresses were still provisioning their nests, but had to be transplanted early to avoid destructive digging by rabbits in and around the buckets at the source site. Foundresses resumed provisioning the day after being transplanted to the University of Sussex. A further six buckets that were not affected by rabbits were transplanted in early June, after foundresses at all nests had ceased provisioning.

6.2.4 Treatments

Treatment manipulations were undertaken throughout the active worker phase. Based on a known distribution of brood sizes from previous years, nests were randomly assigned a post-treatment number of workers ranging from zero to five. For any given brood size, these aimed to create all possible group size configurations. For example, of all the nests where foundresses produced a total of four workers, a proportion would each be randomly assigned as control (assigned treatment of ≥four workers in this case), or as treatment nests with three, two, one or

Figure 6.2
(a) The source site where buckets were embedded and foundresses were marked. The arrow shows the footbath along which the *L. malachurum* aggregation was located.
(b) Five buckets embedded adjacent to the footpath (to the left of the buckets) at the source site, where foundresses naturally initiated nests in spring.
(c) A single bucket containing nests and numbered nails. The arrow indicates a nest entrance.
(d) Eleven buckets that were removed from the source site and embedded at the University of Sussex for treatment manipulations. Buckets are located within the area highlighted by the dashed rectangle.

zero workers post treatment respectively. There were no zero-worker control nests, because foundresses were not seen at nests where no workers emerged. Treatment assignment was necessarily random because (i) the removal of excess individual workers had to be as rapid as possible, and (ii) workers emerge over an extended period so it would not be feasible to assess group size individually in the first one or two days after emergence.

Workers assigned to remain in a given nest were caught with a net upon emerging subsequent to a provisioning event being observed, and were individually marked (Humbrol^{TM} and Revell$^{®}$ enamel paint applied with a pin). With the help of five additional observers, all provisioning events by both marked and unmarked bees were recorded for the duration of activity on every day of suitable weather from 17-26 July 2014. When the number of workers in a nest reached the post-treatment number assigned to it, its nest nail was painted red and any further unmarked bees were caught and permanently removed as soon as they were observed leaving the nest. Workers destined for permanent removal were caught as soon as possible, placed into individual tubes and retained in a cold box. To save time during the experiment, wing lengths of the permanently removed workers were then later measured with digital callipers in the lab. In some sweat bees, a proportion of B1 females mate and enter hibernation instead of becoming workers (e.g. Yanega, 1989). However, in *L. malachurum* this has only ever been recorded in worker-sized females produced in the third brood of nests located in southern Europe (Wyman and Richards, 2003), and therefore all B1 females that were removed are expected to have behaved as workers.

6.2.5 Excavating nests

After workers at all but one nest had finished provisioning the B2 brood, buckets were removed on 25 July 2015 and placed in a cold room (maintained at 5°C). This ensured that B2 offspring would not be able to eclose and disperse before each bucket could be excavated. The soil was removed from each bucket by tipping the bucket upside down whilst holding a square of wood over the open end, which then became the base. The bucket was then carefully pulled off, leaving a freestanding mass of soil. In order to get the soil mass the right way up, the procedure was reversed with a second square of wood held tight on the top. Soil was then carefully scraped away to reveal the brood cells of each nest, and the contents of each cell was removed with forceps, recorded, and placed in ethanol. It was possible to ensure comprehensive sampling of the B2 offspring because cells of *L. malachurum* are arranged in a single cluster. If foundresses were present they were easily caught with forceps and rarely attempted to flee, preferring instead to retreat into the nest. Foundresses were also placed in ethanol.

6.2.6 Statistical analyses

All analyses were conducted in the *R* environment version 3.1.2 (R Development Core Team, 2013), and packages used relate to *R*.

Testing of H1-H3

Hypotheses 1-3 (Fig. 6.1) were tested using a negative binomial generalised linear model (GLM), with B2 productivity as the response variable and the 'number of workers removed' from nests, 'post-treatment group size' (the number of workers present after removal) as explanatory variables, with 'foundress size' included in an attempt to take account of variation in foundress quality. The maximal model initially included an interaction term between post-treatment number of workers and original group size, before stepwise model simplification. H1-H3 would be supported or rejected based on the sign and significance of the relationship between original group size and the number of B2 offspring produced (see Fig. 6.1).

To examine worker quality, I investigated three aspects of worker behaviour: worker longevity (time between first and last observation), the number of days on which a worker was observed provisioning, and foraging effort (mean number of provisioning events observed per day). As multiple bees were recorded from within the same nest, I used generalised linear mixed models (GLMMs) in the 'lme4' package (Bates et al., 2014) with Poisson or normal errors as appropriate, and nest as a random factor. In each analysis original group size and post-treatment group size were included as explanatory variables, and an interaction was initially included between the two. I further examined whether workers that were observed to provision on fewer days worked harder. For this analysis I included foraging effort (mean number of provisioning trips per day) as the response variable, with the number of observed provisioning days as a single explanatory variable.

Finally, I used GLMMs with negative binomial errors to determine whether manipulations caused disturbance to the remaining bees, by examining individual rates of worker provisioning. The number of provisioning events observed on a given day by individual bees remaining in a nest was the response variable, and number of workers removed on a given day was the single fixed effect. Nest and day were included as random effects to take account of variation between nests and across days.

Testing H6

I used a GLMM with normal errors to test whether nests containing more post-treatment workers provisioned larger second brood females. I include head width of excavated female pupae and adult offspring (easily discernible from their fresh appearance) as the response variable, and post-treatment number of workers and offspring type (pupa or adult) as explanatory variables. Nest was included as a random factor. 'Offspring type' was not significant ($p > 0.05$) and therefore I report results from the post-treatment number of workers only.

Testing of H4 and H5

Larger foundresses may derive benefits such as increased longevity, and the production of more or larger B2 offspring from producing larger workers. Additionally, foundresses may trade off number of workers against their size, opting to produce either a greater number of smaller workers or fewer, larger workers. When considering the effects of foundress size and pre-treatment group size on worker size, the raw data for worker wing size was highly non-normal, and it was not possible to transform worker wing data to meet assumptions of normality of residuals for a generalised linear mixed model (GLMM) with normal errors. Therefore worker wing size was averaged within nests to avoid pesudoreplication, and these mean values used as the response variable in a generalised linear model (GLM) with normal errors, with foundress wing length and original group size as explanatory variables. When mean within-nest worker size was plotted against original group size, the relationship suggested non-linearity. Polynomial regression was used to test this objectively, by including a quadratic term for original group size to test whether the addition of these terms significantly improved the model fit (Crawley, 2013).

Size-dependent foundress mortality was examined using GLMs with binomial errors. Binary response variables were (i) whether a foundress produced workers and (ii) whether a foundress was seen again during the worker-provisioning phase, with foundress wing length the single explanatory variable in each respectively. An additional binomial GLM considered whether foundresses that produced more workers had higher mortality rates. In this case pre-treatment group size was the single explanatory variable and whether or not a foundress was seen during worker-provisioning the binary response variable. Foundresses were seen either outside the nest or in the nest entrance and were easily distinguished by their white face mark. Additionally, the impact of the presence and number of workers on colony survival was examined using binomial GLMs with presence of at least one worker and number of post-treatment workers present as explanatory variables, and whether or not a nest produced B2 offspring as a binary response variable.

6.3 Results

6.3.1 H1-H3: Foundress quality, worker provisioning and B2 productivity

Worker provisioning was first observed on 17 June, and, in the vast majority of nests, was finished by 24 July. Successful manipulation and subsequent brood excavation was carried out at 66 nests. To test whether workers of different original group sizes differed in quality, I examined the effects of both the original group size and post-treatment group size on the

number of B2 offspring. There was no interaction between original group size and post treatment group size (X^2_1=0.001, p=0.978). Both original group size and post treatment group size significantly affected B2 offspring productivity. In line with the frequently found trend for productivity to increase with group size in social taxa, I found that B2 productivity increased with increasing post-treatment group size (X^2_1=31.777, p<0.001; Fig. 6.3a). However, worker manipulations also revealed support for H3: original group size had a negative effect on B2 productivity (X^2_1=7.350, p=0.007; Fig. 6.3b).

Provisioning behaviour was recorded for 94 workers at 46 nests. The post-treatment number of workers had no effect on the number of days on which a worker was recorded provisioning (X^2_1=2.574, p=0.109). However, original group size had a significant negative effect on the number of days on which a worker was recorded provisioning (X^2_1=7.295, p=0.006; Fig. 6.4a), suggesting that workers originally from larger nests tended to provision on fewer days overall than those originally from smaller nests. There was also a trend that workers from originally larger nests exhibited reduced longevity (time between a bee's marking and final observation) than workers from smaller nests, although the effect was marginally non-significant (X^2_1=3.498, p=0.061). Workers that were observed to provision on fewer days

Figure 6.3 (overleaf)
Data shown in 6.5 (a) and (b) are the same data shown in two different ways., as detailed below. See Results, section 6.3.2 for statistics). Open circles represent treatment nests, closed circles represent controls

(a) Scatter plots showing the relationship between the post-treatment number of workers and the number of B2 offspring produced for each original group size. Each panel shows results from all nests originally containing the number of workers stated in the panel. Panels 6-9 do not contain control nests because the maximum post-treatment group size was five. The line in each panel is the least squares regression, and is shown for illustrative purposes only. Lines emanating from points represent multiple overlapping observations.

(b) Scatter plots showing the relationship between original group size and B2 offspring productivity for each post-treatment group size. Each panel shows results from all nests containing the number of workers post treatment as denoted by the number written in each panel. In each panel, the furthest left points represent control nests from which no workers were removed. The line in each panel is the least-squares regression, and is shown for illustrative purposes only. Across all nest sizes there was a marginally non-significant trend for nests originally comprising more workers to produce fewer offspring (see text for details).

(a)

(b)
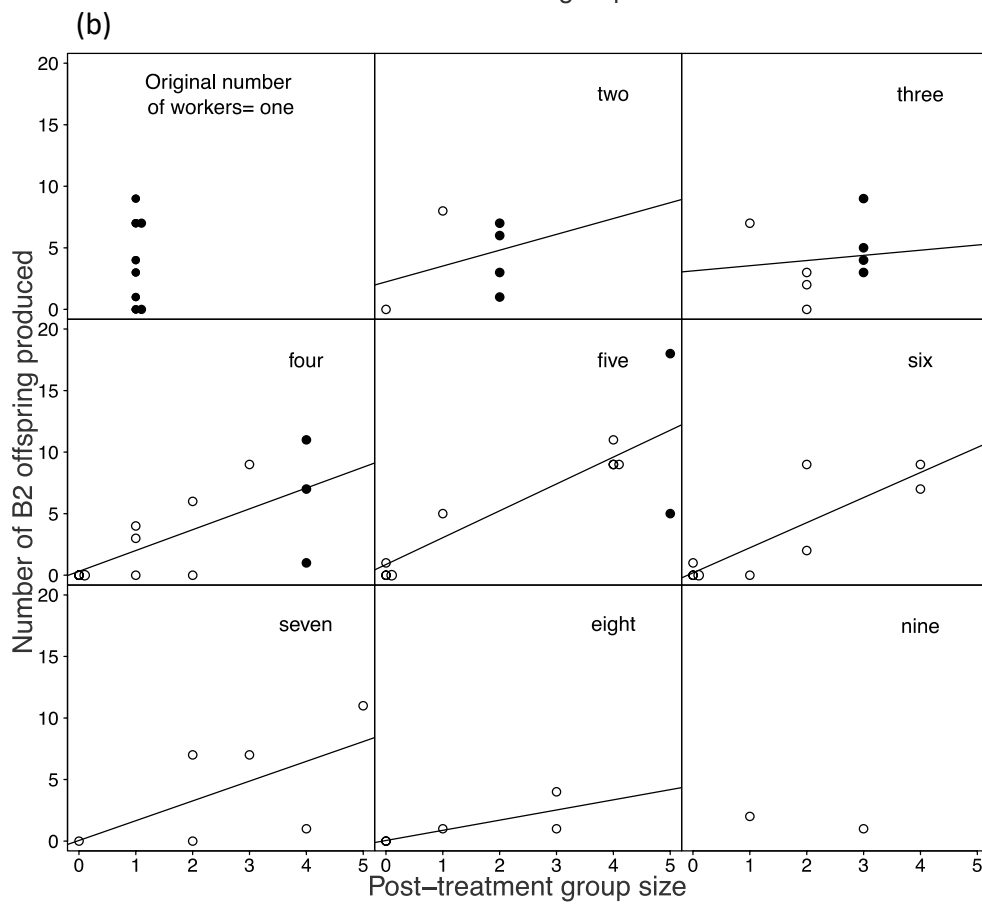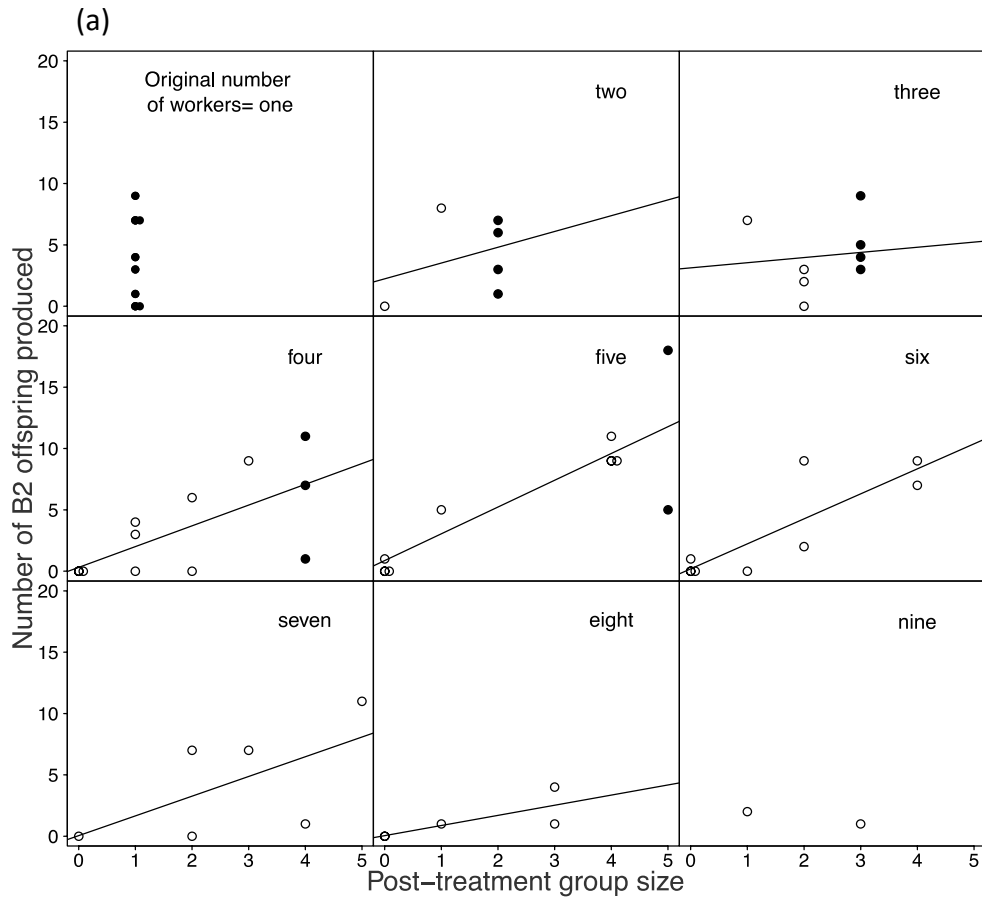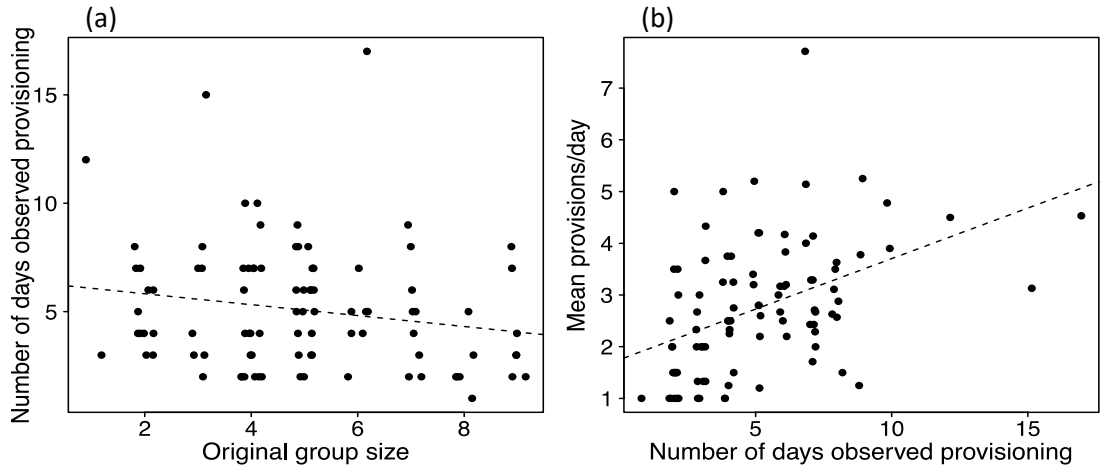
Figure 6.4

Scatterplots showing (a) the relationship between original group size and the number of days on which each (X^2_1=7.295, p=0.006), and (b) the relationship between the numbers of days on which a bee was recorded provisioning and foraging effort (X^2_1=22.001, p<0.001). Points are horizontally jittered to show overlapping observations. The line on each graph represents the least squares regression.

did not work more intensively. In fact, a worker's daily foraging effort increased with the number of days on which it was observed provisioning (X^2_1=22.001, p<0.001; Fig. 6.4b), but was not affected by either the post-treatment or original group size (p>0.4).

6.3.2: H4: Number of workers and B2 female offspring size

It was possible to measure the head width of 55 B2 female pupae at 25 nests, encompassing all post-treatment group sizes from one to five. There was no evidence that the size of female B2 offspring was positively correlated with the number of provisioning workers (X^2_1=0.807, p=0.369).

6.3.3 H5 and H6: Foundress size, group size and worker size

Larger foundresses did not produce larger workers (Fig. 6.5a; $F_{1,25}$=0.045, p=0.834), or more of them (Fig. 6.5b, $F_{1,30}$=1.120, p=0.283). There was a small and marginally non-significant trend for workers from larger original group sizes to be larger ($F_{1,42}$=3.255, p=0.079), and the addition of a quadratic power term significantly improved the model fit ($F_{1,41}$=4.4301, p=0.042; Fig. 6.6). Larger foundresses were more likely to produce workers (X^2=4.343, p=0.037, n=41 producing at least one detected worker, n=16 producing no detected workers), and to be recorded again during or after worker provisioning (X^2=4.921, p=0.027, n=23 seen, n=34 not seen).
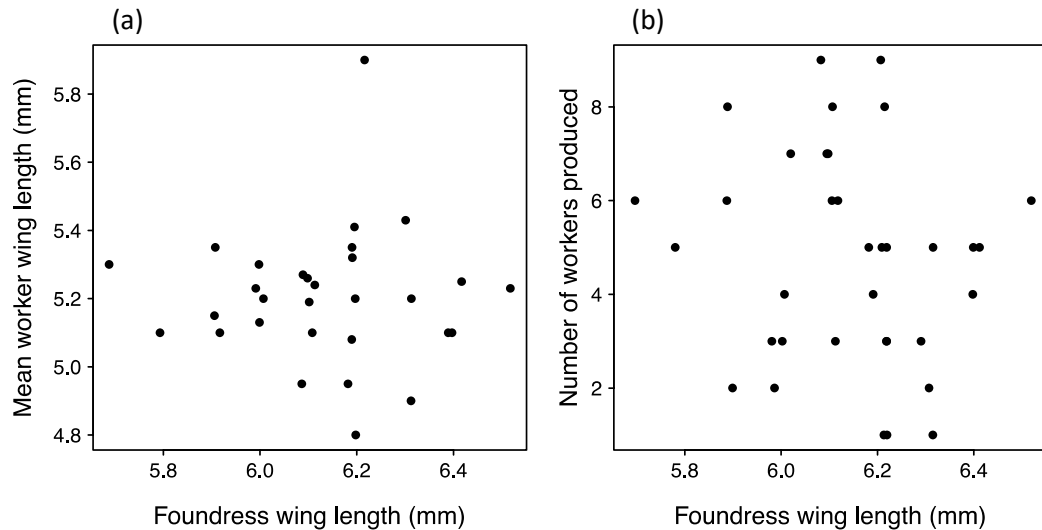
Figure 6.5
Scatterplots showing foundress size, measured as wing length, against (a) mean within nest wing length of workers and (b) the total number of workers produced. Neither relationship is significant (see text for statistics). Points have been horizontally jittered to expose overlapping observations. P>0.05 in (a) and (b).

6.3.4 Colony survival

Sixty two per cent of all control and treatment colonies survived to produce B2 offspring (i.e. 62% of colonies survived to show at least some B1 activity). Post-treatment group size had a highly significant positive effect on whether a nest would survive to produce B2 offspring ($X^2_{1,64}$=27.648, p<0.001), suggesting that the number of workers present in a nest is an important factor determining its survival. Just fewer than 10% of colonies with no workers produced B2 offspring, whereas having just a single worker or two workers boosted the likelihood of survival to nearly 80%. Having three or more workers guaranteed that at least some B2 offspring would be produced. Furthermore, observed productivity in the two zero-worker nests most likely derived from workers not being removed quickly enough, and which therefore had an opportunity to provision one cell (no foundress provisioning was recorded at either nest).

6.3.5 Possible disturbance effects

It is likely that nests from which more workers were removed experienced greater disturbance, so that the reduced productivity of originally larger group sizes after controlling for post-treatment group size could be an artefact of impeded provisioning. However, there was no evidence that the number of workers removed on a given day affected the rate of provisioning (X^2_{1}=1.789, p=0.181, n=72 nests, mean number of provisions per worker per day=3.01±0.09).
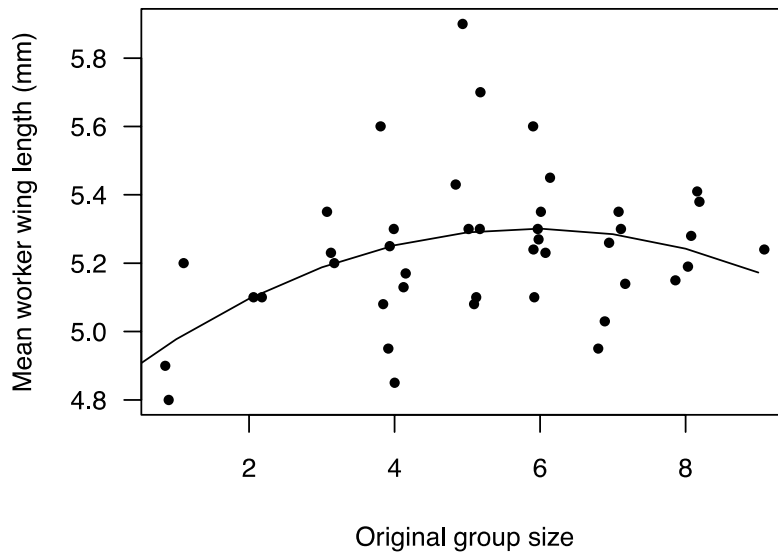
Figure 6.6
Scatterplot showing original group size and mean within-nest worker size. The curve is the quadratic polynomial regression line (see text). Points are horizontally jittered to show overlapping data points. Quadratic curve, $F_{1,41}=4.4301$, p=0.042.

6.3.6 Foundress provisioning at zero treatment nests

Just over 30% of foundresses were seen alive in their nests during the worker-provisioning phase, and 72% of these (23 foundresses) were recorded either provisioning or entering their nests during the worker-provisioning phase. Among foundresses recorded alive, those that provisioned were equally likely to be from a zero treatment nest or one with workers (Fisher's exact test: p=0.608, n=2/10 zero worker nests, n=7/21 nests with workers). Foundresses from zero worker nests provisioned on more days than those from nests with workers (Fisher's exact test: p=0.028, foundress recorded provisioning on more than one day at 2/2 zero worker nests, at 0/7 nests with workers). In general, foundress provisioning appeared lackadaisical when compared with that of workers, and at the two zero-worker nests neither foundress managed to produce any B2 offspring. Most foundresses with workers that provisioned were recorded only once on a single day.

Thirteen foundresses were recorded simply entering their nest without pollen, and most of these (9/13) were recorded before worker activity began in their nest. Most were single observations, but one foundress was recorded entering the nest on three separate days. Nine foundresses (12.5%) were found alive during nest excavations, and four of these had not been recorded during worker phase observations, demonstrating that the figure for foundress survival to the worker-provisioning phase is certainly an underestimate.

6.4 Discussion

Previous work has demonstrated a positive correlation between group size and productivity across diverse taxa, from primitively eusocial bees (Smith et al., 2007) to cooperatively breeding vertebrates (Cockburn, 1998; Russell, 2004), but such relationships are potentially confounded by factors such as the quality of breeders and helpers (Cockburn, 1998). Manipulating the number of helpers can be a powerful method for investigating the quality of individuals, and has been successfully employed in cooperatively breeding birds and wasps (e.g. Dickinson and Hatchwell, 2004; Field and Foster, 1999). Correlations between group size and productivity are also widely reported in primitively eusocial sweat bees (Boomsma and Eickwort, 1993; Strohm and Borden-Hauser, 2003), but manipulation experiments controlling for individual quality are rare. In the present study I used the primitively eusocial sweat bee *Lasioglossum malachurum* to perform the first worker removal experiment to properly control for the quality of foundresses and workers in a sweat bee (cf. Brand and Chapuisat, 2013). I found no evidence that larger foundresses produced more workers, and foundress size did not play a direct role in the number of B2 offspring produced: the number of workers was by far the most important factor influencing B2 productivity (Fig. 6.3a). There was, however, a significant negative effect of original group size on B2 productivity, suggesting that workers from larger nests may individually be of lower quality (Fig 6.3b).

6.4.1 Nest productivity, individual quality and costs of worker production

Productivity in social groups has been positively associated with group size across various taxa, yet there have been few studies in primitively eusocial species directly controlling for other causal factors such as the quality of breeders. I found no evidence supporting the hypothesis that the greater productivity observed in larger groups is directly correlated with foundress quality. If the greater productivity of larger groups was in part due to higher quality foundresses, experimentally reduced groups might still have been more productive than control nests of the same post-treatment group size. In fact, when workers were removed from large groups, productivity tended to be *lower* than in unmanipulated groups containing the same number of workers (Fig. 6.5b). Moreover, the number of workers in a nest after manipulation remained the most significant predictor of productivity (Fig. 6.3a). First, this indicates that overall the number of workers in a nest is an important factor influencing nest productivity, and agrees with studies from cooperatively breeding birds (Brown et al., 1982; Brouwer et al., 2012; Browning et al., 2012) and primitively eusocial bees (Brand & Chapuisat, 2013) in which helpers have been shown to increase productivity. Second, the results presented in this chapter suggest that foundresses do incur costs through the production of more workers.

Strohm and Bordon-Hauser (2003) could not identify costs of worker production incurred by foundresses. This finding was based on the absence of a negative correlation between the number of workers and the number of reproductives produced, or between the mass of successive reproductive broods. However, as Strohm and Bordon-Hauser (2003) point out, this apparent lack of trade-offs could arise because better quality foundresses have more resources *overall* to allocate towards both provisioning workers and laying B2 eggs (von Nordwijk and de Jong, 1986; Reznick et al., 2000). Consequently, these traits might still appear to be positively correlated, and direct manipulations of group size are required to test whether trade-offs actually exist (Lessells, 1991; Strohm & Bordon-Hauser, 2003). The results presented in this chapter suggest that foundresses do incur costs from the production of more workers, because productivity was negatively influenced by the original group size. Costs to worker production could manifest in one of two non-mutually exclusive ways. Higher quality foundresses emerging from hibernation in spring might be able to provision a large numbers of workers, but doing so means that they no longer have more resources than initially lower quality foundresses. Alternatively, or in concert, foundresses that produce more workers may allocate each with fewer resources, such that workers are individually of lower quality (Smith and Fretwell 1974; Kramer and Schaible, 2013).

Strohm and Bordon-Hauser (2003) had no data on worker provisioning. In the present study, however, original group size had a significant negative effect on the number of days a worker was recorded provisioning (Fig. 6.4a), and an almost significant negative effect on observed worker longevity. In contrast, the post-treatment number of workers had no effect. These results indicate that workers from originally larger groups provisioned on fewer days, and that they may have suffered higher rates of mortality. Moreover, workers that provisioned on fewer days did not suffer increased mortality because they worked more intensively (e.g. Schmid-Hempel and Wolf, 1988). The data presented in this chapter show that daily worker foraging effort increased with the number of days on which a worker provisioned (Fig. 6.4b), and that neither the original nor the post-treatment number of workers in a nest had any effect on daily foraging effort. Thus, a possible mechanism causing the negative effect of original group size on productivity is that workers from larger nests exhibit reduced longevity, which would be an indication of lower quality (Kramer and Schaible, 2013). One way this relationship might be mediated is through a frequently observed trade-off between the number of offspring and their size (Smith and Fretwell, 1974; Kim and Thorpe, 2001). However, consistent with the findings of Strohm and Bordon-Hauser (2003) I found no evidence that workers from originally larger nests were smaller (Fig. 6.6); nevertheless, worker quality probably does not equate simply with body size (Strohm and Liebig, 2008). Another possibility is that variation in worker quality reflects variation in levels of fat reserves; relative fat content does not appear to correlate with

body size, at least in sweat bee foundresses (Brand and Chapuisat, 2012; Weissel et al., 2012). Variation in fat content of workers could therefore represent a mechanism mediating variation in longevity independent of body size, and this could be tested by correlating the mass of worker upon emergence with longevity and overall foraging effort. Offspring supplied with more nectar in the pollen ball might lay down more fat reserves during development (Richards and Packer, 1994). Since most nectar is added to the provision mass after the completion of the pollen ball (Plateaux-Quénu, 1983), foundresses could save resources by reducing the number of trips out of the nest to collect nectar, which in turn might result in leaner workers (Richards and Packer, 1994).

The trend for reduced B2 output in originally larger groups could have been due to a disturbance effect (Dickinson and Hatchwell, 2004), but there was no evidence that the removal of workers from nests caused reduced provisioning by the remaining bees. Moreover, because nests were physically very close together, any general disturbance from marking and removing bees was likely to have been shared across all nests. For example, when a bee was caught leaving its nest, the net used to capture it necessarily covered most if not all nests in that bucket, so physical disturbance from catching bees is unlikely to have caused disproportionate disturbance to the nest from which the bee was removed. Another explanation could be that worker removal caused social disruption within the nest. For example, if there is a hierarchy or inheritance queue among workers (e.g. Field et al., 2006), their continual removal could cause disruption if the remaining individuals find themselves frequently changing position. There is currently little evidence that sweat bees form such queues; however, some form of social disruption is still possible.

6.4.2 Foundress size

Foundress size is expected to positively affect traits such as fecundity and survival (Stearns, 1992; Honěk, 1993). If foundress size is an important correlate of quality, larger foundresses might be expected to produce a greater number of workers (Zobel and Paxton, 2007), and workers that are larger. Previous studies of *L. malachurum* have either not reported foundress size or worker size (Strohm and Borden-Hauser, 2003; Zobel and Paxton, 2007), and it has not been possible to determine the importance of foundress size for both traits in the same study. In this chapter I found no evidence that larger foundresses produced more workers, or that their workers were larger (Fig. 6.3a, b). Benefits of large size have been difficult to detect in sweat bees, and studies often report contradictory results. For example, some studies find evidence that larger foundresses produce larger workers (Boomsma and Eickwort, 1993; Richards and Packer, 1996), whilst others do not (Davison and Field, in press, Chapter 2). My result is

surprising, because larger bees might produce larger workers simply because they are able to carry more pollen per foraging trip than smaller bees (Richards, 2004).

Although larger body size may be advantageous during foraging in taxa such as sphecid wasps that specialise in carrying large prey items (Strohm and Linsenmair, 1997; Coelho and Ladage, 1999; Field et al., 2015), the efficient collection of pollen may be less dependent on body size (Strohm and Liebig, 2008). In fact, body size appears not to affect the loading capacity of individual bees (Giovanetti and Lasso, 2005), although this has not been investigated in sweat bees. In consequence there would be little advantage to producing larger workers, and foundresses should invest fewer resources in offspring that are unlikely to ever endure the physiological costs associated with hibernation and nest founding (Strohm and Liebig, 2008; Weissel et al., 2012). Sweat bee queens are thought to inhibit worker reproduction via dominance interactions (Breed and Gamboa, 1977), and the effectiveness of queen control is thought to increase with the relative size difference between queens and their workers (Kukuk and May, 1991; Richards and Packer, 1996). One disadvantage of producing larger workers could therefore be reduced queen dominance within the nest (but see Field et al., 2010), particularly in species such as *L. malachurum* that tend to produce a relatively large number of first brood workers (Wyman and Richards, 2003; Strohm and Bordon-Hauser, 2003).

I also found no relationship between foundress size and the number of workers produced. This result agrees with previous findings by Zobel and Paxton (2007), and suggests that factors other than foundress size are important in determining the number of workers a foundress provisions in spring. Foundresses often do not provision as intensively as workers, and for example do not provision on every available day of suitable weather (Richards, 2004). Foundresses that produce more workers spend more time away from the nest foraging, which both increases the risk of mortality (Kukuk, 1998; Cant and Field, 2001) and leaves the nest more exposed to both inter and intra-specific parasitism (Smith and Weller, 1989; Bogusch et al., 2006). Rates of nest failure in *L. malachurum* and other sweat bees during the solitary foundress phase can exceed 40%, and offspring are significantly more likely to complete development in nests where the foundress remains alive until offspring emergence (Sakagami and Fukuda, 1989; Zobel and Paxton, 2007; Ulrich et al., 2009), which may provide a link with size-based benefits in *L. malachurum*. In the present study larger foundresses were significantly more likely to produce workers and be seen alive during worker provisioning, suggesting they were more likely to lay their own second brood offspring.

The estimate of foundress survival beyond offspring emergence is conservative, however, because four of the nine foundresses excavated from nests at the end of the experiment had not

been observed during the worker-provisioning phase. It could be that larger foundresses were more likely to appear at their nest entrance, although it is hard to see why this would be the case. Nevertheless, larger foundresses were more likely to produce first brood offspring, which probably occurred because foundresses could reduce brood mortality by providing care to developing B1 offspring (Knerer, 1969; Plateaux-Quénu, 2008). Moreover, if larger foundresses survive longer they are more likely to lay a larger proportion of the second brood eggs themselves. Foundresses that lay more of the second brood eggs stand to derive significant genetic gains from producing offspring instead of grand-offspring, to which they are half as related (Hamilton, 1972; Field, 2010). Again, however, other studies find equivocal support for the importance of foundress size in nest founding (e.g. Richards and Packer 1996).

Foundresses that produce more workers could also begin to lose their reproductive monopoly, as suggested by higher levels of worker ovarian development in larger groups (Strohm and Borden-Hauser, 2003). Alternatively, if foraging by larger groups of workers outstrips the rate at which queens can lay eggs, and if foundresses can still effectively suppress worker oviposition in larger groups, a benefit of larger group size could be the production of larger reproductive offspring (Frank and Crespi, 1989; Boomsma and Eickwort, 1993). There was no evidence that the size of second brood pupae increased with the number of post-treatment workers in a nest, although other species show evidence of this being the case (Boomsma and Eickwort, 1993). However, this result might have been different if a wider range of post-treatment group sizes had been available: the mean post-treatment number of workers was small relative to the original number of workers per nest (4.4±0.27 before treatment, 2.4±0.19 after treatment). It is nevertheless likely that *L. malachurum* queens are able to effectively suppress reproduction by their own workers (Paxton et al., 2002), despite high levels of worker ovarian development observed in larger groups (Strohm and Bordon-Hauser, 2003).

6.4.3 Group size and nesting success

In this chapter, I found that a key advantage of more workers was an increased chance of successfully producing B2 offspring. Nests where all workers were removed had about a 10% chance of successfully producing B2 offspring, whereas the presence of one or two workers raised this to almost 80%, and three or more guaranteed successful production of at least some B2 offspring. This result agrees with other studies showing that nests with more helper co-foundresses (Tibbetts and Reeve, 2003; Richards and Packer, 1998) or workers (Shreeves and Field, 2002; Strohm and Borden-Hauser, 2003; Brand and Chapuisat, 2013) are more likely to survive and successfully produce offspring. Successfully producing at least three workers therefore conveyed significant advantages to foundresses, probably because there is adequate

insurance against mortality of single workers. In small nests, the stochastic mortality of workers is likely to have a greater impact on colony survival than in larger nests, probably because there is a greater chance that a foundress will be left workerless (Nonacs, 1991).

Foundresses with no workers had essentially zero productivity. The single B2 offspring produced in two nests from which all workers were removed probably derived from workers provisioning prior to being removed. Some workerless foundresses engaged in a very small amount of provisioning, but others that were observed to be alive in their nests failed to provision at all. Foundresses of other social sweat bees that fail to raise workers have been recorded successfully raising offspring alone (Richards and Packer, 1995; Yagi and Hasegawa, 2012; Brand and Chapuisat, 2013), but to my knowledge this has not been recorded in *L. malachurum*. In more socially specialised species such as *L. malachurum* that tend to raise larger worker broods, failure to produce any workers at all whilst remaining alive may be a relatively rare occurrence. In species that produce smaller worker broods (e.g. Yagi and Hasegawa, 2012; Brand and Chapuisat, 2013), high rates of brood mortality could frequently lead to failure to produce any workers. Foundresses expend a considerable proportion of their fat reserves by the time workers emerge (Weissel et al., 2012), and may be energetically incapable of both provisioning and egg laying, or rapidly become so.

Chapter 7

Concluding remarks

In this thesis, I have focused on environmental components of behaviour and life history attributes in sweat bees. I have presented data on the geographic extent of, and mechanisms underlying, social polymorphism in the sweat bee *Lasioglossum calceatum* (Chapters 2 & 3), and investigated the consequences of social polymorphism for clinal variation in body size in *L. calceatum* and *Halictus rubicundus* (Chapter 4). The environment is thought to strongly influence the geographic extent of social behaviour in sweat bees, and I report results from the first attempt to test this idea experimentally (Chapter 5). Finally, Chapter 6 represents the most comprehensive field manipulation experiment ever conducted on sweat bees.

Despite a long and productive history of laboratory-based work, fewer studies have attempted to experimentally study sociality in sweat bees in the field (see Chapter 1, section 1.6). In this thesis, I have attempted to extend the scope of sweat bee research by utilising innovative experimental methods to address outstanding questions (Chapters 3, 5, 6). I have further attempted to incorporate long-term field observations to describe social behaviour (Chapter 1), and make use of available resources to explore related issues (Chapter 4).

7.1 Summary of results

Below I will briefly summarise my principal findings, and then discuss outstanding questions and future avenues for research.

7.1.1 Social polymorphism in *Lasioglossum calceatum*: distribution and mechanisms

Since the discovery of solitary nests in Japan more than 40 years ago (Sakagami and Munakata, 1972), only one other study has reported on the existence of social polymorphism in *L. calceatum* (Field, 1996). I have presented evidence that *L. calceatum* is highly likely to exhibit latitudinal social polymorphism in the UK: nests studied over three years in the south were mostly social, but those in the north were solitary (Chapter 2). Long term observations of nests in the south also revealed that as well as becoming workers, first brood females can enter directly into hibernation or found their own summer nests solitarily. Reciprocal field transplants of *H. rubicundus* between the north and south of the UK have shown that whether bees nest socially or solitarily depends largely on environmental conditions (Field et al., 2010, 2012). However, I have provided evidence suggesting that social phenotype in *L. calceatum* might only show a limited response to environmental conditions, and that there may be fixed genetic differences between social and solitary populations (Chapter 3). This finding closely matches the results of a common garden laboratory experiment on *L. albipes*, the polymorphic sister species to *L. calceatum* (Plateaux-Quénu et al., 2000).

7.1.2 Social polymorphism and body size in *L. calceatum* and *H. rubicundus*

Body size clines have rarely been investigated in social insects, and studies of Hymenoptera have typically focused on honeybees or ants (Chown and Gaston, 2010). Studies reporting body size from widely separated populations suggest that sweat bees are smaller in areas where the season is shorter (e.g. Field et al., 2012). The latitudinal transition from social to solitary behaviour in socially polymorphic sweat bees is analogous to transitions in voltinism exhibited by some solitary taxa, which can result in saw-tooth size clines (See Chapter 4 for a detailed explanation). In Chapter 4 I examined how body size of nest foundresses changes with season length in *L. calceatum* and *H. rubicundus*, finding support for suggestions that bees are largest where the season is longest. However, although neither species exhibited a saw-tooth cline, body size in *H. rubicundus* was distinctly non-linear. I argue that bees just north of the transition zone cannot capitalise on the longer growing season afforded by solitary nesting. As I argue, collecting pollen is more costly for a lone foundress than multiple workers, and therefore a single foundress cannot provide enough food to increase the size of each offspring.

7.1.3 Environmental constraints on sociality and social plasticity in *L. malachurum*

The geographic distribution of sociality in sweat bees is frequently linked with the length of the active season (e.g. Soucy and Danforth, 2002). I tested this idea by transplanting *L. malachurum*

foundresses to the north of the UK, well beyond their natural range. In Chapter 5 I showed that the considerably later start to the season in the north of the UK would directly preclude the successful expression of sociality in *L. malachurum*. Furthermore, bees showed little sign of changing their behaviour in response to novel environmental cues: when first brood offspring did emerge they behaved as workers, but there was also the *possibility* that transplanted foundresses produced more first brood males.

7.1.4 Individual quality and productivity in *L. malachurum*

The quality of individuals can have hidden implications for productivity in social groups (see Chapter 6). To date, studies reporting group size and productivity in sweat bees have either been correlative (e.g. Boomsma and Eickwort, 1993) or involved manipulations that were limited in scope (Brand and Chapuisat, 2013). In Chapter 6 I used comprehensive manipulations of group size to experimentally test the relationship between group size and productivity, while simultaneously controlling for the quality of foundresses and workers. I show that the number of workers in a nest is the most important factor determining overall productivity, but also that for a given post-treatment group size, originally larger groups tended to be the least productive. I argue that this indicates variation in worker quality, because workers from the largest groups tended to provision on fewer days overall but not more intensively.

7.2 Future directions

The discovery that social polymorphism probably has a large fixed genetic component in *L. calceatum* suggests many avenues of future work, as I outlined in Chapter 3 (section 3.4.3, but see also below). However, I would argue that a particularly useful next step would be to locate the region in which the latitudinal transition from social to solitary nesting occurs. This can be done with careful dissection of bees caught throughout the year at different sites (e.g. Sakagami and Munakata, 1972). However, several of the issues I raise at the end of Chapter 3 and below would be best addressed from the study of a latitudinal series of nesting aggregations, although they are notoriously difficult to find (Richards et al., 2015)

The existence of fixed differences between populations in which individuals express social or solitary behaviour will of particular interest for studies investigating the genetic basis of sociality (e.g. Kocher et al., 2013). Second, few studies have examined the extent to which social polymorphism has promoted population differentiation (e.g. see Soucy and Danforth, 2002; Zayed and Packer, 2002; Soro et al., 2010), or considered whether polymorphism could facilitate ecological speciation (Rundle and Nosil, 2005; Thibert-Plante and Hendry, 2011). It

would be interesting to transplant bees from a solitary population much further south than my far northern site at Inverness (see Chapter 2, Fig 2.1), where selection for plasticity may have persisted. There is also an obvious question; why does *L. calceatum* appear to have largely lost plasticity while *H. rubicundus* has not? Arguments based on separate phylogeographic histories (e.g. Field et al., 2010) likely do not apply because in Europe both species, together with *L. albipes*, occupy a broadly similar area (Pesenko et al., 2000).

In Chapter 4 I investigated body size clines of nest foundresses. However, this study has presented some intriguing questions about how the body size of workers might also vary. As referenced throughout this thesis, Strohm and Liebig (2008) suggest that the reason so few sphecid wasps have evolved eusociality is because their prey is large. Large prey items are costly to carry, and require a large body to transport them (Field et al., 2015). In bees, however, smaller-bodied individuals can specialise as workers because gathering pollen is comparatively cheap, and foundresses could save resources for future reproduction by strategically investing less in workers (Strohm and Liebig, 2008). There is evidence that the body size of sweat bee workers can vary between years (Richards and Packer, 1996), but there has been no systematic study of how worker body size varies with latitude. The hypothesis proposed by Strohm and Liebig (2008) predicts that worker body size should show no latitudinal size cline, because larger workers are not necessarily more efficient, and foundresses should not expend more resources than necessary.

Contrary to studies of *Halictus ligatus* (Boomsma and Eickwort, 1993; Richards and Packer, 1996), data presented in this thesis suggest that larger foundresses do not provision larger workers in either *L. calceatum* or *L. malachurum* (see Chapters 2 and 6). Caste-size dimorphism is therefore variable and seems largely to derive from the size of the foundress: larger foundresses are proportionately greater in size compared with their workers than smaller foundresses. If this pattern were replicated through space then larger southern foundresses would provision workers that were the same size as those further north, and the result would be a north-south cline of increasing caste-size dimorphism. This pattern may occur in *L. calceatum* and *H. rubicundus* (see Chapter 4). A cline in worker size could also be investigated using museum specimens, although it would be less clear that all specimens were workers. Few studies have explicitly examined how body size affects patterns of foraging among sweat bee workers (see Richards, 2004 and references therein); however, as discussed in Chapter 6, the factors affecting the longevity and patterns of provisioning among workers may be complex (see also Richards et al., 2015).

Nevertheless, as briefly discussed in Chapter 4, the body size of workers, or investment into each worker by a foundress, is likely to be shaped by a series of other factors: survival to lay B2 eggs (mortality during provisioning and the maintenance of resources for the production of second brood eggs), the number of workers produced (see Chapter 6), the availability of floral resources and reducing kin conflict are all likely to be important considerations. Therefore, the size of workers cannot be considered in isolation from these factors, and a multifaceted study examining each at various latitudes would be exceptionally illuminating. Nevertheless, a major problem with conducting detailed studies of sweat bees in multiple locations is the difficulty of locating nesting aggregations (Richards et al., 2015). This kind of study may be best suited to a species such as *L. malachurum*, large nesting aggregations of which can readily be found in large numbers (S. Kocher pers. comm.; T. Wood, pers. comm.).

As emphasised throughout this thesis the production of workers must be costly for foundresses. Although sweat bee life history has received theoretical treatment (Metisser et al., 2006; 2007), there is currently a lack of experimental evidence demonstrating what the costs of worker production are and how investment in workers is traded off against the production of subsequent broods (e.g. see Strohm and Bordon-Hauser, 2003; Weissel et al., 2012). Results presented in Chapter 6 provided evidence that producing workers is costly to foundresses, but it is necessary to manipulate provisioning effort of foundresses during spring (e.g. Strohm and Marliani, 2002) to test how this affects a foundress' subsequent fecundity or longevity. Trade-offs are likely to be different at different season lengths; for example, a *L. malachurum* foundress in southern Europe might lay more than three times as many eggs in a lifetime than in northern Europe (Richards et al., 2005; Davison, unpubl.).

7.2.1 Sociality and phenology

At simplest, the problem of how a newly emerged offspring becomes a worker, or behaves independently, is a question of phenology. When viewed in this way the origin of eusociality merely involves flexibility in the timing of behaviours, which then later become more fixed (Rehan and Toth, 2015). The mechanisms underpinning phenology are largely the same as those discussed in the context of the origins of eusociality: genes, photoperiod, temperature and precipitation (Forrest et al., 2010): in essence, genetic and environmental factors which cause individuals to perform alternative behaviours. However, although "Phenology affects nearly all aspects of ecology and evolution" (Forrest et al., 2010, pg. 3101), implications for sociality are often not considered (e.g. Forrest et al., 2010; Gallinat et al., 2015). As discussed in Chapter 4, the behaviour exhibited by socially polymorphic sweat bees is analogous to solitary taxa that exhibit transitions in voltinism. Moreover, the principle that a species' phenology in large part

limits its geographic range (Chuine, 2010) also underpins our current hypotheses with respect to the geographic distribution of alternative social phenotypes in sweat bees, and the conditions under which they might be expressed (Field et al., 2010; Kocher et al., 2014; Chapters 3 and 5). In both examples sociality adds an interesting twist, but our understanding of how the environment shapes the expression of sociality would benefit from more integration with mechanistic studies of phenology.

Different polymorphic sweat bees appear to show either high (e.g. Field et al., 2010), or low (e.g. Chapter 2; Plateaux-Quénu et al., 2000), responsiveness to environmental cues in determining social phenotype. Fully understanding how such different outcomes are mediated requires disentangling the precise roles of environmental and genetic factors, and the subsequent physiological processes that result in the observed social phenotype (Visser et al., 2010). For example it is critical to understand how the timing of gene expression is affected by cues such as photoperiod and temperature (Bradshaw and Holzapfel, 2007), and how this differs between species exhibiting different degrees of social plasticity. Such issues have already been addressed in plants and other insects (e.g. Van Dijk and Hautekéete, 2007; Sandrelli et al., 2007), but controlled experiments have not yet been performed in sweat bees where it has been possible to precisely control for both temperature and photoperiod.

In Chapter 5 I showed how the social life cycle of *L. malachurum* is almost certainly temporally precluded in the north of the UK. The questions addressed are partly those of what limits the range of a species (Sexton et al., 2009). This is intrinsically linked with phenology, because foundresses and their subsequent offspring must emerge sufficiently early in the season for both broods to be completed successfully (Hirata and Higashi, 2008). The reasons why natural selection does not increase a species' ability to tolerate limiting environmental variables can be unclear (Angert et al., 2008). For example, is there a trade-off between becoming socially specialised and maximum northern range? Data from Chapter 5 suggests that *L. malachurum* is behaviourally inflexible. Moreover, the near-absence of worker-sized spring foundresses (Davison, unpubl.) implies that early-diapause appears to be uncommon or even absent in *L. malachurum* (but see Yanega, 1997; Wyman and Richards, 2003). This suggests that reproductive output derives almost exclusively from the second brood, which in a marginal environment is a risky enterprise (Packer et al., 1989; Fu et al., 2015).

In common with other primitively eusocial sweat bees, *L. malachurum* has recently undergone a rapid northwards expansion in the UK (Falk and Lewington, 2015), possibly in response to

Figure 7.1
Map of the UK and Ireland showing the control (South) and transplant destination (North) study sites in Chapter 5. The arrows point to areas it may be particularly fruitful to transplant (solid arrows) and additionally study (dashed arrow) *L. malachurum* phenology, behaviour and productivity. The dashed line shows the limit of the known distribution of *L. malachurum* in the UK (Falk and Lewington, 2015).

recent climate warming. Conducting a more comprehensive transplant of bees to areas much closer to the expanding range limit (Fig. 7.1) and gathering more precise phenological, behavioural and productivity data, could provide a more detailed understanding of how environmental factors act to constrain sociality.

One factor that is thought to play a critical role in the geographic distribution of social nesting is development time (Kocher et al., 2014). Available data suggest that sweat bees do not exhibit countergradient variation in developmental rate (Conover and Schultz, 1995), and that the time taken to reach maturity is largely dependent on temperature (Field et al., 2012; Chapter 3). Since other taxa exhibit more rapid development in northern areas (e.g. Kivelä et al., 2011), the reasons why sweat bee development has not, or cannot, evolve to be more rapid are worthy of attention. This may be an important issue because developmental rate may be a key factor limiting the northern extent of social species or populations.

The latitude or elevation at which the season becomes too short to express sociality is not necessarily consistent across species. In France, social *L. calceatum* nest in sympatry with a solitary population of its polymorphic sister species *L. albipes*, and apparently also emerge around one month earlier (Plateaux-Quénu et al., 2000). Given that populations are likely to track the environmental conditions in which they can persist (e.g. Pfenninger et al., 2007) it is possible that this discontinuity represents biological differences between the two species. For example, the different emergence times could be linked with subtle differences in pollen diet requirements (e.g. Sedivy et al., 2011) Alternatively this pattern could represent range boundary disequilibrium, where a species does not occupy its full potential range (Sexton et al., 2009).

Uncovering the factors underlying the different spring emergence times in these two species, and whether this is important for the timing of offspring emergence, could provide further insights into circumstances facilitating social nesting and how sociality evolves differently in different species.

One related and particularly interesting question arising from Chapters 3 and 4 is whether there are differences in foundress phenology and patterns of provisioning between populations of *L. calceatum* just either side of the transition zone. For example, just north of the transition, foundresses may be able to emerge slightly later. Furthermore, as body size appears not to change across the transition zone (Chapter 4), foundresses whose offspring will not, or are unlikely to, become workers should at least spend more time provisioning each brood cell. This is because social foundresses provision workers, which are smaller than offspring of foundresses in solitary populations.

7.3 Final remarks

There is currently considerable interest in addressing the genetic mechanisms underlying the transition to eusociality (Kapheim et al., 2015b; Rehan and Toth, 2015). Together with previous studies, data presented in this thesis show that sociality in sweat bees represents an important continuum of increasing genetic influence over social behaviour. In UK *H. rubicundus* the expression of genes causing offspring to behave as workers is contingent on environmental cues (Field et al., 2010). In *L. calceatum* (Chapter 3) and *L. albipes* (Plateaux-Quénu et al., 2000), however, social phenotype appears respond in only a limited way to environmental cues. Finally, in *L. malachurum* sociality is obligate and the ability to nest solitarily has probably been lost completely (Chapter 5). Moreover, some other sweat bees are solitary bivoltine and therefore represent the likely antecedent condition to primitive eusociality (e.g. Seger, 1983; Hunt and Amdam, 2005), providing an important and under-exploited reference for studies of sociality (see Chapter 4). Sweat bees therefore represent several important rungs on the 'social ladder' put forward by Evans and West Eberhard (1970), and will likely prove to be a critical taxon for future hypothesis testing (Rehan and Toth, 2015).

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Appendix A

Microsatellites and PCR protocol

New microsatellite markers were developed for *Lasioglossum malachurum* (Parsons and Field, in prep), and utilised for assigning the sex of *L. malachurum* larvae (Chapter 5). These microsatellite markers were also used for conducting a preliminary investigation of genetic relationships within *L. calceatum* nests (briefly reported in Chapter 2) and confirming the population of origin of offspring excavated from nests initiated by transplanted *L. calceatum* foundresses (Chapter 3). These new microsatellites will be published elsewhere (Parsons and Field, in prep); below I give the primer sequences (Table A.1) and briefly outline details of the multiplexes, PCR protocol and scoring procedure followed.

A.1 PCR protocol and allele scoring

DNA was extracted following the ammonium acetate precipitation method outline by Nicholls et al. (2000). Loci were amplified in two multiplexes (Table 1). Multiplexes were amplified in a 2μl Qiagen Multiplex reaction under the following profile: 95°C for 15 min, followed by 44 cycles of 94°C for 30 s, 57°C for 90 s and 72°C for 60 s, then 60°C for 30 mins. PCR products were genotyped using an ABI 3730 48-well capillary DNA Analyser using LIZ size standard (Applied Biosystems Inc.), and alleles were scored using GENEMAPPER v3.7 software.

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Table A.1 Characterisation of twelve microsatellite loci developed for *Lasioglossum malachurum*[1]

Locus	Ht	Multiplex	No. alleles	Annealing T (C°)	Minimum allele	Forward Sequence	Reverse Sequence
Primer02	0.786	1	7	57	133-157	CCGAGTTCATCAACATCCTC	TTGATTATCAGCGAGATGAGC
Primer03	0.692	1	9	57	139-165	AAAGCGTTGCGAGACACC	AGCATAAATGGAAACCCAACG
Primer12	0.286	2	5	57	129-137	CCAACCGAACACCAACTTTC	CTCCCGGGTTGTCATGTAAG
Primer24	0.357	2	4	57	143-153	TCCTCGGACAAGGAGATACG	TTCGGGTACCGTTCAGTCTC
Primer27	0.333	2	5	57	183-199	GCTGGCAGCTCTGGAGAAG	TGACGGCCATTTAGTTCGTC
Primer29	0.385	2	6	57	197-211	CTCGTCCCTCGTGTGACTC	GTATCGTGCGTGCGTGTC
Primer36	0.417	1	7	57	171-199	GGCCCTTGACTTTGTTG	GAATCTCTGGGTGCTCTAACG
Primer40	0.833	2	7	57	149-175	CGTTCGTTCGTTCGTTACTG	CAGAGTGCGTCGCTTGTTAG
Primer51	0.231	1	3	57	230-237	GAGAAATTGCCAGCAAACATC	AGTTTCGTGGAAGGGAACG
Primer53	0.462	1	5	57	223-233	ACGCGGGATTACTTTCAATC	CCAATTATCGGGTGAAGGAG
Mala09	0.714	2	3	57	87-91	GTTCCGCCAGCTTCTACC	GCAAACTAGTCCGTTAACTCGT
LM20	0.182	1	5	57	80-94	TGTTCCCTCTTGCCTCC	AACCTTGAGACCGGTGC

[1]Data pertains to preliminary testing on 12 specimens from two sites in the United Kingdom (n=6 from Sussex in 2012, n=6 from Inverness in 2015, see Chapter 2 for site details).

Table A.2 Characterisation of twelve microsatellite loci developed for *Lasioglossum malachurum*[1]

Locus	Heterozygozity	Multiplex	Number of alleles	Allele size range	Forward Sequence	Reverse Sequence
LMA02	0.87	1	10	139-185	CCGAGTTCATCAACATCCTC	TTGATTATCAGCGAGATGAGC
LMA03	0.816	1	7	137-167	AAAGCGTTGCGAGACACC	AGCATAATGGAAACCCAACG
LMA29	0.868	1	12	201-231	CTCGTCCCTCGTGTGACTC	GTATCGTGCGTGCGTGTC
LMA36	0.737	1	8	185-199	GGCCCTTCGACTTTGTTG	GAATCTCTGGGTGCTCTAACG
LMA51	0.475	1	4	237-243	GAGAAATTGCCAGCAAACATC	AGTTTCGTGGAAGGGAACG
LMA53	0.675	1	9	217-241	ACGCGGGATTACTTTCAATC	CCAATTATCGGGTGAAGGAG
LM20	0.583	2	5	77-105	TGTTCCCTCTTGCCTCC	AACCTTGAGACCGGTGC
LMA12	0.667	2	10	131-181	CCAACCGAACACCAACTTTC	CTCCCGGGTTGTCATGTAAG
LMA24	0.925	2	13	141-181	TCCTCGGACAAGGAGATACG	TTCGGGTACCGTTCAGTCTC
LMA40	0.947	2	14	155-189	CGTTCGTTCGTTCGTTACTG	CAGAGTGCGTCGCTTGTTAG
Rub06	0.67	2	15	238-284	GTCTGGCGGAAGTCTACGTGC	CAAGTTCGGTGCGTTAGATAATG

[1]Data pertains to preliminary testing on 30 specimens caught at Denton Downs in 2013 (see Chapter 5 for site details).

Appendix B

Preliminary analysis of brood relatedness in *Lasioglossum calceatum*

Nests were excavated at the end of the season in 2015 (see chapter three) and all foundresses, workers and second brood offspring were successfully collected and genotyped at ten loci from 14 native nests. Preliminary analysis of relatedness between second brood female offspring was performed using the software RELATEDNESS v5.0.8 and KINSHIP v1.3.1 (Queller and Goodnight, 1989). Detailed results will be presented elsewhere (Davison and Field, in prep).

Appendix C

Supplementary data for Chapter two

Below I present two tables detailing the data used in the analysis of social level in the *Lasioglossum* subgenus *Evylaeus* (Table A3), and the results from pairwise comparison of *L. calceatum* foundress size among different sites in the UK (Table A4).

Table C.1

Details of species, mean number of workers, caste-size dimorphism and method of measurements used for data included in the *Evylaeus* social level analysis

Species	Mean number	Caste-size dimo	Environment st	Measurement	Method of cou	Reference
L. laticeps	4.1	7.3	wild	wing	census	Packer and Knerer, 1985
L. pauxillum	4	14.5	wild	wing	census	Packer and Knerer, 1985
L. lineare	4.1	15	wild	wing	census	Packer and Knerer, 1985
L. lineare	6.3	21	wild	wing	census	Packer and Knerer, 1985
L. malachurum	6.7	14.3	wild	wing	census	Packer and Knerer, 1985
L. malachurum	6.8	18	wild	wing	census	Packer and Knerer, 1985
L. malachurum	5.1	17.5	wild	wing	census	Packer and Knerer, 1985
L. malachurum	6.5	18.1	wild	wing	census	Packer and Knerer, 1985
L. malachurum	4.5	15	wild	wing	census	Unpublished
L. malachurum	4	14.3	wild	wing	census	Richards, 2000
L. malachurum	7	10.3	wild	head	census	Wyman & Richards, 2003
L. nigripes	7.2	10.3	wild	head	census	P&K 1985
L. marginatum	3.5	0.1	wild	wing	census	Packer and Knerer, 1985
L. calceatum	2	6.9	wild	wing	census	Present study
L. calceatum	3.5	6.5	wild	wing	census	Present study
L. duplex	4.6	9.6	wild	head	census	Packer and Knerer, 1985
L. duplex	1.3	8	wild	head	census	Hirata et al. 2005
L. duplex	4.1	4.5	wild	head	census	Hirata et al. 2005
L. duplex	3.6	6.12	wild	head	census	Hirata et al. 2005
L. baleicum	1.7	4.5	wild	head	census	Cronin & Hirata, 2003
L. baleicum	1.17	0	wild	head	census	Yagi & Hasegawa, 2012
L. baleicum	4.4	9.3	wild	head	census	Hirata and Higashi, 2008
L. albipes	3	5.6	lab	head	census	Plateaux-Quénu, 1992
L apristum	6.9	7.7	greenhouse	head	census	Miyanaga et al. 1999

Table C.2
Results from Tukey's HSD individual
pairwise comparisons of foundress size from
different populations

Comparison	p-value
Hexham-Dartmoor	0.002
Inverness-Dartmoor	0.994
Sussex-Dartmoor	0.001
Inverness-Hexham	0.002
Sussex-Hexham	0.818
Sussex-Inverness	0.002

Appendix D

The Other Sweat Bee Sub-families

D.1 Rophitinae

The Rophitinae comprise some 257 species in four tribes and 13 genera, all of which are solitary and oligolectic (Patiny et al., 2008; Dumesh and Sheffield, 2012), and do not exceed 10mm in length (Michener, 2007). They are primarily Holarctic in distribution, with a small number of species in Southeast Asia, East Africa and the Neotropics (Niu et al., 2005). Most specialise on a narrow range of closely related plant host species, but some may be monolectic (Patiny et al., 2008). For example, *Conanthalictus conanthi* exclusively specialises on *Nama hispidum* (Rozen, 1993). Reflecting this narrow specialisation, several genera have morphological specialisations for collecting pollen from their hosts. Species in the genus *Rophites* possess facial spines (modified hairs) for gathering pollen from nototribic [12] flowers. Pollen is first gathered on the head by rubbing these spines, possibly accompanied by buzzing, and subsequently scraping it onto their legs (Patiny et al., 2008; Müller, 1996). The genus *Systropha* has specialised metasomal scopa (abdominal hairs) for transporting the pollen from their host *Convolvulus* (Thorp, 1979; Michener, 2007), and the genus *Conanthalictus* has a specialised long head and mouthparts for reaching nectar and pollen within narrow tubular flowers of *Nama* (Patiny et al., 2008).

Despite the apparently close relationship between bee and host plant, host switching has occurred frequently and co-evolution does not seem to have occurred (Patiny et al., 2008). The

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[12] Flowers where pollen is positioned such that it comes into contact with the forager's dorsal surface (Mueller, 1996)

Rophitinae are basal and therefore the oldest halictid subfamily, diverging some 119mya with major groups starting to appear around 90mya[13] (Danforth et al., 2004).

D.2 Nomiinae

Nomiinae is a significant subfamily, represented in the paleotropics[14] and Australia by more than 500 species in 11 genera (Astafurova and Pesenko, 2006; Michener, 2007). Around 20 species occur in North America and they are absent from South America (Wcislo and Engel, 1996). Size is highly variable between genera, ranging from 4mm in *Halictonomia* to 2.3mm in *Dieunomia* (Michener, 2007).

Nests are typically constructed in sandy, alkaline soil with sparse vegetation cover (Parker et al., 1986), and can form large aggregations. For example, aggregations of *Dieunomia triangulifera* can contain well over 50,000 densely packed nests (Minckley et al., 1994). A few Nomiinae construct cell clusters as do some augochlorines and halictines, (Danforth and Eickwort, 1997). Equal numbers of species studied are solitary and communal with some hints of social structure, but the details are 'sketchy' (Vogel and Kukuk, 1994; Wcislo and Engel, 1996).

Although not eusocial, some species may be quasisocial[15] (Batra, 1966; Vogel and Kukuk, 1994), a more complex trait than communality where individuals must do more than merely tolerate each other. It is likely that females in such nests are sisters. The number of females in communal or putatively quasisocial nests is variable, ranging from very few (two or three) in the majority of species (Wcislo and Engel, 1996), up to 20 in *Nomia tetrazonata* (Wcislo, 1993).

Breadth of host plant preference is largely related to being either solitary or communal/quasisocial (Wcislo and Engel, 1996). *Dieunomia triangulifera*, for example, is solitary and oligolectic (Minckley et al., 1994), whereas *N. tetrazonata* is communal and polylectic (Wcislo and Engel, 1996). Some *Lipotriches* species in southern Africa specialise on collecting grass pollen, and are active only early in the morning (Immelman and Eardley, 2000). Grass pollen is rapidly lost to the wind, precluding all day foraging (Immelman and Eardley, 2000). Where grasses produce pollen at different times of the day, *Nomia* have been observed to switch from the early flowering *Panicum coloratum*, to the later flowering *Chloris gayana* and so can gather grass pollen all day (Bogdan, 1962).

[13] Danforth et al. (2004) suggest halictids originated in Africa, but a more recent analysis suggests that the New World is more likely (Hedtke et al., 2013)
[14] The tropical regions of Africa, Asia and Oceania
[15] Members of the same generation use the same nest and cooperate in brood care (Wilson, 1971)

Nomiinae is notable for containing the only solitary, ground-nesting bee commercially managed for pollination: *Nomia melanderi*, or the so-called alkali bee (Bohart, 1972; Danforth et al., 2008), brought in to bring forward the timing of pollination in the Western USA (Bohart, 1972).

Another notable feature of Nomiinae is the diversity of male morphologies and courtship behaviour (Wcislo and Buchmann, 1995). Males of *Nomia triangulifera* have expanded hind tibiae, with which to grip females and prevent dislodging by up to 8 other males (Wcislo et al., 1992). Pre-copulatory behaviour involves an elaborate combination of fore and mid-leg tapping, abdominal drumming, antennal waving and buzzing, on average lasting more than a minute (Wcislo et al., 1992).

D.3 Nomioidinae

Nomioidinae is a small subfamily containing almost 90 recognised species comprising three genera, with most inhabiting arid regions of Africa and Central and Southern Asia (Pesenko and Pauly, 2005). Nomioidinae is the least speciose of the halictid sub-families (Danforth et al., 2008). These small, metallic bees range from 2.5-6mm in length, and all species investigated are solitary and polylectic, although some may be communal (Batra, 1977; Radchenko, 1979; Pesenko and Pauly, 2005; Michener, 2007). Despite being polylectic across the provisioning period, pollen balls within single cells may contain up to 99% of pollen from the same plant (Rust et al., 2004). Nests are typically dug in sandy or stony soil, and form aggregations of varying size (Rust et al., 2004). Where the season length allows, they are bivoltine or multivoltine, but the few species that occur in Europe are univoltine (Pesenko and Pauly, 2005). Two unique features of nomioidine bees are that both sexes overwinter, and do not emerge in the autumn, instead overwintering in their cells as adults and emerging for the first time the following spring (Rust et al., 2004; Michener, 2007); all other halictids emerge and overwinter (Michener, 2007) either beneath their natal burrow or elsewhere (e.g. Bonelli, 1965), and males never overwinter.