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Why do bumblebee workers vary in size?

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ABSTRACT

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WHY DO BUMBLEBEE WORKERS VARY IN SIZE?

by James W Peat

This thesis investigates size variation in the worker caste of social bumblebees (*Bombus* spp.) by examining various morphological and behavioural characteristics in relation to body size. My primary study species is *Bombus terrestris*, common throughout Europe. However, other common and rare *Bombus* species also feature. Like many bees, wasps and ants, *Bombus* species are eusocial. Eusociality, the ultimate in cooperative breeding, is strongly associated with high sibling relatedness. It is in a worker's genetic interests to raise sisters rather than daughters. Bumblebee workers vary dramatically in size within a colony. This variation is due to differential food provision during the larval stage. Ultimately, there must be an adaptive explanation, as unnecessary production of large workers would be wasteful. Bumblebees also exhibit division of labour, whereby foragers are larger, on average, than in-nest workers. Here, I confirm size variation in the worker castes of 23 *Bombus* species and that foragers are, on average, larger than in-nest bees and that workers of pocket-making species vary more than pollen-storers. It is believed that larger bees are suited to foraging tasks and small bees to in-nest tasks, large bees being able to collect more forage per trip. I confirm that larger workers gather nectar more efficiently. Workers show behavioural plasticity: smaller workers occasionally forage and larger bees often perform in-nest tasks and are superior brood carers. By manipulating the worker size distributions in artificial nests, I show that a workforce of large bees may be superior to one of small workers in terms of colony growth. Larger workers are supposedly better adapted to colder conditions than smaller workers. Here, I show that smaller workers lose heat more rapidly, have shorter thoracic setae and proportionally larger heads, legs and abdomens. I show that bumblebees from cold regions have longer thoracic setae than those from hot regions and that, within species, foraging workers of races from cooler regions are often found to be larger than those from warmer regions. I also show that, in southern England, ambient temperature has no influence on nectar foraging efficiency or on the size of forager, but that pollen foraging trips are made in warmer conditions. Bumblebees obtain food from flowers. I show that different plant species attract foragers of different mean size, that larger bees have longer tongues and handle deeper flowers more quickly and shallower flowers more slowly than smaller workers. That size variation enables a colony to efficiently harvest nectar and pollen from a variety of floral resources is the most encouraging adaptive explanation for size variation in bumblebee workers thus far.

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Chapter 1:

Introduction

If asked which insects I prefer,
To bumblebees I shall refer,
They walk, buzz and fly,
They're soft on the eye,
Plus they're social and covered wi' fur.
- James Peat

1.1 Introduction

When hearing the word 'bee' you are likely to imagine a huge furry insect, buzzing amongst garden flowers during the lush spring days. This is a bumblebee queen (plate 1.1), recently emerged from hibernation, collecting nectar and pollen to feed her first brood of daughters tucked away, under ground or in a tussock of grass, in a recently founded nest. For many of us, bumblebees are the insects of our childhood. Unlike most insects they spend much of their time out in the open, a behaviour to which they owe their familiarity. They appeal to us for their striking colouration, fluffy (therefore friendly) appearance, loud buzzing and charming work ethic. The popular phrase "as busy as a bee" is no doubt attributable to them and their honeybee cousins, who share their interest in floral harvesting and cooperative brood care. However, bumblebees differ from honeybees in a number of ways, the most striking of which is in the within-nest worker size variation; within a single colony, bumblebee workers can vary in size up to ten-fold (plate 1.2), whereas honeybee workers (plate 1.3) are all virtually identical (Waddington *et al.* 1986; Roulston and Cane 2000). It is in this phenomenon that my interest lays.

1.1.1 The Order Hymenoptera

The insect order Hymenoptera, literally meaning 'membrane wing', consists most notably of bees, wasps and ants. Like the orders Diptera, Lepidoptera, Coleoptera and others, they are endopterygotes, so develop as larvae before undergoing complete metamorphosis to become adults, after which they no longer grow. The Hymenoptera contains well over 100,000 known species split into two sub-orders: the primitive Symphyta, which have no 'waist', and the Apocrita, which have a narrow 'waist' (petiole; figure 1.1) between the thorax and abdomen.

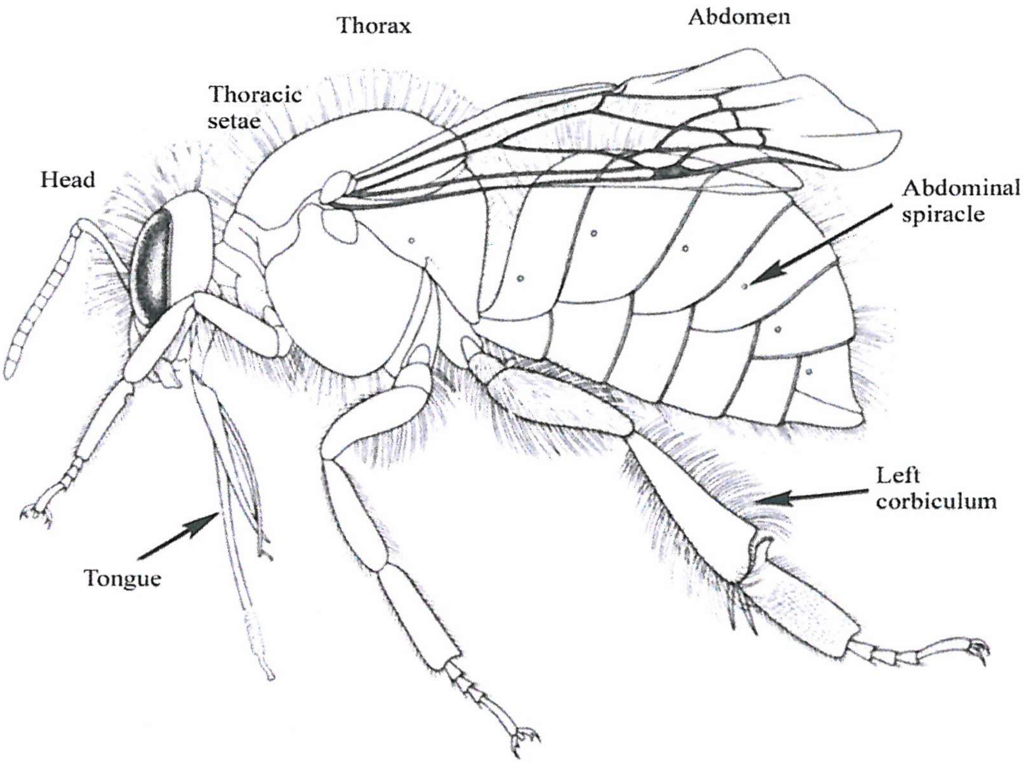

Figure 1.1 Diagram of a bumblebee. Note the long tongue or glossa, the covering of setae, the narrow waist or petiole and the corbiculum (taken from Heinrich 1979).

1.1.2 Eusociality in the Hymenoptera

Bees, ants, wasps, ichneumons and others belong in the Apocrita and it is only in this group (and amongst termites (Division Exopterygota; Order Isoptera) plus some rare exceptions in the Homoptera, Coleoptera and Thysanoptera (Crozier and Pamilo 1996)), shrimps (Duffy *et al.* 2002) and mammals that we find eusocial behaviour. Eusociality in the Hymenoptera is based upon haplo-diploidy, whereby males are haploid and females are diploid. Unfertilised, haploid eggs develop into males and fertilised, diploid eggs become females (figure 1.2). Consequently, females are, on average, 75% related to each of their sisters but only 50% related to their potential offspring. Therefore, according to kin selection theory, it is in the best interests of a female to aid the development of her sisters rather than attempt to produce offspring of her own, all else being equal. In some cases this has resulted in eusociality as defined by the presence of continued care of young, cooperative brood care, reproductive division of labour and colonies with at least two adult generations (Crozier and Pamilo 1996). By no means are all the Hymenoptera eusocial. A majority of bee species are solitary. However, aside from honeybees (*Apis* spp.) and stingless bees (Meliponae), bumblebees (Bombinae) are one of the most advanced eusocial bee subfamilies.

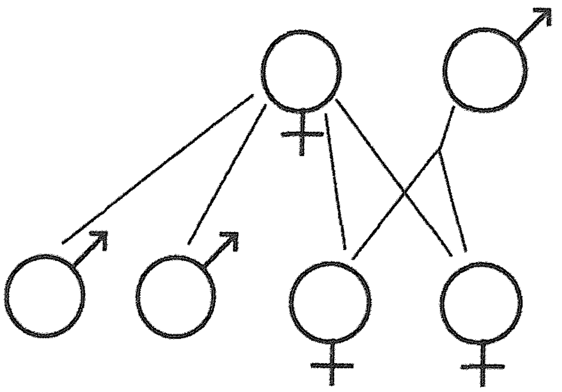

Figure 1.2 A simple male-haploid pedigree. Males arise from unfertilised eggs and females from fertilised ones. A male passes on identical genes to all his daughters causing them to be very closely related (taken from Crozier and Pamilo 1996).

1.1.3 Bumblebees

Bees are members of the Superfamily Apoidea. Unlike most other Hymenopterans, bees feed exclusively on nectar and pollen. In addition to the standard Hymenopteran biting mouthparts, bees have evolved an extended glossa and labial palps (figure 1.3), enabling efficient gathering of nectar. On their hind legs, corbicula hairs (pollen baskets; plate 1.4; figure 1.1) are used to trap pollen, the protein needed for larval growth. Although the social bees aren't the only insect pollinators, as they are so numerous and forage so systematically, they are arguably vital. Some plants, such as the Solanaceae (tomatoes and potatoes), require buzz pollinating by bumblebees (i.e. the bee has to shake the anthers to extract the pollen). Honeybees are incapable of this (Rick 1950).

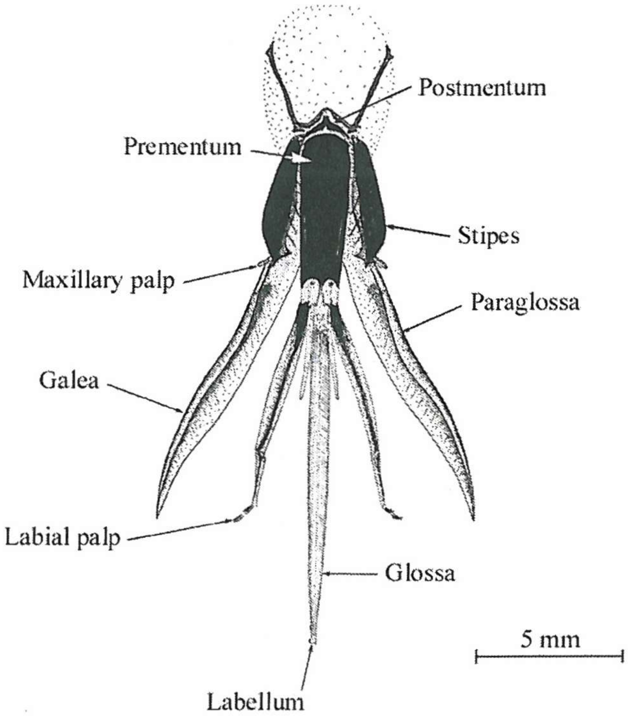

Figure 1.3 A bumblebee tongue. Long and slender, it is adapted to extract nectar from flowers (taken from Alford 1975).

Worldwide, there are estimated to be approximately 250 species of bumblebee, predominantly in the northern hemisphere in either temperate areas or areas of tundra. Species richness peaks in the mountains of central Asia (Williams 1994; plate 1.5). In the UK we have 23 species of bumblebee, although three of these (*B. pomorum, B. cullumanus,* and *B. subterraneus*) are thought to be extinct and a further eight (*B. distinguendus, B. muscorum, B. humilis, B. soroeensis, B. ruderarius, B. monticolla* and *B. sylvarum*; plate 1.6) are in decline with uncertain futures. Their decline is thought to be due to a loss in arable floral diversity and perhaps also of nest and hibernation sites, both a result of habitat destruction through intensive farming (Williams 1986; Osborne and Corbet 1994; Goulson 2003). From the remaining twelve, six are common and widespread (*B. terrestris, B. lucorum, B. pascuorum, B. lapidarius, B. pratorum,* and *B. hortorum*; plate 1.7) and six are parasitic (cuckoo bumblebees, parasites of *Bombus* species; plate 1.8). *B. hypnorum,* a recent immigrant from the continent has settled successfully all across southern England (Goulson, pers. com.) so may be considered an additional UK species.

1.2 The bumblebee life cycle

The life-cycle of the bumblebee is well described by Alford (1975, 1978), Goulson (2003), Heinrich (1979) and Prys-Jones and Corbet (1991). These descriptions are summarised below. As a general rule, bumblebees have an annual life-cycle (figure 1.4). In the UK, however, *B. pratorum*, an early emerging species, has a partial second brood and other exceptions occur in climates warm enough to permit a second nest cycle during the winter months. In most cases however, and especially in the UK, queen bumblebees hibernate from autumn until spring. Typically, during this time the queens are the only surviving bumblebees: all the males (drones) and workers die. In the spring queens emerge from hibernation, the exact time depending upon the species. Early emergers include *B. jonellus, B. pratorum* and *B. terrestris*, whereas *B. humilis, B. lapidarius, B. pascuorum* and most parasitic *Bombus spp.* (Plate 1.8) usually emerge later.

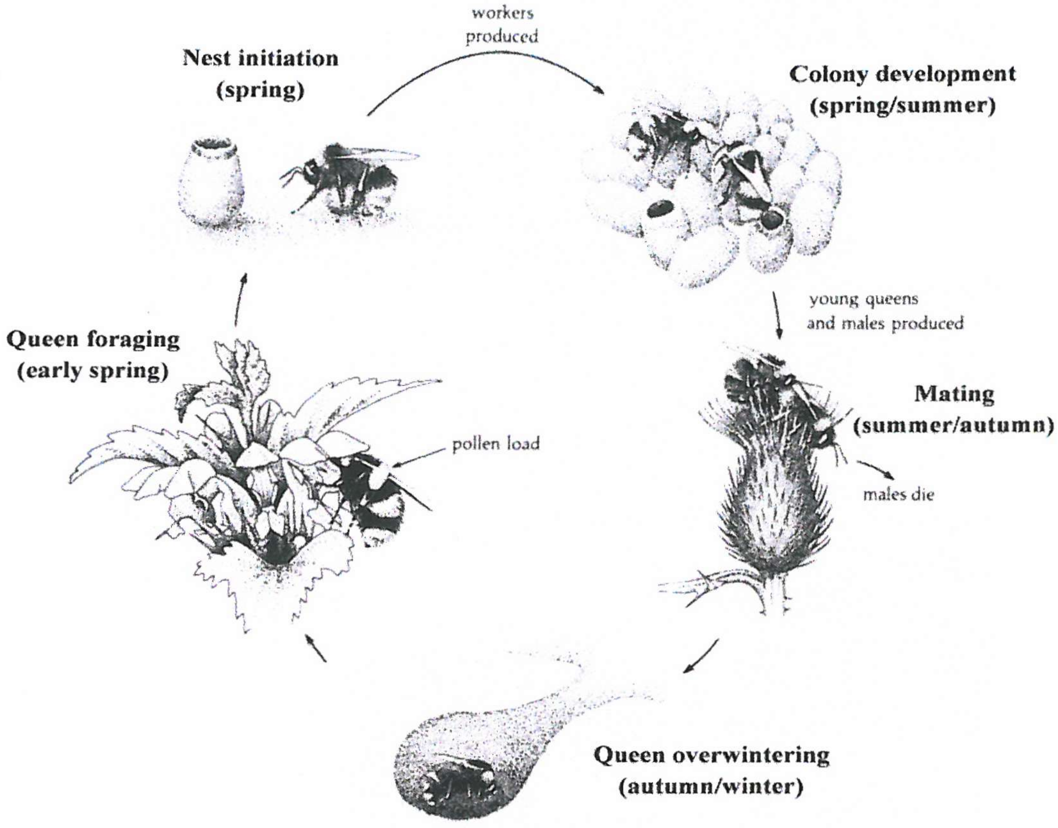

Figure 1.4 The Life Cycle of temperature *Bombus* species (taken from Prys-Jones and Corbet 1991).

The newly emerged queens forage for nectar and pollen whilst their ovaries develop, then seek a viable nest site. Typically, bumblebee nests are established in the vacated nests of small rodents, although colonies of some species, for example *B. pratorum, B. muscorum* and *B. pascuorum,* can be found nesting on the surface of the ground amongst material such as moss or loose grass. Queens often die competing for favourable nest sites. When a nest site is established and the queen has prepared a small chamber within, she is able to produce her first batch of workers.

Figure 1.5 The brood chamber. Here, a queen sits upon the brood clump containing the eggs of her first batch of workers. She incubates the clump by vibrating her wing muscles and replaces lost energy by drinking nectar from a prepared pot placed in front of her (taken from Goulson 2003).

Between 8-16 eggs are laid in a mixture of pollen and wax (figure 1.7a), secreted from the queen's abdominal gland, and incubated by the queen, who lies on top of the brood clump in the incubation groove (figures 1.5 & 1.7d) vibrating her wing muscles to provide heat whilst replenishing her energy supply from a pot of nectar. Eggs take between four and six days to hatch and become larvae. The queen forages intermittently for nectar to fuel incubation and pollen to feed the larvae. It is possible to categorise bumblebees by what method of larval feeding they employ. Odontobombus (pocket-makers), such as *B. pascuorum* and *B. hortorum*, force fresh pollen underneath the brood clump (figure 1.7b). The larvae then graze upon this collectively. Later, holes are pierced in the wax covering by the queen who then regurgitates a mixture of pollen and nectar through these holes onto the larvae, who continue to feed collectively. In the Anodontobombus (pollen-storers), such as *B. lapidarius, B. lucorum, B. terrestris* and *B. pratorum*, the brood clump disintegrates and each larva builds itself an individual cell from wax and silk. They remain separate and are fed individually with regurgitated pollen and nectar. In all species, the larval stage consists of five instars and lasts for between ten and twenty days, depending on temperature. Centrally placed larvae grow larger as they are fed more frequently and develop in a more consistently optimum temperature. When fully fed, the larvae spin their own silken cocoons (figure 1.7e-f) in which they pupate. Pupal cells are constructed with no obvious overall pattern (plate 1.8 & figure 1.6). They vary in size depending upon the size of the larvae that constructed them. The pupal stage takes roughly two weeks. In total, it takes about five weeks for a worker to develop from egg to adult. Having bitten out of the cocoon, a worker is pale and soft-winged, but after a few hours its full colours appear and after a day the wings have hardened. Soon after the first brood of workers become adult, they relieve the queen of her foraging duties allowing her to concentrate on laying eggs. Old pupal cells are reused as nectar pots and a wax cover is often built, covering the nest. The colony grows rapidly with the peak workforce typically numbering two or three hundred, this number varying greatly between species.

Figure 1.6 A bumblebee brood in a mature nest. Larvae have been revealed in various stages of development. The layout of larval cells is disorganised (taken from Alford 1975).

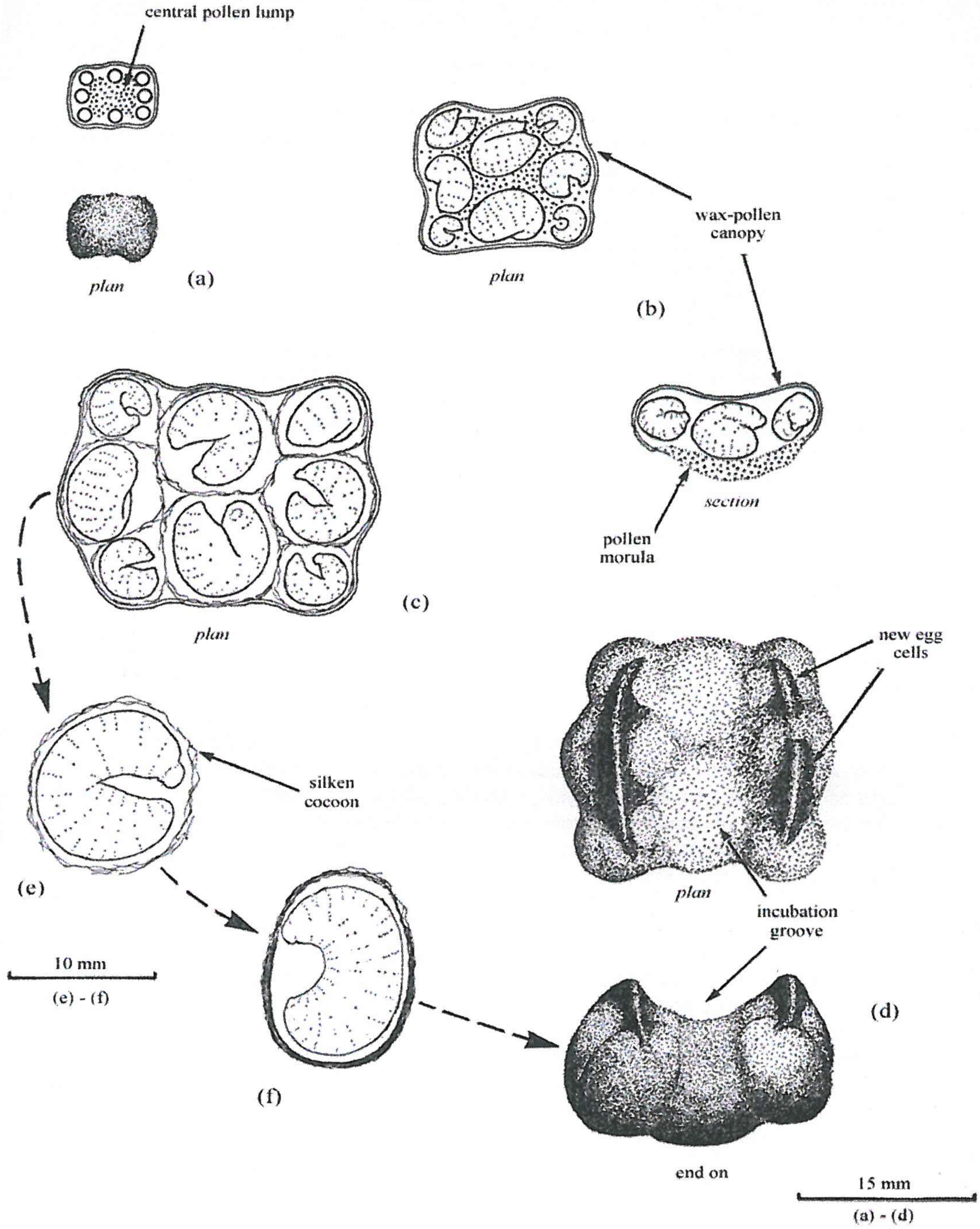

Figure 1.7 The development of the incipient brood clump of *Bombus pascuorum*. (a) egg clump; (b) young larval clump; (c) advanced larval clump; (d) pupal clump; (e) lateral aspect of fully fed larvae; (f) larva entering pre-pupal stage, having spun final cocoon (taken from Alford 1975).

Towards the end of the season, offspring production switches to males and new queens, the main cue for this probably being worker density (Goulson 2003). Inter-nest production of sexuals are generally intra-specifically synchronised (Pomeroy and Plowright 1982; Muller and Schmid-hempel 1992) so there may also be some seasonal cues or sharing of information between nests. The production of sexuals is influenced by nest size. Small nests often produce no sexuals at all, medium sized nests often only produce males, whilst only the biggest nests produce both males and queens (Schmid-Hempel 1998). New queens leave the nest to forage although they do not forage for their sisters but for themselves. By consuming pollen and nectar they build up large fat reserves in preparation for over-wintering. Likewise, males do not work, but leave the nest after a few days without returning, feed on flowers (plate 1.9) and search for a mate. Typically, to acquire a mate, males regularly patrol an area containing a number of prominent objects, such as leaves, stones, hedges and tree trunks, on which they have previously deposited a pheromone. Often a number of males will patrol the same route, the height of which varies between species. Queens are presumably attracted to these pheromones, although no evidence exists to prove this.

With the exception of some species, queen bumblebees mate only once. Once they have mated, queens soon begin searching for a suitable site in which to over winter. These sites are different from potential nest sites and generally, in the U.K., queens are thought to prefer north facing banks with loose soil. When a location is chosen, the queen digs down a few centimetres and creates a small oval chamber in which to lie dormant until the following spring, surviving on the fat reserves accumulated in her abdomen prior to mating. The old nest, meanwhile, soon degenerates. All occupants including the departed males become lethargic and eventually expire and the wax structure is consumed by parasites and commensals.

1.3 Thermoregulation in bumblebees and other insects

Queen bumblebees are amongst the first flying insects in the air after winter, indeed it is not too unusual to spot one during the cold winter months themselves. To be able to fly in cold temperatures, bumblebees and other insects have had to evolve some special features.

Traditionally, insects have been considered exothermic, relying on external heat sources to heat their muscles enough to allow movement. Currently, many insects are recognised as being far more active in their thermoregulation (Heinrich 1996b). To fly, an insect needs to beat its wings, the frequency being positively correlated with the insect's body size, negatively with wing size and also influenced by lifestyle. Acrobatic, small winged fliers, such as hoverflies and bees, tend to have higher frequency wing beats, whereas large winged fliers, such as dragonflies and butterflies, beat their wings more slowly (Heinrich 1996b). To beat its wings fast enough to hover, a bumblebee's flight muscles need to be above a certain temperature (Heinrich 1979). As in many other insects, attainment of this temperature can be done metabolically by vibrating these muscles thus generating heat and possibly through a biochemical reaction known as substrate cycling whereby heat is generated in the flight muscles without them contracting (Newsholme *et al.* 1972). To sustain flight in cool weather, especially with regular stops on flowers, mechanisms to prevent heat loss are essential. Moths, commonly active at night, insulate themselves with scales. For bumblebees, a covering of setae performs the same function. In addition to this, air pockets and a constricted waist prevent heat flow from the thorax to the outside world via the abdomen (figure 1.1). A third important factor, especially for queens and large workers, is size itself. Larger objects lose heat less quickly than smaller objects due to the relationship between body mass and surface area. In the UK, some of the bulkiest insects are those that fly in cool conditions, either at night (*e.g.* hawk moths and stag beetles) or at a cold time of year (*e.g.* bumblebee queens). The variation in worker size must have significant consequences upon the thermoregulatory capabilities of different individuals (Chapters 3 & 4).

1.4 A comparison of bumblebees and honeybees

Although bumblebees and honeybees are from the same family (both are eusocial and both rely on flowering plants for sustenance), there are a number of distinctions between them (table 1.1) which may be informative when trying to understand worker size variation in bumblebees.

Table 1.1 Key differences between bumblebees and honeybees

Bumblebees	Honeybees
Workers vary enormously in size	Workers all virtually identical size
Larval and pupal cells haphazard and varied in size	Cells tessellated and uniform
Larval cells flexible and constructed by larvae	Cells rigid and constructed by workers
Covered in thick pile	Relatively (but not entirely) hairless
Generally an annual species	Colonies last for several years
<400 workers at peak	>10,000 workers at peak
Short term storage of forage	Produce honey in large quantities
Sting lacking barbs	Sting barbed
Generally not aggressive (with exceptions)	Can be aggressive
Queens not morphologically differentiated	Queens morphologically differentiated
Can buzz pollinate	Cannot buzz pollinate

Honeybees are in the subfamily Apinae, which includes six advanced eusocial species. Bumblebees are in the subfamily Bombinae, globally consisting of approximately two hundred and fifty primitively eusocial species (advanced eusocial species have morphologically differentiated queens and primitively eusocial species do not (Crozier and Pamilo, 1996)).

Amongst the social bees, in terms of worker size variation, bumblebees and honeybees represent the extremes. Bumblebee workers vary in size considerably (Alford 1975; Heinrich 1979; Goulson *et al.* 2002; plate 1.2 & Chapter 2) whereas honeybee workers exhibit virtually no size variation at all (Roulston and Cane 2000). Correlated with this, we find the organisation of honeybee larval and pupal cells to be greater than that of bumblebees. In a honeybee hive, pupal cells are tessellated hexagonally and are uniform in size (plate 1.3). Perhaps this regular pattern allows an efficient feeding regime, ensuring equal provision for all honeybee larvae. A bumblebee nest, however, has no obvious pattern, with cells of varying size dotted around apparently at random (plate 1.10). Perhaps the regular pattern of honeycomb limits the potential for the development of varying sized offspring. A key difference is that workers construct honeybee cells, whereas silken bumblebee cells are spun by the larvae themselves and expand during growth. Honeybee larvae grow to fit their cell; bumblebee cells grow to fit the larvae. Honeybee eggs are laid singly on the bases of the cells, whereas bumblebee eggs are laid communally, providing them with the opportunity to compete for resources (Free 1977). After three days honeybee eggs hatch into larvae, up to twice as quickly as in bumblebees. In bumblebees, larvae of the Odontobombus feed communally and are likely to compete for resources. Apart from the earliest stages of larval development, the Anodontombus larvae are fed individually, thus the size they attain is under direct control of the queen or workers. Honeybee workers feed each larva individually and frequently, providing a total of about 125 mg of honey and between 70 and 150 mg of pollen per larva (Free 1977). Before each feed, larvae are inspected closely. It is likely that a honeybee worker is able to assess age of a larva and then feed it accordingly (Free 1977). Honeybee queen cells are of larger diameter than larval cells and queen larvae are fed more frequently. So the differences between bumblebees and honeybees in size variation may be proximately explained by their different larval rearing systems. However, these systems have probably evolved in response to some ultimate cause of size variation or lack thereof. Other differences may offer explanations as to what those ultimate causes are.

Bumblebees possess a thick layer of setae (plate 1.11), which covers their thorax and abdomen. Although honeybees possess setae, it is far more sparse (plate 1.12). This suggests that bumblebees have needed to insulate themselves against cold conditions and that honeybees have not, at least not to such a degree. Maybe this can be explained by the climates in which each has evolved. Bumblebees are said to be adapted to tundra (Heinrich 1979), which occurs in and around the Arctic Circle. They are also adapted to mountainous regions, according to their global distribution. Indeed, we find the highest diversity of bumblebee species in the mountains east of Tibet and the mountains of central Asia (Williams 1994; plate 1.4), cold places where insulation is essential for any animal to prevent rapid heat loss. However, honeybees probably spent most of their evolutionary history at lower latitudes as three of the four honeybee species are confined to South-eastern Asia (Free 1977).

Bumblebees are generally annual in their colony development with queens living for just one year, whereas honeybee colonies may survive for several. This may be reflected by the climates in which they originated. If bumblebees evolved in cool temperate climates with distinct seasons, during the winter months there would have been few or no flowers from which to feed and therefore little opportunity for sustaining a colony during that period. As honeybees probably evolved nearer the equator where, despite distinct wet/dry seasonality, seasonal temperature variation is dramatically reduced, colonies have been able to survive for many years. Consequently, honeybee colonies have been able to grow to large sizes with thousands of workers, whereas bumblebee colony growth is limited to the spring and summer seasons during the one year in which any particular colony exists. Having a huge number of individuals in a colony has probably been the selective pressure on honeybees to store honey, although it could be argued that an ability to store honey has led to the development of large worker numbers. A huge workforce is a huge potential loss. If, when resources were poor (due to adverse weather conditions, for example), there were no stored food, many of the workers would die. Bumblebees store nectar and pollen but in relatively small quantities, reflecting the small size of the colony.

As honeybee colonies have such vast stores of honey and pollen, they attract many predators (Free 1977). When defending the nest, a stinging honeybee leaves an 'attack' pheromone on its target which attracts the aggression of other workers. The aggression that honeybees show is essential for the colony's survival. In addition, honeybees are armed with barbed stings. Bumblebees lack barbs on their sting and are generally relatively placid (defensive behaviour usually involves lying on their backs and exposing their sting; plate 1.13), with exceptions (species of the subgenus Fervidobombus, found in tropical America, are noted for their aggression and have been named 'fervid' accordingly). The barbs on a honeybee's sting prevent the sting from being easily withdrawn from mammal flesh. Moreover, when a stinging honeybee is removed, it leaves the seventh abdominal segment complete with poison sacs and pumping muscles attached, enabling the sting to inject further toxins. Consequently, the mammal, a potential threat to a hive, receives a stronger aversive stimulus. So why might honeybees have evolved barbed stings but bumblebees not? With up to several thousand workers in a hive and a large brood to protect, economically, it makes sense to send out kamikaze workers if that results in a stronger deterrent. A honeybee colony can afford to lose a few individuals for the sake of remaining undisturbed. A bumblebee worker is more precious as there are relatively few of them. Although they can sting, the poison they deliver is likely to be less because, unless the bee is buried deep in the fur, the sting can be easily removed. A bumblebee colony does not need to employ such aggressive defensive tactics as they are relatively small and have relatively few individuals entering and exiting the nest so are therefore less likely to attract the attention of a predator. The traffic going in and out of an active honeybee hive is so vast it is easily spotted. A high profile calls for high protection.

1.5 Alloethism and worker size variation

Alloethism, a division of labour dependant upon differing sizes of individuals, can be found among the hymenopterans, especially in many ant species (Wilson 1980), and also in termites (Crosland *et al.* 1998). Social bumblebees (*Bombus* spp.; Hymenoptera; Apidae) also display a degree of alloethism within the worker caste (Alford 1975). Size varies both within caste and, in the case of the Anodontobombus (pollen storers; see below) species, between castes (queens are larger than other castes; plate 1.2) and each caste performs a distinct function. Bumblebees can be categorised into three main castes (queens, drones and workers) and, within the worker caste, we find tasks vary between in nest (larval feeding and incubating, maintenance of nest ambient temperature and nest structure) and extra nest (foraging for pollen and nectar) tasks (Alford 1975). Within the worker caste, a great deal of size variation exists in many bumblebee species (as much as a ten fold difference in mass (Alford 1975)). Discounting many ant species, this phenomenon is rare in other insects and much less extreme in other social bees (Roulston and Cane 2000). In *Bombus terrestris* L., it has been found that the larger workers most commonly forage for nectar and pollen, leaving the smaller workers in the nest (Colville 1890; Cumber 1949; Free 1955; Goulson *et al.* 2002). Only in times of resource shortage or reduction in the number of the normal foraging workforce do the smallest workers emerge to forage (Brian 1952, Pendrel and Plowright 1981).

The proximate causes of this size variation may be explained by the differential food provision during the larval stage (Ribeiro 1994). A well-nourished larva matures into a larger adult than one that is poorly fed. As mentioned in sections 1.2 and 1.4, social bumblebees can be roughly categorized into two groups: the Odontobombus (or pocket makers) and the Anodontobombus (or pollen storers). Size variation is greater amongst the workers of Odontobombus species (Pouvreau

1989), probably because the larvae feed communally for a greater proportion of their development than the Anodontobombus. Anodontobombus larvae are fed individually except during the earliest stages, thus their size is under greater control of the workers. Despite this, size variation is still very large and seems likely to be adaptive (Goulson *et al.* 2002). Alloethism must also be adaptive (making large bees is expensive) and broadly related to foraging efficiency. Cnaani (1994) showed that manipulated nests with artificial food provision containing a number of larger than average workers produced a greater number of emerging workers than manipulated nests containing the same number of smaller workers. This indicates that larger workers are superior brood carers but leaves the question of foraging ability unanswered.

Objects with a higher ratio of volume to surface area (I.e. bigger) retain heat more than those with a lower ratio (I.e. smaller). This has been applied as partial explanation of the differences in the size and shape that we see between diurnal equatorial animals (needing to lose heat) and comparable arctic animals (needing to retain heat). Putting behavioural and physiological adaptations aside, we often find that the volume to surface area ratio is greater in cold climate animals than in hot climate animals (Ray 1960). There is no reason why these principles should not be applied to invertebrate species, as they require high thoracic temperatures to allow flight. Heinrich (1975) demonstrated that the flight of worker bumblebees is limited to warmer temperatures (>10°C) compared to the larger queens. He also showed that larger bumblebees lose heat at a relatively slow rate (Heinrich and Heinrich 1983) and that bumblebees of all sizes maintain similar average thoracic temperatures whilst foraging. Bumblebees can raise their thoracic temperatures by biochemical means (Newsholme *et al.* 1972) and by vibrating their wing muscles, both requiring energy. Whilst foraging, workers spend some time flying and some pausing on flowers. During flight, the thoracic temperature is maintained through the action of the flight muscles. All the while, heat is lost by convection to the surrounding air at a rate dependent on flight speed, the air temperature and the size of the bee (this shall be tested in Chapter 3). Therefore, a smaller bee must use more energy per unit mass than a larger bee in maintaining a specific thoracic temperature.

Foraging is a more dangerous task than working in the nest (Van Doorn 1987; O'Donnell *et al.* 2000). Larger foragers may be less vulnerable to predation, being better equipped to tear itself free from a spider web, for example. One might assume that the larger a prey item the more conspicuous it is, therefore the more likely it is to be preyed upon. However, many bumblebee species appear to advertise the fact they are dangerous to potential predators through aposematic colouration: it is conceivable that a large size may offer greater protection because a larger pattern gives a stronger signal (larger patterns have been shown to enhance the protective value of warning patterns possessed by artificial butterflies (Forsman and Merilaita 1999)).

Müller *et al.* (1996) found that bigger *B. terrestris* foragers were more at risk to conopid parasitoid infection than smaller foragers. This is not evidence for active host choice by the parasite and might be due to the fact that big bees forage more frequently than small bees and thus have a greater chance of coming into contact with the parasitoids. They also showed that conopid fly larvae develop more successfully inside larger hosts. If there were an active host choice by the flies it would be to parasitise bigger bees, a factor potentially contrary to bigger bees being advantageous as foragers.

1.6 The aims of this study

It is the aim of this study to elucidate the causes of size variation in the worker caste of *Bombus* species by studying size related aspects of morphology and behaviour and how they influence thermoregulation, foraging efficiency, flower choice and colony development.

Specifically, the aims of this thesis are:

1. To confirm that size variation exists amongst workers of *Bombus* species, that it is more extreme in species of Odontobombus and that foraging workers are, on average, larger than nest bound bees (Chapter 2).
2. To investigate the consequences of size variation for thermoregulation in foraging workers by examining differential thoracic cooling rates, thoracic insulation and morphology (Chapter 3), also the impact of climate on the evolution of body size (Chapter 4) and thoracic insulation (Chapter 3)
3. To investigate the nectar foraging ability of different sized workers in a variety of climatic conditions (Chapter 5).
4. To investigate forage preferences and flower handling ability amongst different sized workers (Chapter 6).
5. To compare colony productivity between nests with manipulated workforces in order to establish whether a varied workforce is preferable to one of uniform size (Chapter 7).

Colour Plates

Plate 1.1 A *Bombus terrestris* queen grappling with a Crane's Bill flower (picture: James Peat).

Plate 1.2 Size variation in *Bombus terrestris*. The two workers represent the extremes of size variation within the worker caste. The queen is significantly bigger than even the largest workers (picture: James Peat).

Plate 1.3 Honeybee workers on the brood comb. There is virtually no size variation within the honeybee worker caste (picture: P. O. Gustafsson).

Plate 1.4 A *Bombus lapidarius* worker foraging on knapweed. Note the pollen sac on the left corbiculum (picture: James Peat).

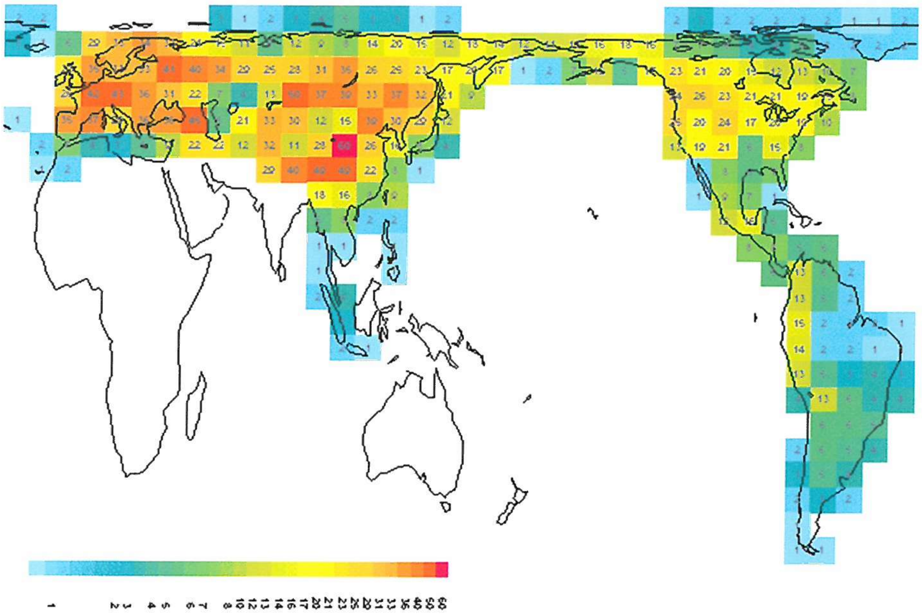

Plate 1.5 The global distribution of bumblebee species. They are predominantly found in temperate and tundra regions of the Northern Hemisphere. Species richness peaks in the mountains of South-east Asia (taken from Williams 1994).

1	*Bombus soroeensis* (Q)
2	*B. soroeensis* (M)
3	*B. distinguendus* (Q)
4	*B. muscorum* (Q)
5	*B. jonellus* (Q)
6	*B. jonellus* (M)
7	*B. ruderatus* (Q - pale)
8	*B. ruderatus* (Q - dark)
9	*B. ruderatus* (Q - intermediate)
10	*B. humilis* (Q)
11	*B. subterraneus* (Q)
12	*B. subterraneus* (M)
13	*B. ruderarius* (Q)
14	*B. ruderarius* (M)
15	*B. sylvarum* (Q)
16	*B. monticola* (Q)

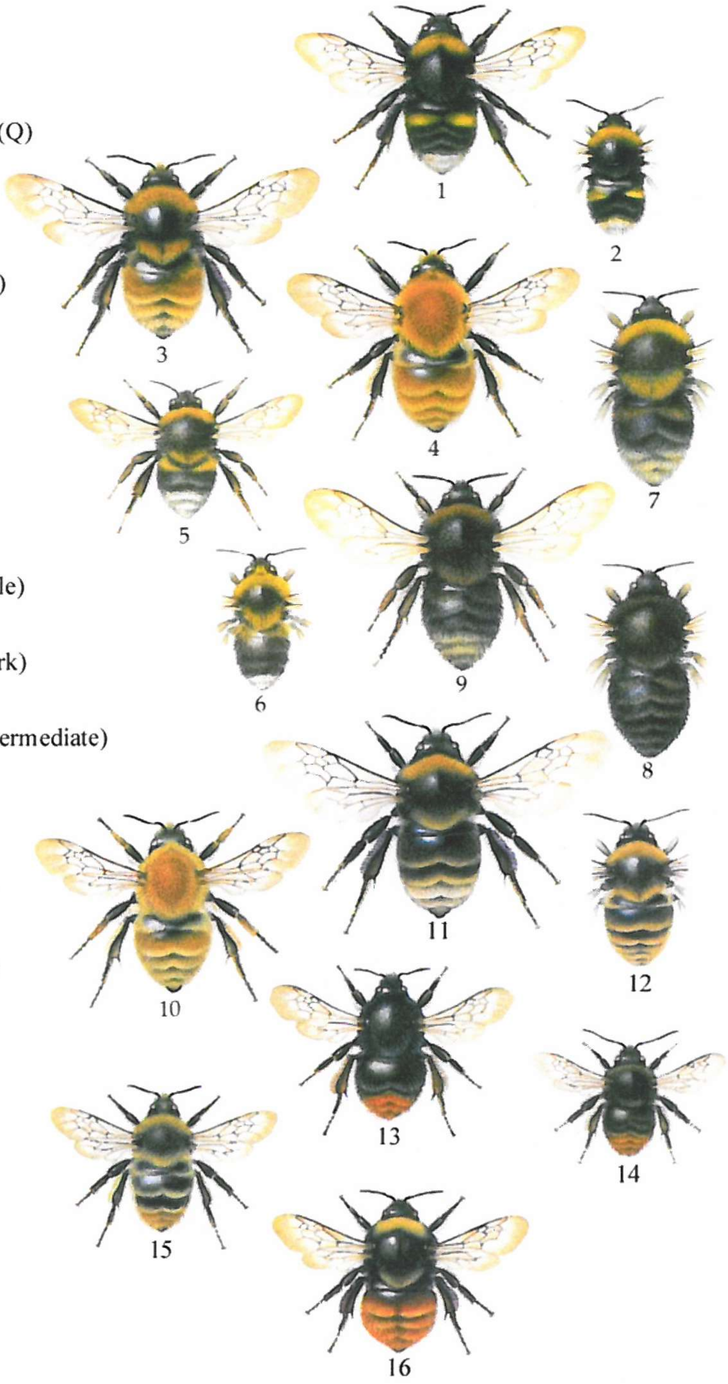

Plate 1.6 The rare UK *Bombus* species. *B. subterraneus* is thought to be extinct (taken from Prys-Jones and Corbet 1991).

1 *Bombus lucorum* (Q)

2 *B. lucorum* (M)

3 *B. terrestris* (Q)

4 *B. terrestris* (W)

5 *B. Hortorum* (Q)

6 *B. lapidarius* (Q)

7 *B. lapidarius* (M)

8 *B. pratorum* (Q)

9 *B. pratorum* (M)

10 *B. pascuorum* (Q)

11 *B. pascuorum* (W)

12 *B. pascuorum* (Q)

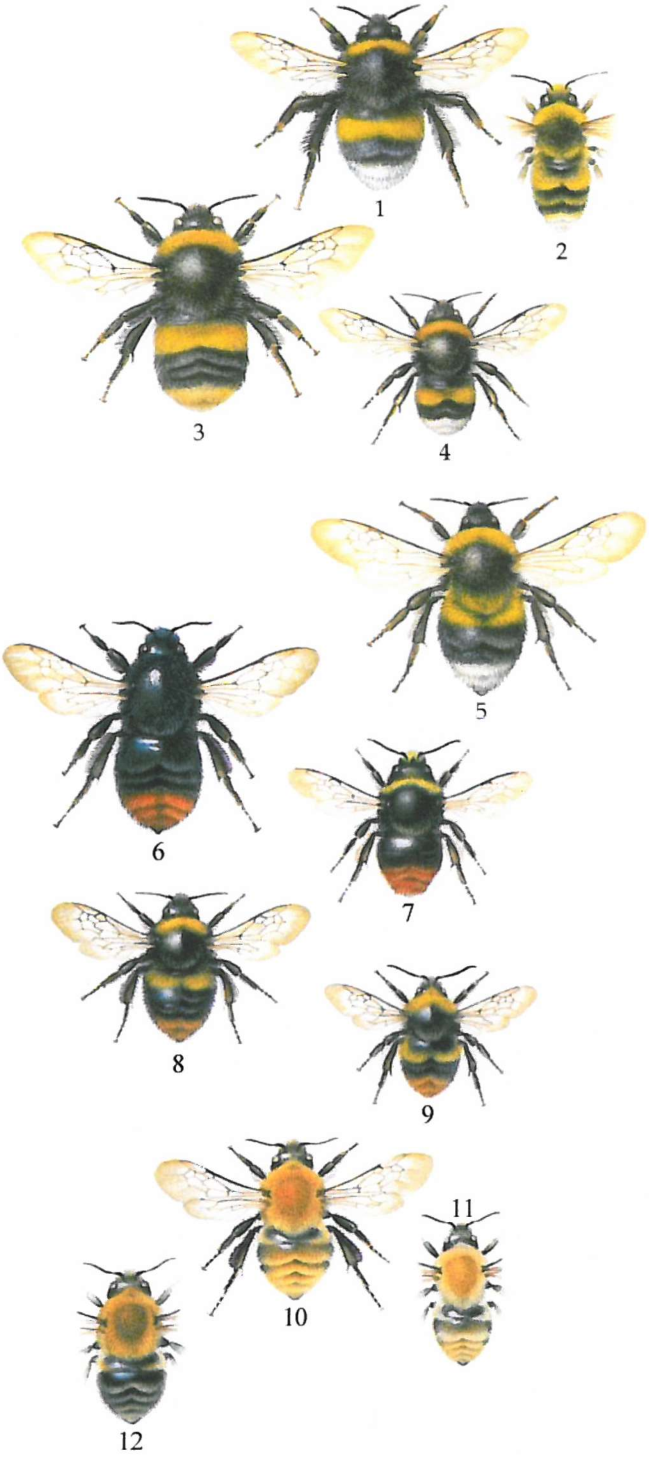

Plate 1.7 The common UK *Bombus* species (taken from Prys-Jones and Corbet 1991).

1 *Bombus rupestris* (F)
2 *B. rupestris* (M)
3 *B. sylvestris* (F)
4 *B. sylvestris* (M)
5 *B. bohemicus* (F)
6 *B. bohemicus* (M)
7 *B. vestalis* (F)
8 *B. vestalis* (M)
9 *B. barbatellus* (F)
10 *B. campestris* (F)
11 *B. campestris* (M - dark)
12 *B. campestris* (M - light)

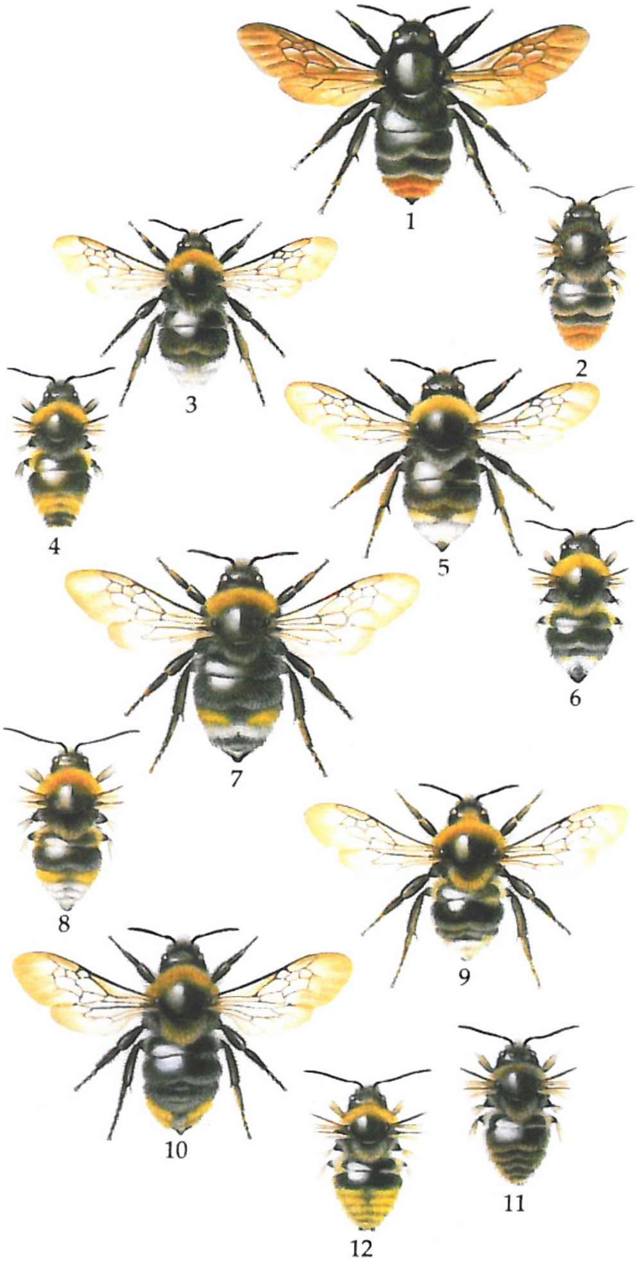

Plate 1.8 The UK parasitic bumblebee species. Otherwise known as cuckoo bumblebees, these species parasitise social *Bombus* species by usurping the queen and laying their own eggs to which the host workers then attend (taken from Prys-Jones and Corbet 1991).

Plate 1.9 A male *Bombus pratorum* found on a daisy in a state of torpor. Aside from differences in markings, males can be distinguished from females by their long antennae and by their lack of sting (picture: James Peat).

Plate 1.10 Inside a *Bombus terrestris* nest. Note how haphazardly the cells are arranged compared to the honeybee brood in Plate 3. Note the cells containing pupating larvae and the old cells reused as nectar and pollen pots (picture: Dave Goulson).

Plate 1.11 A large *Bombus terrestris* worker foraging on thistle. Note the dense, insulating pile covering the thorax and abdomen (picture: James Peat).

Plate 1.12 A honeybee. Note the comparative lack of setae (picture: Jim Staples).

Plate 1.13 A *Bombus terrestris* worker, understandably distressed by the presence of an ecologist, adopts a defensive posture and ejects venom (picture: James Peat).

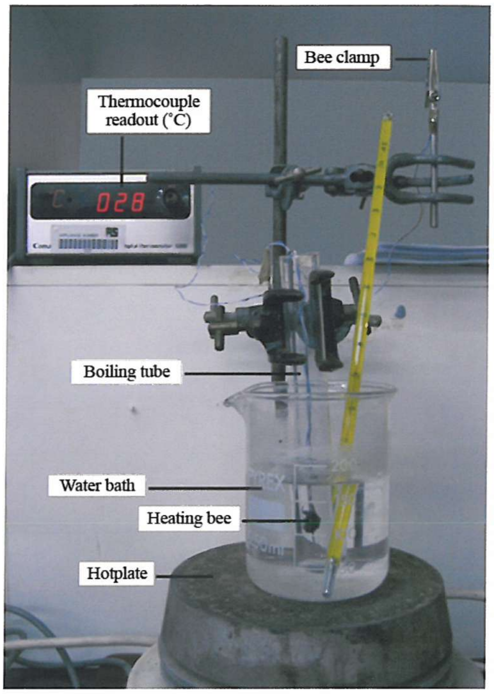

Plate 3.1 Experimental set-up for bumblebee cooling. The fan is out of view (picture: James Peat).

Plate 3.2 A crocodile clip holding the thermocouple measuring the thoracic temperature of the dead *Bombus terrestris* worker. Note the lower thermocouple used to measure the ambient temperature (picture: James Peat).

Plate 3.3 Examples of bumblebees from cold climates. Compare this with Plate 3.4 below. Even with the naked eye, we can see that they have longer thoracic setae (pictures: James Peat).

Plate 3.4 Examples of bumblebees from hot climates. Compare this with Plate 3.3 above. Even with the naked eye, we can see that they have shorter thoracic setae (pictures: James Peat).

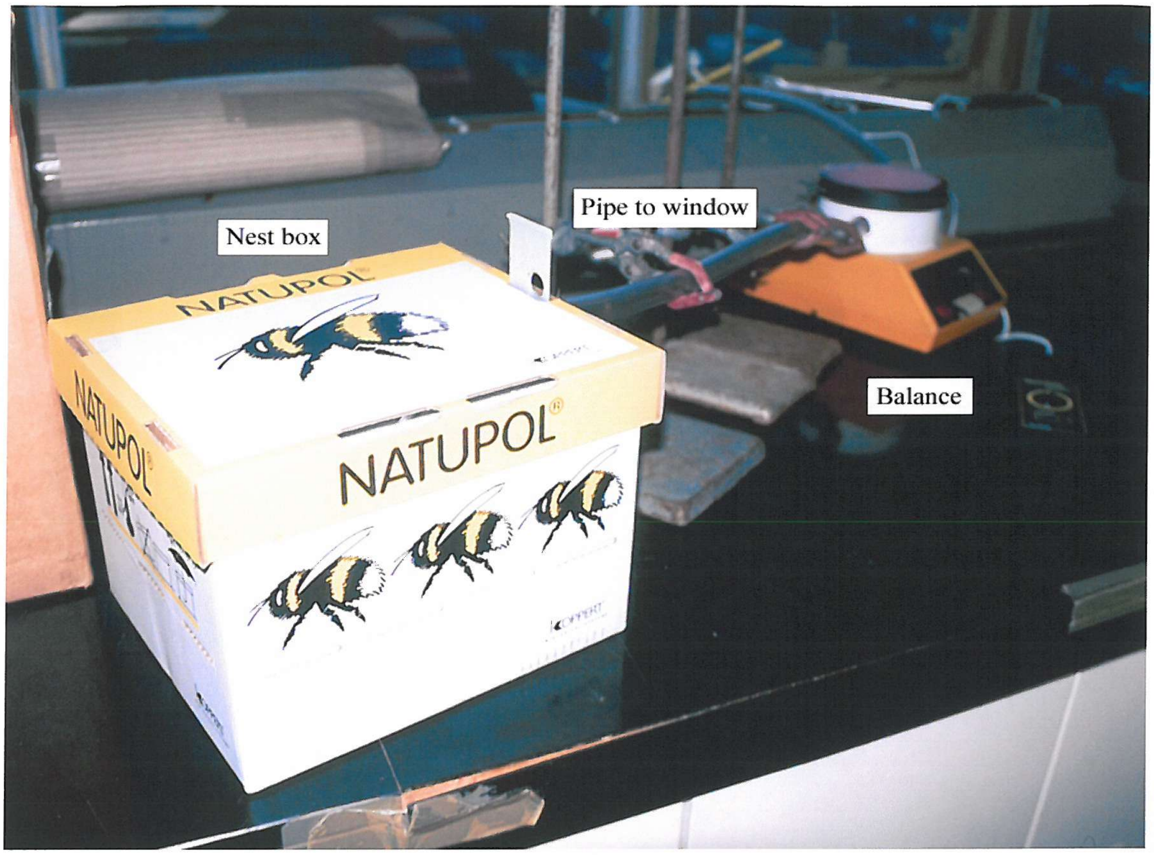

Plate 5.1 Experimental set up for nectar foraging efficiency experiment. Labelled bees were recorded and weighed leaving and returning to the nest over a period of one month. This was repeated with three nests and the relationship between worker size and foraging efficiency was examined (picture: James Peat).

Plate 7.1 A nest-box containing a bumblebee nest. 1 of 12 placed outside in sheltered positions for 3 weeks during the summer of 2002. Each nest was placed on bricks to prevent upward moisture ingress, had a rain-preventative cover pinned down with a brick and the immediate area received a regular application of slug pellets (picture: James Peat).

Chapter 2:

Worker size variation in bumblebees

What bumblebees cannot disguise,
Is that worker bees vary in size,
To a degree that's terrific,
And inter-specific,
Why did this phenomenon arise?
- James Peat

2.1 Abstract

Amongst the worker caste, the social bumblebees (*Bombus* spp.) exhibit a dramatic intra-colony variation in size. This variation is known to be greater in Odontobombus (pocket-making) species than in Anodontobombus (pollen-storing) species. Bumblebees also exhibit size dependent division of labour, or alloethism, whereby larger workers forage more frequently, leaving the smaller workers to perform in-nest tasks such as brood caring. Here, I confirm that considerable worker size variation exists in 23 *Bombus* species that it is greater amongst the Odontobombus than the Anodontobombus and that mean worker size differs between species. I confirm that foraging workers of *Bombus terrestris* are larger, on average, than in-nest workers, but that there is a large overlap in their size distributions.

2.2 Introduction

The insect order Hymenoptera is noted for an unusually high incidence of eusociality. Many wasp, ant and bee species live in great numbers and exhibit cooperative brood care. Workers, closely related daughters of the queen (except in unusual polyandrous species such as *B. hypnorum* (Goulson 2003)), help raise their sisters by foraging for food and feeding the larvae that the queen produces. Commonly, social ant species exhibit a large range of sizes within the worker caste, this variation being linked to division of labour: size-polyethism. Size variation has not been reported in wasps and is not so extreme in stingless bees (Waddington *et al.* 1986; Ramalho *et al.* 1998; Roulston and Cane 2000). Variation amongst honeybee workers (*Apis mellifera*) is very small (Roulston and Cane 2000). However, the existence of worker size variation within bumblebee colonies is well known (Alford 1975; Heinrich, 1979). Here, I seek to quantify size variation within the worker caste in several different *Bombus* species. Bumblebees also exhibit a degree of labour division, or 'alloethism': it has been reported frequently and in several different *Bombus* species that, on average, foraging workers are larger than those that remain nest-bound (Colville 1890; Sladen 1912; Meidell 1934; Richards 1946; Cumber 1949; Brian 1952; Free 1955). The differences in size between foraging and nest-bound workers of Greek *Bombus terrestris* are examined. Size variation in Odontobombus species (such as *B. hortorum* and *B. pascuorum*) is greater than in Anodontobombus species (such as *B. terrestris* and *B. pratorum*) (Alford 1975). This difference is thought to be because the Odontobombus larvae are fed in groups and are likely to compete for food whereas the Anodontobombus adults feed their larvae individually. Here I examine the difference in mean workers size of some UK Odontobombus and Anodontobombus species.

2.3 Methods

Bombus terrestris were obtained from Koppert UK Ltd. (Haverhill, Suffolk), their place of origin (Greece) confirmed by Paul Schmid-Hempel (pers. com. 2004). Foragers were distinguished from nest bees through exhaustive monitoring. UK bumblebee foragers were caught in Southern England (Shaftesbury, Dorset; Canvey Island, Essex; Margam Moors, Glamorgan; Chilworth, Itchen Valley and Winchester, Hampshire; Harpenden, Hartfordshire; Dungeness and Elmley, Kent; Castlemartin Tank Range, Pembrokeshire; Somerset Levels and Wincanton, Somerset; Salisbury Plain, Wiltshire) from May 22nd to August 27th and in Scotland (Hebridean Isles: Barra and associated islands, Canna, Coll, Eigg, Muck, Rum and Tiree) from June 15th to August 10th 2003. The lateral distance across the widest point of the thorax was measured (using Vernier Callipers) as a reliable indicator of body size (Goulson *et al.* 2002). The mean sizes of different UK *Bombus* species were compared using a 1-way ANOVA fitted in Statview 5.0. Samples of other species, to which I shall refer as ‘global’, were measured in the Department of Entomology, Natural History Museum, London (see table 2.1 for the species sampled). Coefficients of variation (a) were calculated for each species (taking place of capture into account where appropriate) using the equation $a=b/c$ where c = the mean thorax width and b = the standard deviation of that mean. The mean coefficients of variation for the Odontobombus and Anodontobombus species were compared using a 1-way ANOVA fitted in Statview 5.0.

2.4 Results

Size variation in the worker caste of *Bombus* species

Mean thorax width varies significantly between the 13 UK *Bombus* species collected ($F_{12, 2803}=91.47$; $p<0.0001$; fig. 2.1; table 2.1). Figure 2.2 shows the size variation that exists amongst foraging workers in six common UK species collected in Scotland (*B. pascuorum, B. lucorum* and *B. hortorum*) and southern England (*B. pratorum, B. lapidarius* and *B. terrestris*). All species display considerable size variation and appear to broadly follow a normal distribution (the data have not been tested for normal distributions) except for *B. hortorum*, the distribution of which appears to be bimodal. In the ten 'global' species sampled we also find considerable size variation amongst the worker caste (table 2.1).

Size variation in foraging and nest-bound workers

In Greek *Bombus terrestris*, foraging workers were, on average, larger than workers that did not forage ($F_{1, 471}=16.12$; $p<0.0001$; see fig. 2.3a). However, there is a considerable overlap in size variation (fig. 2.3b).

Size variation in the Odontobombus and Anodontobombus species

On average, foragers of Odontobombus species (Mean= 0.105 ± 0.007) varied in size significantly more than those of Anodontobombus species (Mean= 0.081 ± 0.005) ($F_{1, 15}=9.1$; $p<0.01$; see fig. 2.4). This is in agreement with previous findings. There was no significant difference in mean thorax width ($F_{1, 15}=0.102$; $p>0.05$).

Table 2.1 Mean thorax widths and coefficients of variation in foraging workers from 23 *Bombus* species. Note the diversity of mean thorax widths and size variation. Also, note the difference in variation between *Bombus* spp. and *Apis mellifera* (the honeybee). *taken from Roulston & Cane (2000).

Sub Genus	**Species**	**N**	**Mean Thorax Width (mm ± S.E.)**	**Range (mm)**	**Coefficient of Variation (a)**
UK samples					
Bombus	*B. lucorum*	327	4.76 ± 0.019	3.8 … 5.8	0.074
	B. magnus	122	4.95 ± 0.041	3.3 … 6.0	0.091
	B. terrestris	278	4.99 ± 0.024	3.6 … 6.3	0.081
	B. terrestris (Grk)	216	4.87 ± 0.025	3.6 … 6.3	0.077
Kallobombus	*B. soroeensis*	22	4.22 ± 0.060	3.4 … 4.6	0.067
Megabombus	*B. hortorum*	158	4.74 ± 0.034	3.6 … 5.7	0.091
Melanobombus	*B. lapidarius*	385	4.37 ± 0.017	3.1 … 5.9	0.075
Pyrobombus	*B. jonellus*	283	4.29 ± 0.029	3.0 … 6.2	0.114
Thoracobombus	*B. pratorum*	189	4.21 ± 0.022	3.5 … 5.7	0.072
	B. humilis	96	4.43 ± 0.060	3.2 … 6.5	0.133
	B. muscorum	507	4.87 ± 0.023	3.1 … 6.5	0.105
	B. pascuorum	205	4.48 ± 0.030	3.2 … 6.2	0.095
	B. ruderarius	189	4.70 ± 0.036	3.3 … 6.2	0.106
	B. sylvarum	55	4.11 ± 0.050	3.2 … 5.0	0.091
'Global' samples					
Alpinobombus	*B. alpinus*	32	5.28 ± 0.089	4.3 … 6.6	0.095
	B. balteatus	30	4.59 ± 0.093	3.7 … 5.8	0.112
Pyrobombus	*B. huntii*	23	5.13 ± 0.250	3.9 … 7.6	0.234
	B. jonellus	30	4.18 ± 0.059	3.5 … 4.9	0.077
	B. lapponicus	30	5.31 ± 0.183	3.7 … 6.8	0.189
Fervidobombus	*B. atratus*	30	5.23 ± 0.073	3.6 … 5.8	0.077
	B. dahlbomii	30	6.01 ± 0.125	4.9 … 7.5	0.114
	B. medius	23	5.03 ± 0.146	3.1 … 7.0	0.139
	B. mexicanus	21	4.84 ± 0.082	4.0 … 5.5	0.077
	B. transversalis	30	6.21 ± 0.087	5.3 … 7.4	0.076
The Honeybee					
	*Apis mellifera**	53			0.018

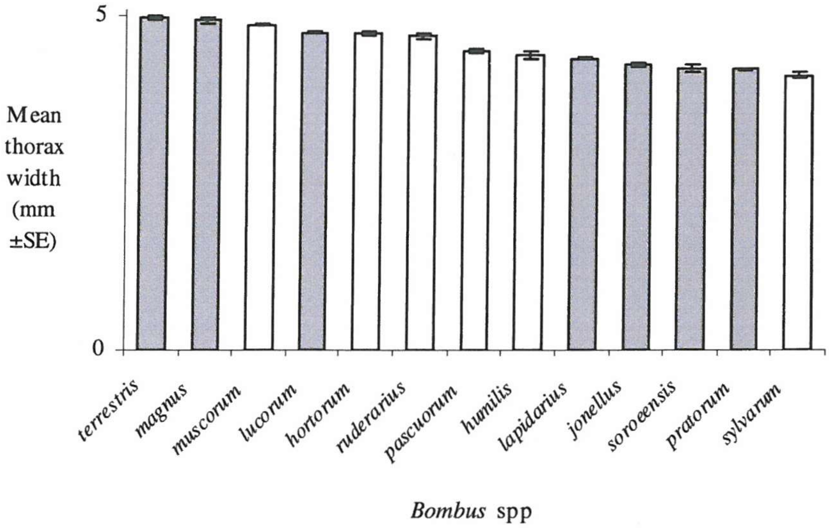

Figure 2.1 Variation in mean forager size in 13 UK *Bombus* species, in order of decreasing mean thorax width. Anodontobombus species are shaded, Odontobombus are white. The mean thorax width varies significantly between species ($F_{12,2803}$=91.47; P<0.0001).

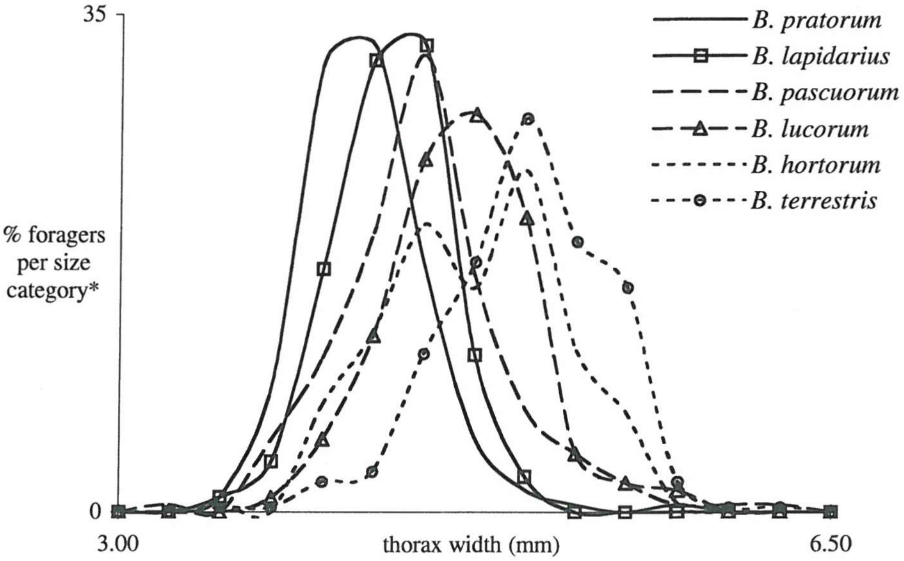

Figure 2.2 Size variation amongst foraging workers in six common UK *Bombus* species. *B. pascuorum, B. lucorum* and *B. hortorum* were collected in Scotland, *B. pratorum, B. lapidarius* and *B. terrestris* in southern England. All species display considerable size variation and appear to follow a normal distribution except for *B. hortorum*, the distribution of which appears to be bimodal. *Size categories were defined by 0.25 mm divisions.

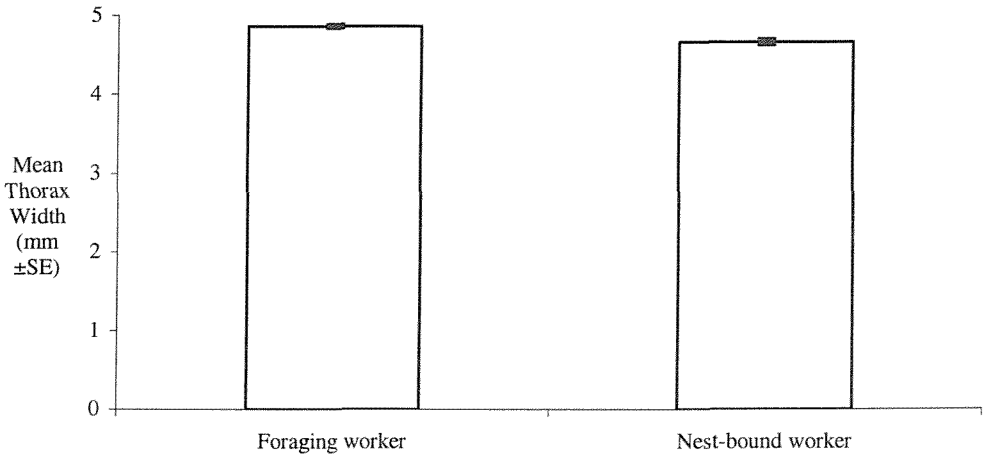

Figure 2.3a Mean size of foraging and nest-bound workers in *Bombus terrestris*. Foraging workers were, on average, larger than nest-bound workers ($F_{1,471}$=16.12; P<0.0001).

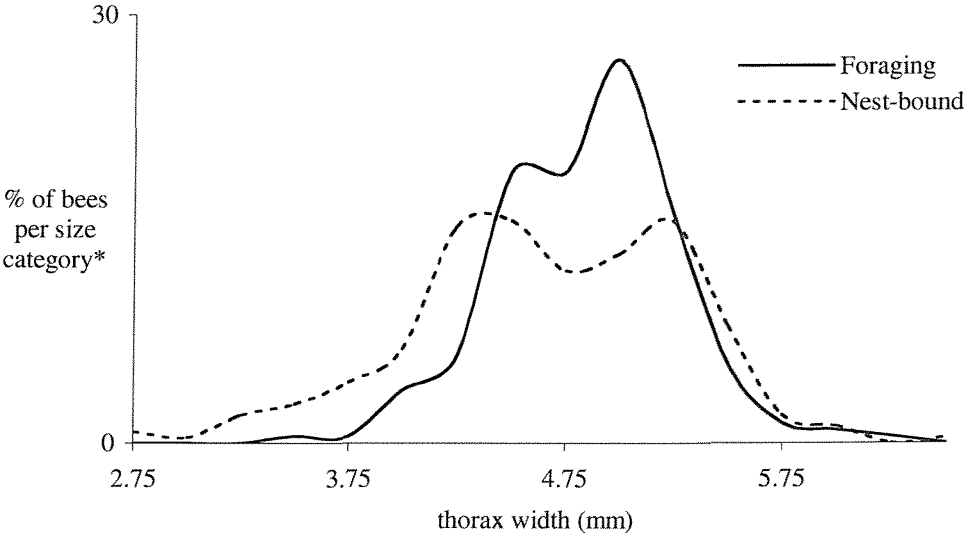

Figure 2.3b The size distributions of foraging and nest-bound workers in *Bombus terrestris*. Although foragers are larger on average, there is considerable overlap. *Size categories were defined by 0.25 mm divisions.

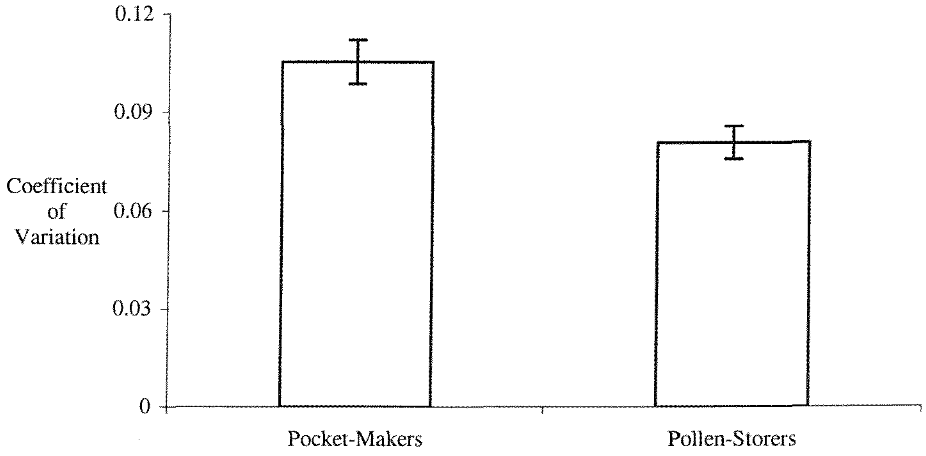

Figure 2.4 Size variation in Odontobombus (pocket-maker) and Anodontobombus (pollen-storer) species. On average, the Odontobombus (Mean= 0.105 ± 0.007) vary in size significantly more than Anodontobombus (Mean= 0.081 ± 0.005) ($F_{1,15}$=9.1; P<0.01).

2.5 Discussion

2.5.1 Summary

I confirm that considerable worker size variation exists in 23 *Bombus* species (table 2.1), that it is greater amongst the Odontobombus than the Anodontobombus (fig. 2.4) and that mean worker size differs between species (table 2.1; figs. 2.1 & 2.2). I also confirm that foraging workers of *Bombus terrestris* are larger, on average, than in-nest workers (fig. 2.3a), but that there is a large overlap in their size distributions (fig. 2.3b).

2.5.2 Size variation in the worker caste of Bombus species

Dramatic size variation can be found amongst the worker caste of at least 13 *Bombus* species from 6 sub genera in the UK and also from at least 10 *Bombus* species from beyond the UK (table 2.1). Not only does this variation occur within one species, but also within a single colony (Alford 1975; pers. obs.). Compared to bumblebees, honeybee workers are all virtually identical to one another in size (Waddington 1986). A proximate explanation for this difference probably lies in the evolved nest structure and method of larval food provision (a comparison of *Bombus* and *Apis* larval development can be found in Chapter 1). Within *Bombus* species exist two different methods of larval provision: Anodontobombus and Odontobombus. This difference appears to influence the extent to which a colony's workers will vary in size (see below). The ultimate reason for worker size variation proves harder to elucidate. Perhaps it is an inconsequential result of a disorganised nest regime and there is no selective pressure driving worker size up or down. Perhaps large and small workers are of equal stature in all duties, be they inside or outside the nest. This seems unlikely as there are likely to be size dependant differences in the ability of an individual to care for the brood, the amount of forage an individual can carry and the weather variables in which an individual can forage effectively. Also, where small workers will suffice, the production of a smaller number of large workers seems a waste of resources. Perhaps large workers, as individuals, are superior to small workers but there is a trade off between worker size and worker number, i.e.

investing valuable resources into a small number of individuals may lead to dramatic losses should a few workers perish. Having a greater number of workers, albeit of smaller average size, would counter such risks. Maybe an advantage is found in producing a workforce of varying sized individuals. Bumblebees forage in a wide range of weather conditions (Heinrich 1979): size variation may be linked to thermoregulation, bigger bees adapted for cold conditions, smaller to warm (see Chapters 3 & 4). Bumblebees pollinate a wide variety of flowers (Goulson 2003): maybe larger bees are better at foraging on large flowers and smaller bees better on smaller flowers (see Chapter 5). These arguments assume that natural selection is capable, in this case, of maximising efficiency. In reality this may not be possible for reasons such as environmental variation, a lack of genetic variation, phylogenetic effects and pleiotropy.

Mean worker size varies significantly between species (fig. 2.1). The reason for this is not known. Workers of Odontobombus species vary more in size than Anodontobombus species (see section 2.5.4) but there is no apparent difference in mean size between the two groups (fig. 2.1). This suggests that the differences in mean size are not determined by differences in brood care. The mean size of workers may be associated with the geographic range of the species, past or present. Evidence exists to suggest a latitudinal determinant of mean worker size within species (chapter 4) whereby workers of races from higher latitudes are generally larger than those nearer the equator. Also, different regions have different floras. Bumblebee species are known to differ in the flowers they exploit (Alford 1975) and flower choice is influenced by body size (chapter 6). Perhaps, different bumblebee species have adapted to the flower species of different regions. Bumblebee species now resident in the same region, but differing in mean size, may have originated from separate regions and have not adapted or needed to adapt any further to their present environment. A more likely explanation is that different *Bombus* species have evolved to occupy different ecological niches through competitive exclusion. Detailed knowledge of forage plant preferences of each bumblebee species would be needed to determine the validity of this hypothesis.

2.5.3 Size variation in foraging and nest-bound workers

As stated in the introduction to this chapter, alloethism has been demonstrated in many species of bumblebee (Coleville 1890; Sladen 1912; Meidell 1934; Richards 1946; Cumber 1949; Brian 1952;

Free 1955). This we can confirm for *Bombus terrestris* (figs. 2.3a & b). What is the reason for this alloethism? Division of labour occurs as a result of selection pressure to maximise reproductive efficiency, in the case of ant species through the harvesting of nutrients, defence of the nest and foraging workforce and rearing of the larvae (Wilson 1980). Broadly speaking, the same is likely to be true of the bumblebees save, perhaps, for the defence (all workers, regardless of size, are equipped with a sting; foraging bees are less prone to predation than ants because of their ability to fly). It seems likely that the explanation is to be found in either foraging duties and/or nest duties. Ant species harvest a broad range of items of varying sizes, such as seeds and dead insects, and the size of the worker is often correlated with the size of the forage item (Wilson 1980). Bumblebees, however, simply harvest nectar and pollen, both of which occur in units so minute that they are tiny to the smallest of bees. Perhaps the reason foragers are large is because, in some way, large size confers a foraging advantage (see Chapter 5). From a reverse perspective, perhaps the reason nest-bound workers are small is because smallness confers an advantage in larval rearing (see Chapter 7).

Although foraging workers are larger, on average, than in nest bees, size variation amongst foragers is still considerable (fig. 2.3b). There are two credible explanations for this. Different sized bees may cope differently in different weather conditions, large bees better in cold conditions and small bees better in hot (Heinrich 1975, 1979; chapters 3, 4 & 5). *i.e.* a varied workforce is equipped to forage in a broad temperature range. Also likely, is that different sized foragers are suited to different forage plants (Harder 1983; chapter 6). The flowers of different plant species differ hugely in their morphology. Bee size is positively correlated with tongue length (Morse 1978; Harder 1982; Peat *et al.* 2004; chapter 6) and this influences a bee's ability to handle different flowers (Harder 1983; Peat *et al.* 2004; chapter 6).

2.5.4 Size variation in Odontobombus and Anodontobombus species

My findings concur with the literature (Alford 1975). Among foraging workers, the Odontobombus (pocket-makers) do vary in size more than the Anodontobombus (pollen-storers) (fig. 2.4), although the variation is great in both when compared to social bee species other than bumblebees. Honeybees, for instance, have a coefficient of variation of only 0.018, a fraction of that exhibited by bumblebees (Roulston and Cane 2000). Many stingless bees display size variation, but not to so great an extent (Waddington 1986). Presumably this difference in variation is linked to the larval feeding strategies. Odontobombus larvae have more opportunity to compete for food than Anodontobombus larvae as the pocket-maker workers provide food for groups of larvae all through their development, whereas the Anodontobombus feed the larvae individually during the later stages of development (Alford 1975). It is perhaps surprising that we do not find a greater difference in variation.

Chapter 3:

Thermoregulation 1: thoracic cooling and morphological adaptations

In the cold, small bees struggle, alas,
But large foragers don't run out of gas,
For their cooling rate,
Isn't nearly as great,
'cos their surface is small for their mass.
- James Peat

3.1 Abstract

Amongst the worker caste, the social bumblebees (*Bombus* spp.) exhibit a dramatic intra-colony variation in size, a variation much greater than in any other social bee species and a phenomenon not adequately explained. Bumblebees also exhibit alloethism whereby larger workers tend to forage and smaller workers attend in-nest duties. However, even within the foraging workforce there is still remarkable size variation. Bumblebees are supposedly tundra adapted by virtue of their relatively large size (especially in the queens) and thick fur (setae). Minimising heat loss whilst foraging in cold conditions may have been a driving force behind these characteristics. Larger bees should cope better in cold conditions than smaller bees thanks to their relatively small surface area to mass ratio. Here I show that thoracic heat loss is more rapid in smaller bees especially when in simulated flight; that thoracic setae length correlates positively with body size; that *Bombus* species from cold climates have longer setae than those species that have settled in hot climates; and that larger bees have relatively reduced extremities. From these results we may conclude that larger workers are adapted to relatively cold conditions and smaller workers to hot conditions and that species adapted to tropical regions have evolved a shorter coat, presumably in response to over heating.

3.2 Introduction

3.2.1 Endothermy in insects

The Oxford Dictionary of Biology (Martin and Hine 2000) defines 'ectotherm' (or 'poikilotherm') as "An animal that maintains its body temperature by absorbing heat from the surrounding environment". It then states that "All animals except mammals and birds are ectotherms..." and that "...they are often described as being cold-blooded and are unable to regulate their body temperature metabolically." A far as insects are concerned, this final statement is not true. To fly, an insect's flight muscles must be of sufficient temperature. Bumblebee workers cannot beat their wings fast enough to stay aloft unless their thoracic temperature is in excess of around 30°C (Heinrich 1975). Such thoracic temperatures can be achieved by basking in solar radiation, but sunlight is not always present, so some insects have evolved a different method: shivering. Endothermic regulation of body temperature is known to occur in some beetles (Auerswald *et al.* 1998; Mena 2001), cicadas (Sanborn *et al.* 1995a; Sanborn *et al.* 1995b; Sanborn 2000; Sanborn 2001; Sanborn *et al.* 2003; Villet *et al.* 2003), dragonflies (Ishizawa 1998; May 1995), butterflies (Maier 1995 (1996)) and moths (Coombs 1993; Heinrich 1987), wasps (Ghazoul and Willmer 1994) and hornets (Schmolz *et al.* 1993), honeybees (Harrison *et al.* 1996; Heinrich 1996a; Stabentheiner *et al.* 2003), solitary bees (Stone 1993a; Stone 1993b; Stone 1994; Stone *et al.* 1995) and, of course, bumblebees (Heinrich 1975; Heinrich and Heinrich 1983).

3.2.2 Thermoregulation in bumblebees: the effects of size, insulation and body proportion

Bumblebee worker size-variation, if adaptive, must be closely related to the tasks that those workers carry out. Worker tasks can be broadly divided into foraging (for nectar and pollen on a variety of flowering plants) and brood caring (feeding larvae, regulating the nest temperature and defending the nest). This chapter does not concern itself with the latter, but deals with an important aspect of foraging, namely the effect of body size on the ability of an individual worker to thermoregulate in a range of weather conditions by measuring the intrinsic cooling rates of dead bees in different simulated weather conditions. The variation in outer insulation, or setae (hairs) both amongst different sized workers and between workers from relatively cool (England) and warm (Greece) climates is examined, as is the relationship of thorax size with abdomen size, head size and limb size amongst different sized *Bombus terrestris* workers. Mammals adapted to a cold climate, such as polar bears, conserve heat by having a large body and reduced extremities. We see if this can be applied to bumblebees based on the theoretical assumption that big bees are cold-adapted and small bees are warm-adapted. Variation in bodily proportions of different sized workers may also suggest a trade off between the thermoregulatory function of thorax size and the functionality of the limbs (grappling flowers, collecting pollen), abdomen (storing nectar) and head (visual ability as related to eye size (Spaethe and Chittka 2003)).

The thoracic temperature of a flying bumblebee varies between upper and lower limits. If the muscle is too cold the wing beat frequency becomes too slow to fly, if too hot the bee is liable to die of heat prostration. *Bombus vosnesenskii* workers cannot fly if their thoracic temperature drops below 30°C or exceeds 45°C (Heinrich 1975). Given that the thermodynamic properties of objects are largely dependant upon size, different sized workers must experience different thermoregulatory constraints. A large bee will have a relatively small surface area to volume ratio and should therefore lose heat from convective cooling relatively slowly, an assumption we test in this chapter. This should enable a large worker to maintain its thoracic temperature in cold ambient temperatures with relative ease. Conversely, a small worker will experience a reduced risk of over-heating in hot ambient temperatures.

Another important determinant of heat loss/retention is external insulation, *i.e.* fur (or setae). Insulation of some kind is a feature common to a majority of animal life. Mammals insulate themselves with layers of subcutaneous fat, fur, hair and, in one rare exception, clothes. Birds have evolved feathers. Various insects insulate themselves with scales, air sacs and/or setae (Heinrich 1996b). Compared to most other insects, bumblebees have a thick coat of hair all over the body, especially around the thorax. Undoubtedly, this coat helps prevent the loss of heat by insulating the thorax from the surrounding air. We might expect bees from a relatively hot climate to have shorter setae than bees from a relatively cold one. This shall be tested by examining differences within a species across a temperature range and by comparing several species from two temperature extremes (plates 3.3 and 3.4).

Allen's Rule (after Mayr 1942) states that "protruding body parts, such as tail, ears, bill, extremities, and so forth, are relatively shorter in the cooler parts of the range of a species than in the warmer parts." This, of course, traditionally applies to mammals and birds and rather than apply it to bees from different parts of their geographical range (see chapter 4) we shall apply it to bees from different parts of their size range. If big bees are adapted to cold weather and small bees to warm weather then we might expect to find that big bees have relatively reduced extremities and small bees to have relatively increased extremities. In this chapter we refer to the head, legs and abdomen collectively as the extremities as in most discussions the thorax is of central interest, and it is the site of heat production. We know that heat can be lost via the abdomen and that this loss is regulated by the petiole (this is the constricted region between the thorax and the abdomen. A bee is able to regulate the flow of heat using a counter current heat exchange as haemolymph passes simultaneously from thorax to abdomen and vice versa) (Heinrich 1979). Losing heat via the head occurs in honeybees (Cooper *et al.* 1985) and Yellow Jacket wasps (Coelho and Ross 1996) aided by the evaporative cooling effects of regurgitated stomach contents. Carpenter bees, common in the tropics, have large heads which facilitate radiative cooling whilst in flight (Heinrich and Buchmann 1986). It seems likely that heat can also be lost via the limbs whether by transfer of haemolymph to the muscles or simply convection through the limb from the thorax. Like the ears of desert and arctic dwelling mammals, the limbs of flying insects will lose heat differentially depending upon their size. Here we see whether or not thorax size has a linear relationship with abdomen size, head size and/or limb size.

The aim of this chapter is to examine various size dependent physical characteristics that may be adaptations to varying weather conditions giving different sized workers different climatic optimums for foraging, ultimately improving the transfer of genes to subsequent generations.

3.3 Methods

3.3.1 Size-dependent thoracic cooling rate

During February 2003, the cooling rates of sixty dead Greek *Bombus terrestris* workers of varied size, randomly selected from 12 commercial nests (bought from Koppert UK Ltd., UK, killed and preserved at –4°C for a period of two years) were measured under four different treatments: 'warm, hover', 'warm, fly', 'cold, hover' and 'cold, fly' conditions. To simulate forward flight, a fan was placed at a distance of 0.5 metres from the subject. Thirty bees were used for 'warm' trials at an average ambient temperature of 21.5°C. Thirty different bees were used for 'cold' trials at an average ambient temperature of 2.6°°C. Each bee was used twice, once with the fan switched on, once without. The order in which the fan was switched on was alternated between trials. In each trial, body size (thorax width) was measured using Vernier callipers. The abdomen was removed and a small thermocouple heat probe fastened to an unfolded paperclip was inserted into the thorax through the petiole (a separate thermocouple was used to measure the ambient temperature (see plate 3.2)). The bee was then placed in a boiling tube resting in a 60°C water bath (see plate 3.1). When the thoracic temperature reached 45°C, the bee was removed and held in the trial position, the paperclip secured in a crocodile clip (see plate 3.2). The time taken for the flight muscle to cool from 40°C (typical flight temperature) to 30°C (roughly the lower limit for hovering flight in workers (Heinrich 1975)) was recorded. For each bee, this information was converted into cooling rate (°C/second). The data were analysed using ANCOVA on StatView 5.0 with cooling rate as the dependent variable, both ambient temperature and wind as nominal independent variables and thorax width as a covariate. Also, conductance (°C/second/(°CT_t-°CT_a), where T_t=thoracic temperature and T_a=ambient temperature) was plotted against thorax radius and analysed using regression and ANCOVA on StatView 5.0.

3.3.2 Size-dependent thoracic setae length

During October 2002, 34 previously collected local English *B. terrestris* workers and 51 Greek *B. terrestris* workers (from Koppert UK Ltd. Ltd.) were measured to examine the relationship between body size (head width (mm)) and thoracic setae (hair) length (mm). Head widths were measured from the outside edge of the right eye to the outside edge of the left using a microscope with a graticule accurate to 0.1 mm. From each bee, thoracic setae were removed from the posterior dorsal region of the thorax using a scalpel. Five thoracic setae were then randomly selected and measured under the microscope. For each bee, the mean length of the five setae was calculated and that value used in the analyses. Data were analysed in StatView 5.0 using ANCOVA (with thoracic setae length (mm) as the dependent variable, sub-species as the independent variable and head width (mm) as a covariate) and ANCOVA (to analyse the means of head width and setae length in each sub-species).

3.3.3 The effect of climate on thoracic setae length

During March and April 2004, the thoracic setae length and thorax width of 286 worker bees from ten different *Bombus* species from four different subgenera were measured using Vernier callipers. Of the ten species selected, five were from cold climates and five from hot climates (Paul Williams, pers. com. 2004). Data were analysed using ANOVA and ANCOVA and the post hoc test Fisher's PLSD in Statview 5.0.

3.3.4 Size-dependent body proportions

During January 2003, the thorax mass, abdomen mass, head mass, and leg mass of 60 dead Greek *Bombus terrestris* were measured using an Oertling NB33 fine balance. To determine the relationship between bee size and relative size of abdomen, head and limbs, the ratio of each, for example abdomen mass/thorax mass, was calculated then related to bee size (thorax width, measured with Vernier callipers). Correlation analyses were carried out in StatView 5.0.

3.4 Results

Size dependent cooling rate and conductance

Cooling rate was negatively correlated with body size ($F_{1,112}$=87.57; p<0.0001; Fig. 3.1), with smaller bees cooling more quickly than larger bees. Cooling rate was also affected by both the ambient temperature ($F_{1,112}$=41.90; p<0.0001; Figs. 3.3 and 3.4), where bees cooled more quickly at colder temperatures, and the presence or absence of a head wind ($F_{1,112}$=13.55; p=0.0004; Fig. 3.2), where bees cooled more quickly with a head wind. Conductance was negatively correlated with body size ($F_{1,112}$=72.38; p<0.0001; fig. 3.3) and positively correlated with a head wind ($F_{1,112}$=9.26; p=0.0029; fig. 3.4) but independent of ambient temperature ($F_{1,112}$=1.87; p=0.174; Fig. 3.4).

Thoracic setae length in English and Greek *B. terrestris*

Thoracic setae length was positively correlated with head width ($F_{1,81}$=13.58; p=0.0004) and was predicted by sub-species ($F_{1,83}$=117.23; p<0.0001): for a given body size, English *B. terrestris* workers had significantly longer setae than the Greek sub-species (see Fig. 3.5). There was no difference in mean head width between the samples of two sub species ($F_{1,83}$=0.13; p>0.05).

The effect of climate on thoracic setae length

In the comparison of 5 cold adapted species with 5 warm adapted species, workers from cold climates have longer thoracic setae than those from hot climates ($F_{1,275}$=17.85; p<0.0001; see table 3.1; fig. 3.6) but did not differ significantly in body size ($F_{1,9}$=0.285; p>0.05; see Chapter 4; table 4.2 and fig. 4.2). This difference was more dramatic in some species than in others. It is worth noting that where subgenera cross climates, differences in mean setae length are often significant. *i.e.* within the subgenus *Fervidobombus,* the cold climate dwelling *B. dahlbomii* has significantly longer setae than the warm climate dwelling *B. atratus* (Fisher's PLSD: p<0.0001), *B. medius* (Fisher's PLSD: p<0.0001), *B. mexicanus* (Fisher's PLSD: p<0.0001) and *B. transversalis* (Fisher's PLSD: p<0.0001) and within the subgenus *Pyrobombus,* the hot climate dwelling *B. huntii* has significantly shorter hair than *B. lapponicus* (Fisher's PLSD: p<0.0001). Given that setae length is often correlated positively with body size (fig. 3.5), it is worth noting that when, across climates, species samples had equal mean body size cold climate species always had significantly longer setae than warm climate species (table 3.2). Also, when, across climates, species samples had equal mean setae lengths, cold climate species always had significantly smaller mean body size (table 3.3).

Size dependent body proportions

Abdomen mass ($F_{1,59}$=16.296; p<0.0001), head mass ($F_{1,59}$=21.641; p<0.0001) and leg mass ($F_{1,59}$=12.039; p<0.0001) were all positively correlated with thorax mass (see fig. 3.7). The abdomen:thorax mass ratio ($F_{1,49}$=6.091; p<0.05; Fig. 3.8) was negatively correlated with body size (thorax width) as were the head:thorax mass ratio ($F_{1,59}$=29.132; p<0.0001; Fig. 3.9) and the leg:thorax mass ratio ($F_{1,59}$=9.257; p<0.01; Fig. 3.10). These linear relationships show that, compared to their thorax size, bigger workers have relatively small abdomens, heads and legs.

Table 3.1 Summary of relative thoracic setae length in bumblebee species from hot and cold climates.

Climate	Subgenus	Species	N	Mean Setae length (mm)	Mean Thorax width (mm)
Cold	*Alpinobombus*	*alpinus*	32	0.86 ± 0.026	5.28 ± 0.09
Cold	*Alpinobombus*	*balteatus*	30	0.75 ± 0.027	4.59 ± 0.093
Cold	*Fervidobombus*	*dahlbomii*	30	1.33 ± 0.042	6.02 ± 0.125
Cold	*Pyrobombus*	*jonellus*	30	0.73 ± 0.024	4.18 ± 0.059
Cold	*Pyrobombus*	*lapponicus*	30	1.07 ± 0.028	5.31 ± 0.183
Hot	*Fervidobombus*	*atratus*	30	0.74 ± 0.018	5.23 ± 0.073
Hot	*Pyrobombus*	*huntii*	23	0.66 ± 0.029	5.13 ± 0.25
Hot	*Fervidobombus*	*medius*	23	0.73 ± 0.025	5.03 ± 0.146
Hot	*Fervidobombus*	*mexicanus*	21	0.43 ± 0.018	4.84 ± 0.082
Hot	*Fervidobombus*	*transversalis*	30	0.53 ± 0.015	6.21 ± 0.087

Table 3.2 Comparison of mean thoracic setae length between species of matched mean body size across climates. For equal mean body size species samples across climates, cold climate species all have longer mean thoracic setae (from Fisher's PLSD Post Hoc tests).

Cold climate spp.	Warm climate spp.	Mean difference (mm)	p
alpinus	*atratus*	0.115	0.0016
alpinus	*huntii*	0.199	<0.0001
alpinus	*medius*	0.127	0.0012
balteatus	*mexicanus*	0.317	<0.0001
dahlbomii	*transversalis*	0.802	<0.0001
lapponicus	*atratus*	0.325	<0.0001
lapponicus	*huntii*	0.409	<0.0001
lapponicus	*medius*	0.337	<0.0001

Table 3.3 Comparison of mean body size between species of matched mean setae length across climates. For equal mean thoracic setae length species samples across climates, cold climate species all have smaller mean body size (from Fisher's PLSD Post Hoc tests).

Cold climate spp.	Warm climate spp.	Mean difference (mm)	p
balteatus	*atratus*	-0.643	0.0002
balteatus	*medius*	-0.439	0.0172
jonellus	*atratus*	-1.048	<0.0001
jonellus	*huntii*	-0.947	<0.0001
jonellus	*medius*	-0.845	<0.0001

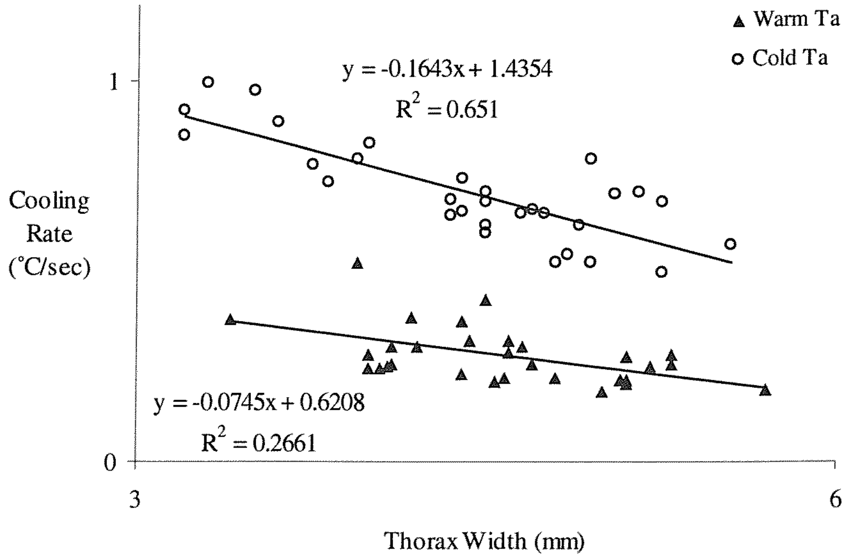

Figure 3.1 Size and temperature dependent thoracic cooling rate in *Bombus terrestris* workers. The rate of thoracic cooling (°C/second) is negatively related to body size (thorax width (mm)) at both warm and cold ambient temperatures (Ta) in still air ($F_{1,112}$=87.57; p<0.0001).

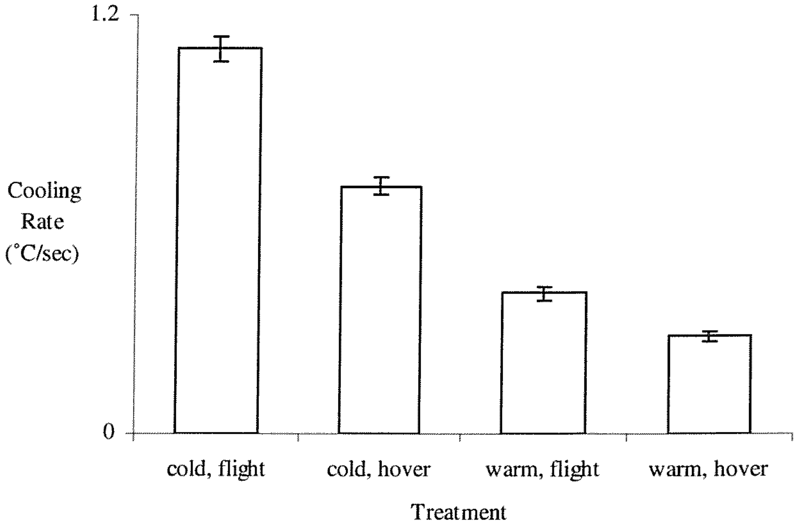

Figure 3.2 Mean cooling rate of *Bombus terrestris* workers in four different treatments: variations on warm and cold ambient temperature and presence and absence of a head wind. There are significant effects of ambient temperature ($F_{1,112}$=41.90; p<0.0001) and head wind ($F_{1,112}$=13.55; p=0.0004).

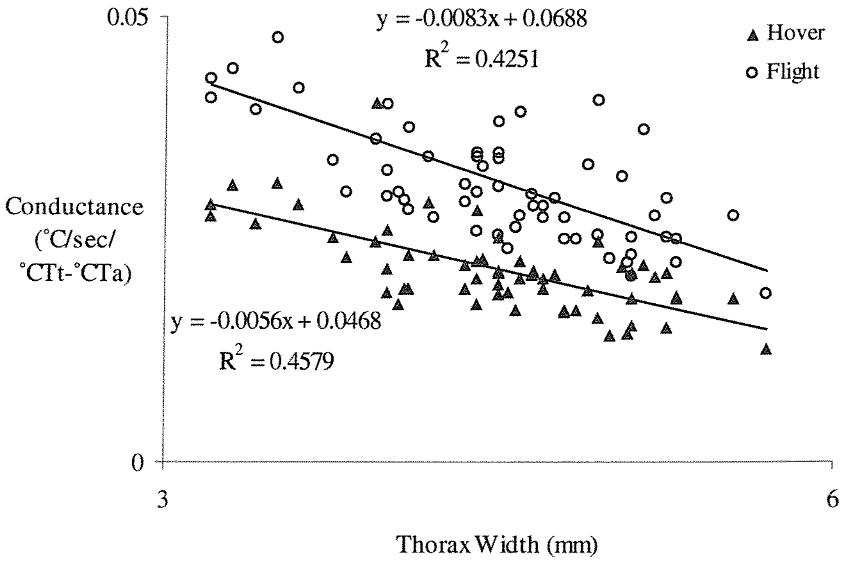

Figure 3.3 Size and wind related conductance in *Bombus terrestris* workers. Conductance (°C/second/(°CTt-°CTa)) was negatively correlated with body size ($F_{1,112}$=72.38; p<0.0001) with and without a head wind.

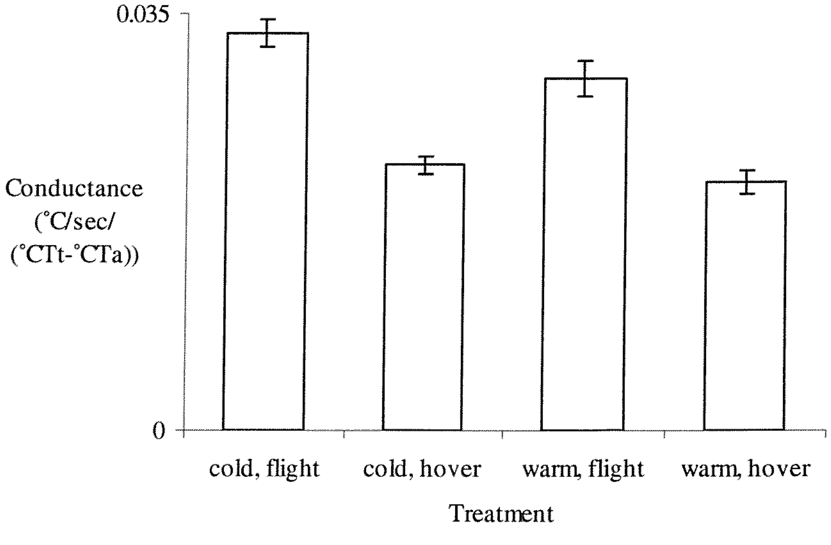

Figure 3.4 The mean conductance of *Bombus terrestris* in four different treatments. It is dependent on head wind ($F_{1,112}$=9.26; p=0.0029) but independent of ambient temperature ($F_{1,112}$=1.87; p=0.174).

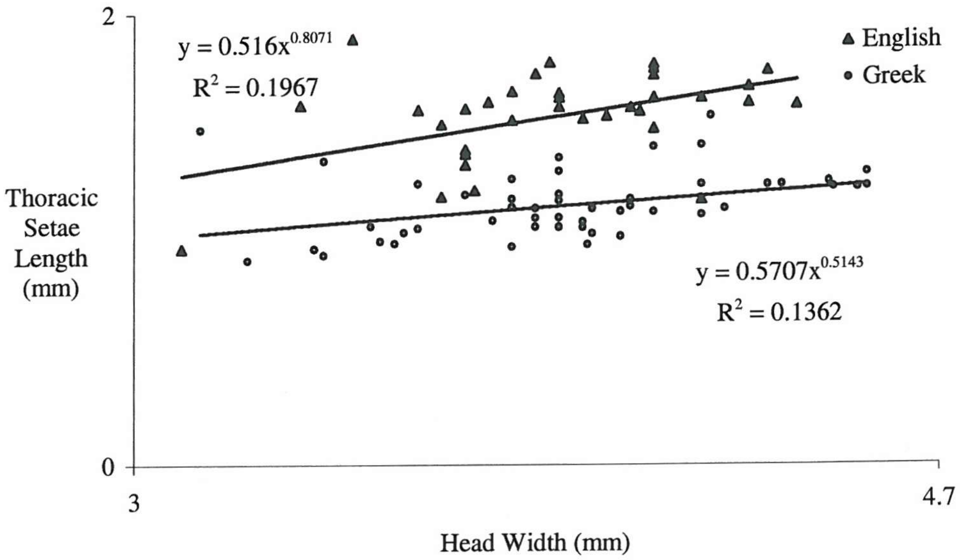

Figure 3.5 Size related thoracic setae length in two sub-species of *Bombus terrestris* (English and Greek). In both sub-species there is a significant positive correlation between body size and setae length ($F_{1,84}$=12.4; p=0.001).

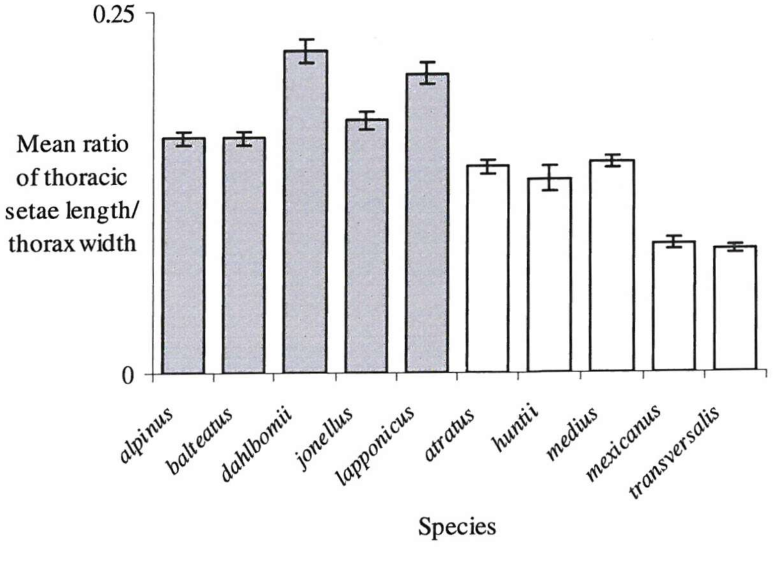

Figure 3.6 Mean thoracic setae lengths in 10 different *Bombus* species from two climatic extremes. Accounting for body size, on average, species from cold climates (in grey) had longer setae than species from hot climates (in white) ($F_{1,275}$=17.85; p<0.0001).

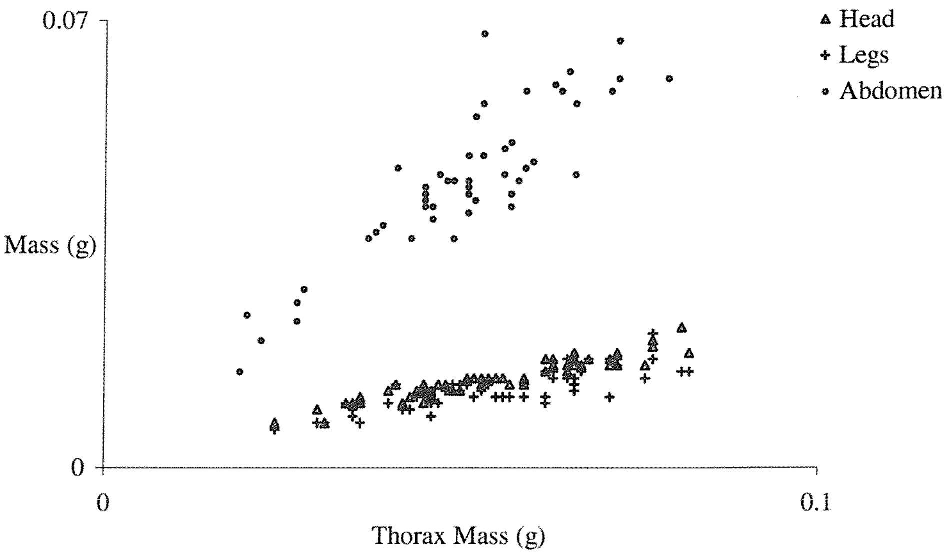

Figure 3.7 Size related head, leg and abdomen mass in *Bombus terrestris*. Thorax mass positively predicts head mass ($T_{1,59}$=21.641; $p<0.0001$), total leg mass ($T_{1,59}$=12.039; $p<0.0001$) and abdomen mass ($T_{1,59}$=16.296; $p<0.0001$).

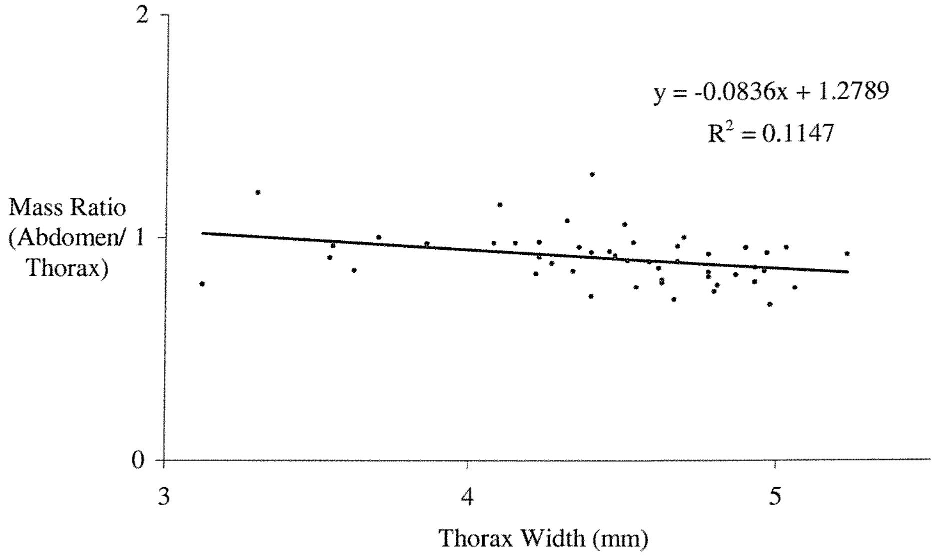

Figure 3.8 The relationship between thorax width and the abdomen:thorax mass ratio in *Bombus terrestris* workers. Relative to the thorax, bigger workers have significantly smaller abdomens ($F_{1,49}$=6.091; $p<0.05$).

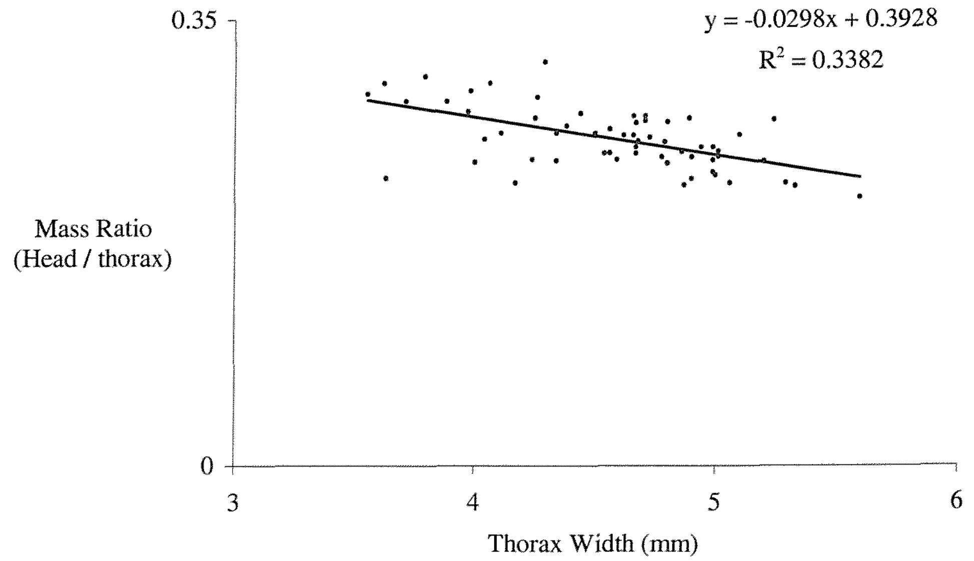

Figure 3.9 The relationship between thorax width and the head:thorax mass ratio in *Bombus terrestris* workers. Relative to the thorax, larger bees have significantly smaller heads ($F_{1,59}$=29.132; p<0.0001).

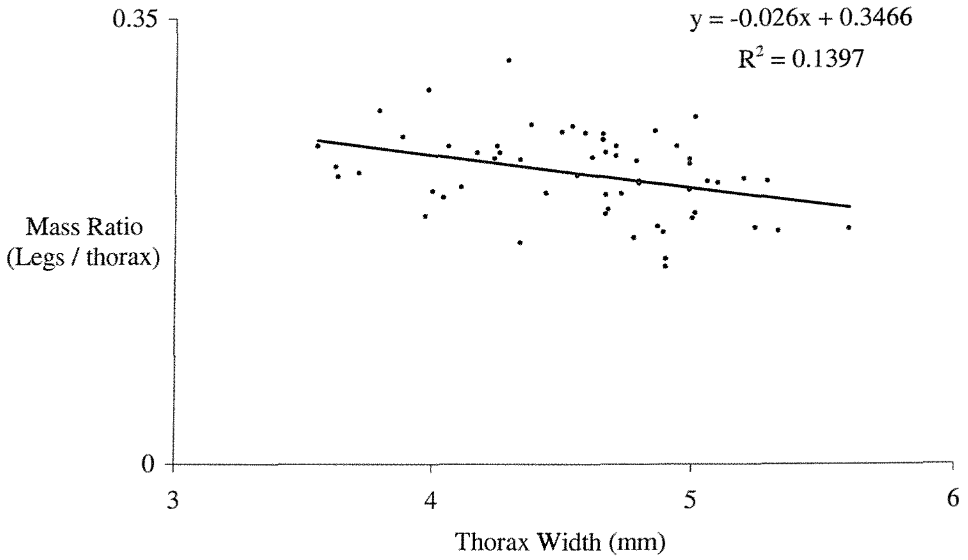

Figure 3.10 The relationship between thorax width and the leg:thorax mass ration in *Bombus terrestris* workers. Relative to the thorax, larger bees have significantly smaller legs ($F_{1,59}$=9.257; p<0.01).

3.5 Discussion

3.5.1 Summary

Here I show that thoracic heat loss is more rapid in smaller bees especially when in simulated flight (figs. 3.1, 3.2, 3.3 & 3.4); that thoracic setae length correlates positively with body size (fig 3.5); that *Bombus* species from cold climates have longer setae than those species that have settled in hot climates (tables 3.1, 3.2 & 3.3; figs. 3.5 & 3.6); and that larger bees have relatively reduced extremities (figs 3.7, 3.8, 3.9 & 3.10).

3.5.2 Size dependent thoracic cooling rate

Heinrich (1975) showed that the ability of small *Bombus vosnesenskii* (a North American species) workers to thermoregulate in continuous flight is restricted to higher ambient temperatures. We have shown that the intrinsic rate of thoracic cooling in *Bombus terrestris* is dependant upon body size, large bees losing heat less quickly than small bees (fig. 3.1), and that that cooling rate is dependant upon the ambient temperature and whether the bee is in 'flight' or 'hovering', the rate being quickest if 'flying' in cold conditions. These results do not come as a surprise. It is well established that larger objects have greater volume to surface area ratios and that such objects are more resistant to heat exchange. It also stands to reason that an object cools more quickly in a colder ambient temperature and that when that object experiences air flow the insulating layer of warm air immediately surrounding that object is carried away, allowing yet more rapid cooling. Trivial though these findings may seem, they do have clear implications for the foraging ability of different individuals.

3.5.3 Size and climate dependent thoracic setae length

We have shown that larger *B. terrestris* workers have longer thoracic setae than small workers (see fig. 3.5). Whilst we cannot claim this as conclusive evidence for large workers being adapted to forage in colder temperatures, long thoracic setae will certainly give them greater insulation than the shorter setae of smaller workers would. We have also shown that workers from a colder climate (England) have, on average, longer thoracic setae than workers from a warmer climate (Greece) (see fig. 3.5), strong evidence of climatic adaptation. Given that globally, bumblebee species have a large latitudinal and altitudinal range (after Williams 1994; see introduction, plate 1.5), it is not surprising to find that the relative setae length (that is, thoracic setae length relative to body size) of bumblebees differs according to the climate in which they are found.

We have shown that, when taking body size into account, workers of species found in cold climates have significantly longer thoracic setae than workers of species found in hot climates (fig. 3.6). Given that this is the case in ten compared species (five from cold and five from hot climates) and that it is also across two subgenera, it is likely that thoracic setae length in bumblebees has evolved in response to ambient temperature. However, I must acknowledge that, amongst my 'cold' samples, four of the five species studied were of the same genus. They may all have long setae because their common ancestor had long setae and not as a recent adaptation to the climate they inhabit.

Parasitic *Bombus* species (subgenus *Psithyrus*) are comparatively lacking in thoracic setae, presumably because they emerge late in the season (Alford 1975) and don't face the same kind of foraging pressures as their needs are taken care of by the *Bombus* workers of the nests they parasitise. Another aspect of insulation is setae density, something I have not investigated. In the mammal world, animals in cold climates generally have finer, more densely packed fur. Further research is required to see if bumblebees follow this trend.

3.5.4 Size dependent body proportions

The size of the abdomen, head and legs were all positively correlated with the size of the thorax (see fig. 3.7). However, these relationships were not directly proportional and in all three cases larger bees, relative to their thorax size, had smaller heads, legs and abdomens (see figs. 3.8, 3.9, & 3.10). i.*e.* larger bees have reduced extremities. This may indicate an adaptation to temperature, allowing larger workers to conserve heat in cold weather and smaller workers to lose heat in hot weather more effectively by further reducing/increasing their surface area to volume ratio. However, this may also reflect a trade off between various size related functions of the thorax, head, legs and abdomen. i.e. large bees may require disproportionately large flight muscles to be able to fly.

From a small worker's perspective, there are potential advantages to having a large head, legs and abdomen. Firstly, larger extremities will facilitate cooling in high ambient temperatures more than small extremities by increasing the body's overall surface area relative to volume. A larger head can also accommodate larger eyes (pers. obs.). Spaethe and Chittka (2003) have shown that in *Bombus terrestris,* larger eyes are superior at resolving objects allowing efficient sighting of floral resources. Secondly, the amount of pollen a bee can carry is probably dependent on the length of the corbiculum. Although there are no data on the relationship between worker size and pollen load size, thorax width in *Bombus terrestris* workers is positively related to corbicula hair length (Goulson *et al.* 2002) so it is possible that small workers can carry a greater amount of pollen than large workers, relative to their mass. Also, longer legs may enhance a bee's ability to handle flowers simply due to increased reach. Finally, a larger abdomen can contain more nectar than a smaller one (pers. obs.; chapter 5), therefore maybe small workers can carry more nectar, for their size, than large workers which may be useful whilst foraging or when performing nest duties.

From a large worker's perspective, there are also potential advantages to having reduced extremities. Small extremities should minimise heat loss in low ambient temperatures by reducing the overall surface area of the body. Making smaller extremities reduces the nutritional input required of the queen or sisters, so more workers, males and new queens can be produced. It is possible that these various conflicting factors have driven workers to evolve the proportions we find in them today.

3.5.5 Conclusions

Regarding the evidence in this chapter, it seems likely that the bumblebee workforce of *Bombus terrestris* and, by inference, all other social bumblebees, are adapted for foraging in variable temperatures. Larger workers appear to be adapted for conserving thoracic heat as they have longer setae, reduced extremities and cool relatively slowly. Smaller workers appear to be adapted for losing heat as they have shorter setae, enlarged extremities and cool relatively quickly. Also, both within and between species, evidence shows that bumblebee workers from colder climates tend to have longer setae, presumably a trait evolved in response to mean ambient temperatures. Amongst other things, the research dealt with in chapter 5 searches for a relationship between worker size and ambient temperature during foraging, a field test of this chapter's inferences.

Chapter 4:

Thermoregulation 2:
climate dependent mean worker size

If small bees are good in a sweat,
Then worker size, I'm happy to bet,
Will correlate quite tightly
With latitude, despite the
Differential seasonal onset.

- James Peat

4.1 Abstract

Amongst the worker caste, the social bumblebees (*Bombus* spp.) exhibit a dramatic intra-colony variation in size. From the previous chapter, it seems likely that in bumblebees, large size is an adaptation to foraging in cool temperatures. Globally, temperature is correlated negatively with both latitude and altitude. If large bumblebee workers are adapted to cold temperatures and small adapted to hot, then we would expect their size to correlate positively with latitude and altitude. To test this, I compare relatively northern and southern races of five common European bumblebee species. Also, I compare the workers of five cold dwelling species with those of five hot dwelling species. Within four common European species, northern workers have a larger average size than southern workers. *B. lucorum* is a curious exception to this: southern English workers are larger, on average, than those from Scotland. When comparing the workers of species from the two extremes of temperature, we find there to be no significant difference in mean worker size. Results indicate that tropical bumblebees are larger, on average, than those from cold climates. Possible explanations are discussed.

4.2 Introduction

Bumblebee (*Bombus* spp.) workers exhibit considerable variation in size within colonies (Alford 1975). As well as varying in size within a colony, workers mean size also varies between species (chapter 2). A possible adaptive explanation for this is that species whose workers are, on average, larger are suited to foraging in relatively cold climates. A negative correlation exists between worker size and cooling rate (chapter 3). Consequently, small foragers will have to work harder to keep warm and are unable to fly if the temperature is too low (Heinrich 1975). Ambient temperature varies locally and on a global scale. Mean temperatures decrease with increasing latitude and altitude. It seems likely that mean bumblebee worker size will decrease with increasing mean temperature and, therefore, correlate positively with latitude and altitude, in accordance with Bergmann's Rule: "The smaller-sized geographic races of a species are found in the hotter parts of the range, the larger-sized races in the cooler districts." as stated by Mayr (Mayr 1942) and applied to 'poikilotherms' by Ray (1960).

To test this hypothesis, I compared the mean sizes of foraging workers from relatively northern and southern races of five European *Bombus* species, *B. lapidarius, B. lucorum, B. muscorum, B. pascuorum* and *B. terrestris*. Theory predicts the workers of the northern (cold adapted) races to be, on average, larger.

Although bumblebees are said to be tundra adapted (Heinrich 1979), their global distribution stretches to the tropics (plate 1.5). It is clear that both hot and cold adapted species have evolved differential thoracic setae lengths as adaptations to their respective climates (chapter 3; plates 3.3 and 3.4). As a further adaptation conferring heat loss, we might expect tropical bumblebee workers to be, on average, relatively small.

4.3 Methods

In the UK the mean annual temperature ranges from 46 °F (8 °C) in the Hebrides to 52 °F (11 °C) in southwestern England. (www.worldsurface.com 2004). UK bumblebee foragers were caught in Southern England from May 22nd to August 27th and in Scotland from June 15th to August 10th 2003. To calculate the effect of climate on mean size, data from Scottish *B. lapidarius, B. lucorum, B. muscorum* and *B. pascuorum* were combined with English *B. terrestris* into a "Cooler" category (there are no *B. terrestris* in Scotland) and compared (using a 2-way anova fitted in Statview 5.0) with English *B. lapidarius, B. lucorum* (species status confirmed by molecular analysis, pers. com. Mairi Knight, 2003), *B. muscorum* and *B. pascuorum* and Greek *B. terrestris* in a "Hotter" category.

Samples of species from hot and cold regions of the planet, which I shall refer to as 'global', were measured in the Department of Entomology, Natural History Museum, London. Advice on which species to select was given by Paul Williams (pers. com. 2004). Cold-dwelling species include *B. alpinus, B. balteatus, B. dahlbomii* (although this species was sampled in Peru, it was at high altitude in the Andes mountains), *B. jonellus* (although *B. jonellus* is found in the UK, these individuals were sampled from Scandinavia) and *B. lapponicus.* Hot-dwelling species include *B. atratus, B. huntii, B. medius, B. mexicanus* and *B. transversalis.* It is assumed that these bees were sampled without size bias aside from the fact that they are all likely to have been foraging workers. Mean worker sizes were calculated for each species. These 10 samples (5 cold and 5 hot) were then compared with a 1-way ANOVA calculated by hand.

Coefficients of variation (a) were calculated for each species (taking place of capture into account where appropriate) using the equation $a = b / c$ where c = the mean thorax width and b = the standard deviation of that mean.

4.4 Results

On average, bumblebee foragers found in cooler climates are bigger than their conspecifics in hotter regions ($F_{4,\ 1908}$=29.38; p<0.0001), with the exception of *B. lucorum* foragers, which were found to be larger in the hotter region (table 4.1; fig. 4.1). Amongst the 'global' samples, there was no overall difference in mean size of forager between the two climatic extremes ($F_{1,\ 9}$=0.285; p>0.05 (table 4.2; fig. 4.2)).

Table 4.1 Latitude related mean body size in foraging workers of five *Bombus* species. With the exception of *B. lucorum*, body size is negatively correlated with latitude.

Species	Location	N	Mean Thorax Width (mm ± S.E.)	Range (mm)	Coefficient of Variation (a)	Mean date of capture
B. lapidarius	Scotland	105	4.46 ± 0.041	3.1 … 5.9	0.094	July 30th
	England	280	4.35 ± 0.017	3.5 … 5.1	0.064	June 13th
B. lucorum	Scotland	193	4.68 ± 0.025	3.8 … 5.8	0.076	July 2nd
	England	134	4.87 ± 0.027	3.9 … 5.6	0.065	June 14th
B. muscorum	Scotland	451	4.94 ± 0.022	3.5 … 6.5	0.094	August 4th
	England	56	4.35 ± 0.074	3.1 … 6.5	0.127	August 4th
B. pascuorum	Scotland	193	4.50 ± 0.031	3.2 … 6.2	0.095	July 4th
	England	12	4.17 ± 0.047	3.9 … 4.4	0.039	July 20th
B. terrestris	England	278	4.97 ± 0.025	3.6 … 6.1	0.079	June 26th
	Greece	216	4.87 ± 0.028	3.6 … 6.3	0.084	-

Table 4.2 Summary of mean thorax width in foraging workers of bumblebee species from cold and hot climates.

Climate	Subgenus	Species	N	Mean Thorax width (mm)	Range (mm)	Coefficient of Variation (a)
Cold	*Alpinobombus*	*B. alpinus*	32	5.28 ± 0.09	4.3 … 6.5	0.095
Cold	*Alpinobombus*	*B. balteatus*	30	4.59 ± 0.093	3.7 … 5.8	0.112
Cold	*Fervidobombus*	*B. dahlbomii*	30	6.02 ± 0.125	4.9 … 7.5	0.114
Cold	*Pyrobombus*	*B. jonellus*	30	4.18 ± 0.059	3.5 … 4.9	0.077
Cold	*Pyrobombus*	*B. lapponicus*	30	5.31 ± 0.183	3.7 … 6.8	0.189
Hot	*Fervidobombus*	*B. atratus*	30	5.23 ± 0.073	3.6 … 5.8	0.077
Hot	*Pyrobombus*	*B. huntii*	23	5.13 ± 0.25	3.9 … 7.6	0.234
Hot	*Fervidobombus*	*B. medius*	23	5.03 ± 0.146	3.1 … 7.0	0.139
Hot	*Fervidobombus*	*B. mexicanus*	21	4.84 ± 0.082	4.0 … 5.5	0.077
Hot	*Fervidobombus*	*B. transversalis*	30	6.21 ± 0.087	5.3 … 7.4	0.077

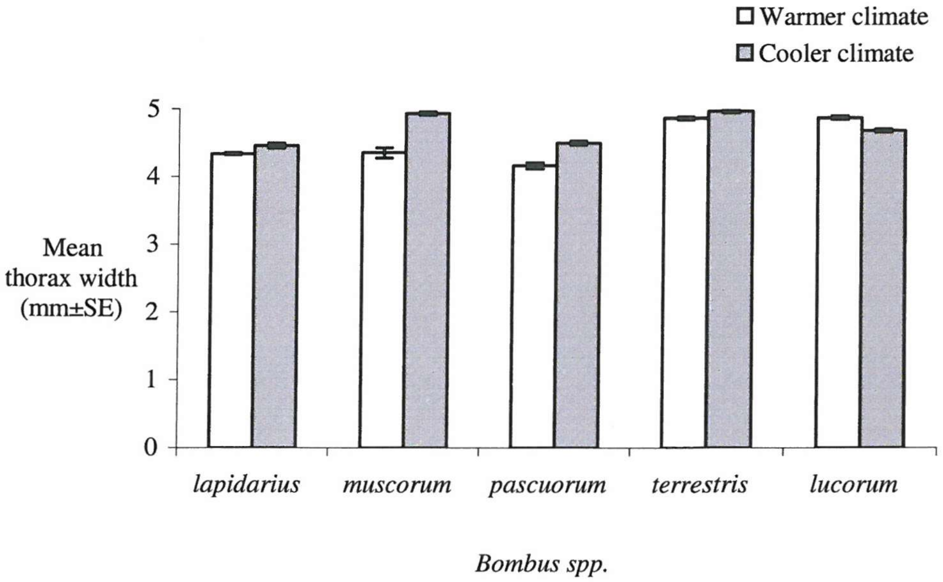

Figure 4.1 Latitude related mean forager size in five *Bombus* species. On average, foragers found in cooler climates are bigger than their conspecifics in hotter climes ($F_{4,1908}$=29.38; p<0.0001), with the exception of *B. lucorum* foragers, which were found to be larger in the hotter region.

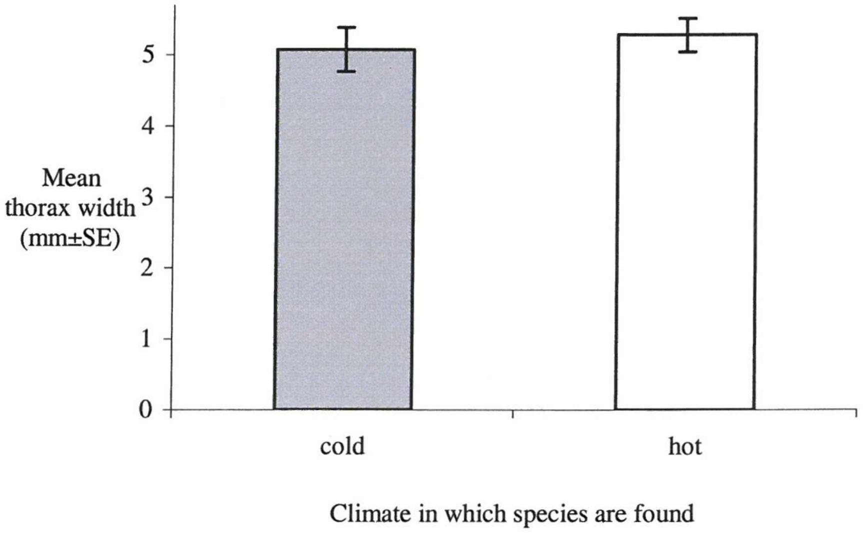

Figure 4.2 The mean thorax widths of workers of species from cold and species from hot climates. There was no significant difference between these means ($F_{1,9}=0.285$; $p>0.05$).

4.5 Discussion

4.5.1 Summary

Bombus lucorum aside, amongst the sampled European bumblebee species, we find that within species, the foraging workers of northern races are significantly larger in size than southern races (table 4.1; fig. 4.1). Amongst species sampled from hot and cold regions of the planet, we find no significant difference in mean size of foraging worker (table 4.2; fig. 4.2).

4.5.2 Latitude dependent intraspecific size variation in European samples

Despite the fact that the largest terrestrial invertebrates are found in the tropics, Ray (1960) found that 75% of 36 invertebrate study species obeyed Bergmann's Rule. *i.e.* in each species, individuals found/bred in cooler areas/conditions were generally larger than those found/bred in hotter areas/conditions. With the curious exception of *B. lucorum*, there is a clear trend of size increase with increasing latitude, as shown from the comparison of English and Scottish races and English and Greek *B. terrestris*. This complies with Bergmann's rule (Mayr 1942) and may be explained by a greater need to conserve heat in colder climates: a larger bumblebee has a smaller surface area to volume ratio so loses heat by convective radiation less rapidly than a smaller bumblebee. This means a larger bumblebee can save energy by reducing the frequency of heat-up by shivering (Heinrich 1979). From another perspective, a bumblebee living in a hot climate is more prone to over-heating and therefore cannot afford such great body size (or such long fur - see chapter 3). Although there are significant differences in mean size depending on latitude, the size variation overlap is considerable. This is not surprising as there is also a considerable climatic overlap in the two regions.

Why does *B. lucorum* show the opposite effect? At higher latitudes, summer is both delayed and reduced in length. During a season, as the colony grows in size it is able to harvest more resources and increase the food provision for developing larvae. Given that races at higher latitudes are likely to start their season later and have less time in which to develop a large workforce, this might conceal evidence of adaptation to a colder climate. This may be one explanation for the anomalous trend found in *B. lucorum* workers. However, despite the effect of differential seasonal onset and length, all four other species showed the expected trend. Another explanation may lie in the difficulty in distinguishing *B. lucorum* and *B. terrestris* workers. If *B. terrestris* workers are generally larger than *B. lucorum* workers and a number of them were accidentally included in the Southern sample, this may have distorted the results.

4.5.3 Samples from hot and cold regions of the planet

Looking further afield, beyond species level, we find no effect of latitude on the mean body size of *Bombus* workers. Indeed, the workers of tropical species are at least as big as Palearctic species. Large size in tropical invertebrates is a common phenomenon and is probably due to the lack of seasonality ensuring a constantly productive ecosystem. With a relatively unlimited time available for larval growth and plentiful resources on which to feed, such tropical creatures are able to grow larger. It is remarkable that such large bees can cope with the high temperatures of the tropics but it is clear that one way to avoid overheating is to reduce insulation by having short setae (see chapter 3).

4.5.4 Conclusions

This chapter provides at least some evidence that mean bumblebee forager size may be dependent on latitude. We cannot be certain, however, whether the different mean sizes found at different latitudes is genetically determined or a response to the environment. One way to test this would be to capture northern queens and rear them in the south and vice versa. This was done in part for one species for, although the Greek *B. terrestris* queens were caught in Greece, the workers were reared in captivity here in the UK. Therefore, despite not being influenced by the Greek climate, they still had a smaller average size than native *B. terrestris*, a fact that adds weight to the hypothesis.

However, we cannot even be sure that the differences are related to mean ambient temperature. The available forage plants in any particular region may influence the size distribution of bumblebee workers. We find that the average size of a forager on a plant is dependent upon that plants species (see chapter 6). Plant species-dependent sampling bias may have played a part here, but without knowing exactly which plants the workers were sampled from, we cannot possibly factor that in (many samples were collected by colleagues and identity of plant species was not always recorded). Effects of competition may also feature. For example, if nest density differed between regions resulting in differential levels of competition then pollen availability may be region dependent. If pollen availability is limited it may result in the production of a workforce of smaller average size.

Chapter 5:

Nectar foraging rate: the effects of body size and microclimate

The thing that I wanted to test,
Was if larger bees foraged the best,
Each one had a marker,
And when it got darker,
The workers returned to the nest.
- James Peat

5.1 Abstract

Workers of social bumblebees vary dramatically in size within colonies. They also exhibit alloethism, a division of labour where foraging workers are, on average, bigger than nest bound bees. Therefore, we expect larger workers to be superior foragers. It has been shown that larger bumblebees, as well as being the primary foragers, are adapted to colder weather than smaller bees. Indeed, evidence suggests that a bumblebee workforce is adapted to forage in a large range of temperatures. This being the case we might expect to find that the behaviour of different sized workers will vary according to the local climatic conditions. Maybe larger workers avoid hot conditions and smaller workers avoid cold conditions. Perhaps hot and cold weather negatively affects the foraging ability of larger and smaller workers respectively. To test these hypotheses, we labelled *B. terrestris* workers with individually numbered marker discs and allowed them to forage freely in Southampton. Using three nests over a total period of three months we show that nectar foraging efficiency is positively correlated with worker body size. Larger workers took the same length of time to make a foraging trip but collected significantly more nectar. However, contrary to expectation, we find no relationship between foraging behaviour and microclimate in different sized workers. Although, overall, foraging took place less in more humid conditions, both foraging frequency and foraging efficiency were independent of temperature. In separate observations, variable size workers visiting bramble flowers in variable weather conditions were recorded. Again, forager size was independent of ambient temperature. It is possible that the ambient temperature in southern England rarely reaches the extremes necessary to exclude individuals from foraging at either end of the size scale. The implications of these findings for worker size variation are discussed.

5.2 Introduction

5.2.1 Worker size variation

Alloethism, a division of labour dependant upon differing sizes of individuals, can be found among the hymenopterans, especially in many ant species (Wilson 1980), and in termites (Crosland *et al.* 1998). Social bumblebees also display a degree of alloethism within the worker caste (Alford 1975). Size varies both within and between castes (queens are distinctly larger than other castes in the Anodontobombus although there is considerable overlap in Odontobombus species (Plowright and Jay 1968)) and each caste performs a distinct function. Bumblebees can be categorised into three main castes (queens, drones and workers) and, within the worker caste, we find tasks vary between in nest (larval feeding and incubation, maintenance of nest ambient temperature and nest structure) and extra nest (foraging for pollen and nectar) tasks (Alford 1975). Within the worker caste, a great deal of size variation exists in many bumblebee species (as much as a ten fold difference in mass (Alford 1975)). Discounting many ant species, this phenomenon is rare in other insects and much less extreme in other social bees (Roulston and Cane 2000; Waddington *et al.* 1986). The proximate causes of this size variation may be explained by the differential food provision during the larval stage (Ribeiro 1994). A well-nourished larva matures into a larger adult than one that is poorly fed (once adulthood is reached, further growth ceases as it is limited by the exoskeleton). The ultimate causes are harder to establish. Assuming body mass reflects the cost of rearing, larger workers may be up to ten times as expensive to produce. The sustained production of large workers at such a cost suggests that their existence is adaptive. The success of the nest critically relies upon the harvesting of nectar and pollen with which to feed the larvae of workers and sexuals. If larger workers are specialized foragers, it is reasonable to assume that foraging ability is positively related to size. Since this experiment was initiated work has been published showing that large *Bombus terrestris* workers gathered nectar at a greater rate to smaller workers (Spaethe and Weidenmuller 2002).

5.2.2 Alloethism

In *Bombus* species, it has been found that the larger workers most commonly forage for nectar and pollen, leaving the smaller workers in the nest (Colville 1890; Cumber 1949; Free 1955; Goulson *et al.* 2002). Only in times of resource shortage or reduction in the number of the normal foraging workforce do the smallest workers emerge to forage (Brian 1952, Pendrel and Plowright 1981). As larger workers are specialized foragers, we might expect them to be superior to the smaller foragers in terms of foraging efficiency. From an alternative perspective, rather than thinking of foragers as being large, perhaps nest bees are small because that allows them to be superior brood carers. Cnaani and Hefetz (1994) showed that manipulated nests with artificial food provision containing a number of larger than average workers produced a greater number of emerging workers than manipulated nests containing the same number of smaller workers. This indicates that, as individuals, larger workers are superior brood carers but does not show whether larger workers are superior in terms of the mass invested in them as larvae, i.*e.* perhaps the combined efforts of two small workers, half the mass of one large worker, are more effective. Also, it leaves the question of foraging ability unanswered.

5.2.3 Bergmann's Rule and size related thermoregulation

Objects with a higher ratio of volume to surface area (*i.e.* bigger) retain heat more than those with a lower ratio (*i.e.* smaller). This has been applied as partial explanation of the differences in the size and shape that we see between diurnal equatorial animals (needing to lose heat) and comparable arctic animals (needing to retain heat). Putting behavioural and physiological adaptations aside, we often find that the volume to surface area ratio is greater in cold climate animals than in hot climate animals (Ray 1960). There is no reason why these principles should not be applied to invertebrate species, as they require high thoracic temperatures to fly. Heinrich (1975) demonstrated that flights of *B. vosnesenskii* workers were limited to warmer temperatures (>10°C) compared to the larger queens (>2°C). It has also been shown that larger bumblebees lose heat at a relatively slow rate (chapter 3) and that bumblebees of all sizes maintain similar average thoracic temperatures whilst foraging (Heinrich and Heinrich 1983). Bumblebees can increase their thoracic temperatures by biochemical means (Newsholme *et al.* 1972) and by vibrating their wing muscles, both requiring energy. Therefore a smaller bee must use more energy per unit mass than a larger bee in maintaining a specific thoracic temperature. The previous chapter describes two size dependent morphological features of thermoregulatory consequence in *Bombus terrestris*: setae length being longer in bigger workers (and in colder climates) and bigger workers having relatively reduced extremities relative to smaller workers. The insulation provided by setae depends partly upon their length, and extremity reduction is a well-known adaptation to cold climates in mammals (Allen's Rule). This being the case, we would expect larger bumblebees to be able to forage in colder temperatures than smaller bumblebees.

5.2.4 Size and climate related foraging efficiency

By measuring the foraging efficiency of *Bombus terrestris* foragers of a range of sizes at changing ambient temperatures and other climatic variables, we aim to establish whether larger workers are better able to forage in adverse weather conditions than smaller workers. It is reasonable to suppose that *B. terrestris* provides an adequate model for other social bumblebee species, especially as the size variation found in *B. terrestris*, a pollen storing species, is smaller than some, namely the pocket making species (Alford 1975).

5.3 Methods

5.3.1 Foraging Efficiency

The experiment was carried out from early June to late August 2001. Three nests of *B. terrestris* were purchased from Koppert UK Ltd. For each nest, individual workers were labelled with numbered honeybee queen marker discs (purchased from E.H.Thorne Ltd., Wragby, Linconshire, UK.) glued to the upper thorax. Thorax widths were measured as an indicator of bee size. Water filtered CO_2 was used to anaesthetise each bee for labelling and measurement. Consecutively, each nest was placed in the lab with clear plastic tubing linking the nest entrance to the outdoors via a balance (accurate to 0.01 grams), over which the bees walked. The balance was enclosed with a card wall topped with a clear, red light filter to discourage individuals from flying above the balance. A small glass barrier was placed midway across the scales to further discourage flying. A white plastic funnel was used as a recognisable landing platform at the end of the tube. A trap door was cut into the tubing to allow the removal of new individuals for labelling, measuring and reintroduction during the experimental period. A thermo-hygrometer placed beneath the funnel was used to record outside temperature and relative humidity.

The nectar bags supplied with the nests were removed from each nest and the bees were allowed to forage freely. Two people observed the outgoing and incoming bees regularly for roughly three weeks per nest. When possible, observations were continuous during each day. For each bee that left or entered the nest the following was recorded: date, time, bee number, direction of travel (in/out), bee mass, possession of pollen (or not), temperature, relative humidity, estimated wind strength and whether it was sunny or overcast, raining or otherwise. Wind speed was divided into four categories (0 to 3), 0 representing still air, 1 a gentle breeze when leaves were observed to move, 2 a stronger breeze when small branches were seen to be in motion and 3 anything stronger than that. Commonly the two people observed the nest at once, so perceptions of wind speed were quickly calibrated across observers.

Nectar-foraging efficiency for each trip was calculated by pairing appropriate outgoings and incomings then dividing the difference between in and out mass by the time taken for that trip. Minimum mass of a worker was defined as the lowest recorded mass of a worker leaving the nest to forage, this being the closest measure to the intrinsic mass without carrying nectar. For any one trip, the number of previous trips recorded was used as a measure of experience. To avoid pseudo-replication, only one trip was analysed from each bee. These trips were selected randomly. To reduce noise in the data, bees with less than five recorded trips were excluded from the analyses. Also disregarded were trips of less than ten minutes and more that three hours in length. Foraging efficiencies of negative value were ignored but trips where nothing was collected were included. Data recorded 30 minutes prior, during and up to 30 minutes after periods of rain were excluded, as rain appeared to disrupt foraging trips. It was assumed that, if foragers were concentrating nectar during flight, that they did so at the same rate, regardless of size.

Bees foraging for pollen commonly gather nectar during the same trip (Goulson *et al.* 2002). No measure of amount of pollen gathered was recorded, merely its presence or absence. Without removing and weighing each and every pollen load, we had no way of knowing how much forage mass was pollen and how much was nectar. It is necessary to distinguish the trips in which only nectar was collected ('nectar trips') from those in which pollen was also collected ('pollen trips') because we cannot assume that pollen and nectar are equal either in terms of quality or handling time; only 'nectar trips' were analysed in terms of foraging efficiency (grams of forage gathered per unit time).

Analyses were carried out using one-way ANOVA, linear regression and univariate general linear models, all fitted in SPSS 11.0 (see table 5.1).

5.3.2 The effect of ambient temperature on B. terrestris forager size on Rubus fruticosus agg.

Six replicate patches of *Rubus fruticosus* agg. at Chilworth Manor, Chilworth, Hampshire, U.K. were observed periodically from June 14th until July 7th 2003. The number of relatively large and relatively small *Bombus terrestris* workers that visited a patch during a period of five minutes was recorded. Workers were classified as large/small depending on their size relative to an average sized pinned worker (4.9mm thorax width, Goulson *et al.* 2002). This was repeated for all six patches on thirty-two separate occasions during the trial period. The ambient temperature was recorded during each visit. After all data were collected, the mean ambient temperature was calculated. All temperatures above the average were considered to be 'hot', all below, 'cold'. The sum of counts for each patch in each temperature and size category was calculated. A two-way analysis of variance was fitted on Statview 5.0 with ‘counts’ the dependant variable and ‘temperature category’ and ‘relative bee size’ the independent variables.

5.4 Results

5.4.1 Foraging Efficiency

One hundred and eighty one of the four hundred and seventy three labelled bees were included in the analyses. The remaining bees were excluded either because they foraged too infrequently, left without returning or never emerged from the nest. Foraging bees qualifying for analysis had an average thorax width of 4.9mm, ranging from 3.6 to 6.3mm and, prior to foraging, an average mass of 0.15 grams, ranging from 0.05 to 0.3 grams. Overall, the mean length of time taken to make a foraging trip was 59 minutes, 49 minutes when collecting nectar alone, 69 minutes when collecting pollen/nectar. The mean mass of forage collected per trip was 0.09 grams regardless of whether pollen was collected.

Nectar Foraging Rate: the influence of worker size, experience and microclimate.

The mean foraging rates of foraging workers were positively correlated with their size ($y=0.07x-0.19$; $F_{1,124}=9.117$; $p=0.003$; see fig. 5.1). The mean duration of foraging trips was independent of worker size, but the mean mass of nectar gathered per trip was positively correlated with worker size both in terms of thorax width ($y=0.03x-0.05$; $F_{1,124}=19.892$; $p<0.001$; see fig. 5.2) and minimum mass when leaving the nest ($y=0.22x+0.05$; $F_{1,101}=7.305$; $p=0.008$). The maximum mass of nectar gathered in a trip was weakly positively correlated with the minimum mass of the worker when leaving the nest ($y=0.22x+0.10$; $F_{1,101}=3.955$; $p=0.0495$). Foraging rate was independent of experience, temperature, relative humidity, wind strength and cloud cover (see table 5.1). The varying foraging rate of different sized workers was independent of both temperature and relative humidity (see table 5.1).

Foraging frequency: the influence of worker size and microclimate.

Overall, foraging frequency was positively correlated with worker size ($y=3.16x-1.37$; $F_{1,133}=4.368$; $p<0.05$). More precisely, when foraging for nectar alone, foraging frequency was independent of worker size (see table 5.1), but when foraging for both nectar and pollen, foraging frequency was positively correlated with worker size ($y=2.79x-6.24$; $F_{1,122}=9.118$; $p=0.003$; see fig.

5.3). Foraging took place less in humid conditions (y=-0.11x+73.35; $F_{1, 36}$=7.518; p=0.01: see fig. 5.4) and under cloud cover (y=-0.29x+72.22; $F_{1, 36}$=4.251; $p<0.05$) but was independent of both temperature and wind strength (see table 5.1). On average, when foraging for nectar, the size of forager was independent of temperature, humidity, wind speed and cloud cover (see table 5.1). *I.e.* foragers across the size range foraged in all weather conditions as much as each other.

A comparison of Nectar trips and Pollen and nectar trips

Pollen and nectar trips occurred at higher temperatures (Nectar trips: 20.66±0.279°C; Pollen and nectar trips: 21.63±0.282°C; $F_{1, 246}$=6.057; p=0.015) and lower relative humidity (Nectar trips: 66.89±0.886%; Pollen and nectar trips: 63.82±0.821%; $F_{1, 246}$=6.434; p=0.012) than nectar trips. There was no difference in mean wind strength or mean cloud cover between nectar trips and pollen trips (see table 5.1). On average, larger workers foraged for pollen at higher temperatures (y=1.54x+14.31; $F_{1, 122}$=9.928; p=0.002), lower wind speeds (y=-0.14x+1.79; $F_{1, 122}$=5.034; p=0.027) and under reduced cloud cover (y=-12.80x+111.73; $F_{1, 122}$=6.010; p=0.016) than smaller workers, whereas large and small bees foraged for nectar at the same mean temperature, wind strength and cloud cover (see table 5.1). Forager size was independent of relative humidity whether foraging for nectar or pollen and nectar (see table 5.1). Both nectar and pollen foraging trip duration were independent of forager size (see table 5.1).

5.4.2 The effect of ambient temperature on B. terrestris forager size on Rubus fruticosus agg.

The total number of *B. terrestris* workers observed was greater on hot days than on cold days ($F_{1, 20}$=4.49; $p<0.05$). Overall, a greater number of large workers were observed, although this difference was not significant. The number of large workers versus the number of small workers observed was independent of the ambient temperature (see table 5.1; fig. 5.5).

Table 5.1 Summary of results. *f* = frequency. UGLM = Univariate General Linear Model

Dependent Variable	**Explanatory Variable**	*Test*	**Slope**	*N*	**F**	**p**	**Sig.**
5.4.1 Foraging effic.							
Trip Duration	Body Size (Thrx Width)	Regression		124	2.71	>0.05	
Mean mass per trip	Body Size (Thrx Width)		+	124	19.89	<0.001	***
Mean mass per trip	Body Size (Min. mass)		+	101	7.31	0.008	**
Mean mass per trip	Body Size (Min. mass)		+	101	3.96	0.049	*
Nectar Foraging Efficiency	Body Size	UGLM	+	124	9.12	0.003	**
	Experience			124	2.68	0.104	
	Temperature			124	0.003	>0.05	
	Relative Humidity			124	2.99	>0.05	
	Wind Strength			124	0.06	>0.05	
	Cloud Cover			124	0.09	>0.05	
	Body Size * Temperature			124	0.83	>0.05	
	Body Size * R.Humidity			124	0.15	>0.05	
Foraging *f* (Total)	Body Size	Regression	+	133	4.37	<0.05	*
	Temperature			36	0.01	>0.05	
	Relative Humidity		-	36	7.52	0.01	**
	Wind Strength			36	0.05	>0.05	
	Cloud Cover		-	36	4.25	<0.05	*
Trip *f* (Nectar)	Body Size	Regression		124	0.01	>0.05	
Trip *f* (Pollen)			+	122	9.12	<0.01	**
Mean Body Size of Nectar Forager	Temperature	Regression		124	0.44	>0.05	
	Relative Humidity			124	1.41	>0.05	
	Wind Speed			124	3.72	0.06	
	Cloud Cover			124	0.01	>0.05	
Mean Body Size of Pollen Forager	Temperature	Regression	+	122	9.93	<0.01	**
	Relative Humidity			122	1.09	>0.05	
	Wind Speed		-	122	5.03	<0.05	*
	Cloud Cover		-	122	6.01	<0.05	*
Relative *f* of Nectar & Pollen Trips	Temperature	ANOVA	Pollen +	246	6.06	<0.05	*
	Relative Humidity		Nectar +	246	6.43	<0.05	*
	Wind Speed			246	1.87	>0.05	
	Cloud Cover			246	0.44	>0.05	
5.4.2 Temp. & size							
No. workers observed	Ambient Temperature	ANOVA	Hot +	20	4.49	<0.05	*
	Worker size	ANOVA		20	4.18	>0.05	
Worker size	Ambient Temperature	ANOVA		20	0.97	>0.05	

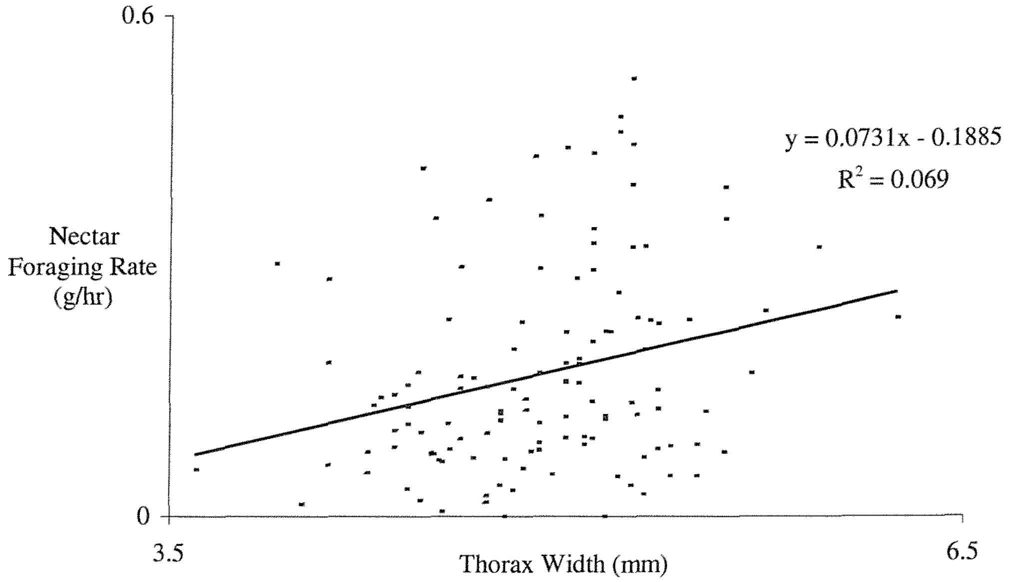

Figure 5.1 Size related nectar foraging efficiency. Mean nectar foraging efficiency correlates positively with worker size ($F_{1,124}$=9.117; p<0.01).

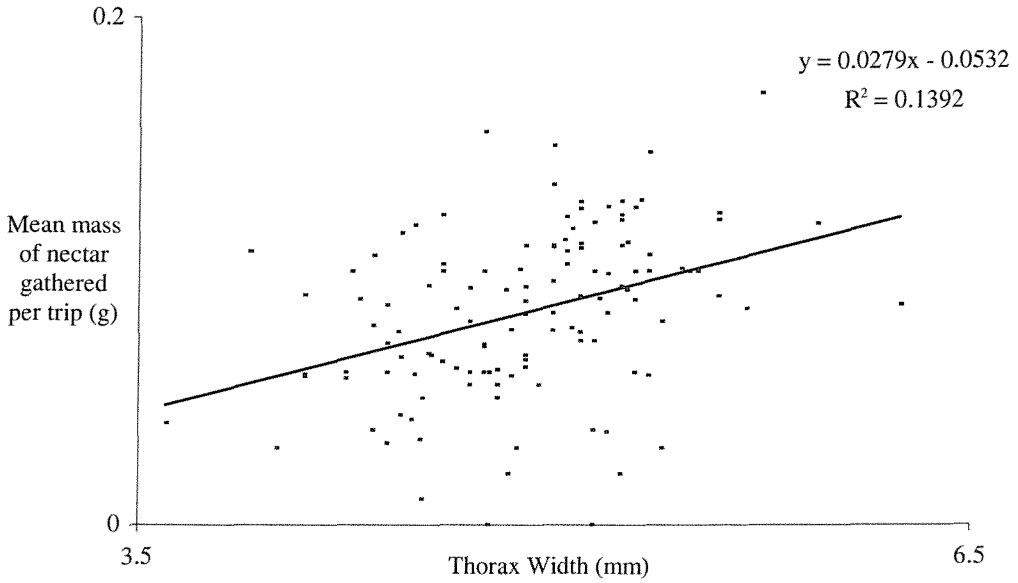

Figure 5.2 The relationship between body size and the mean mass of nectar per trip. When foraging for nectar alone, the mean mass of nectar foraged per trip was positively correlated with worker size ($F_{1,124}$=19.892; p<0.001).

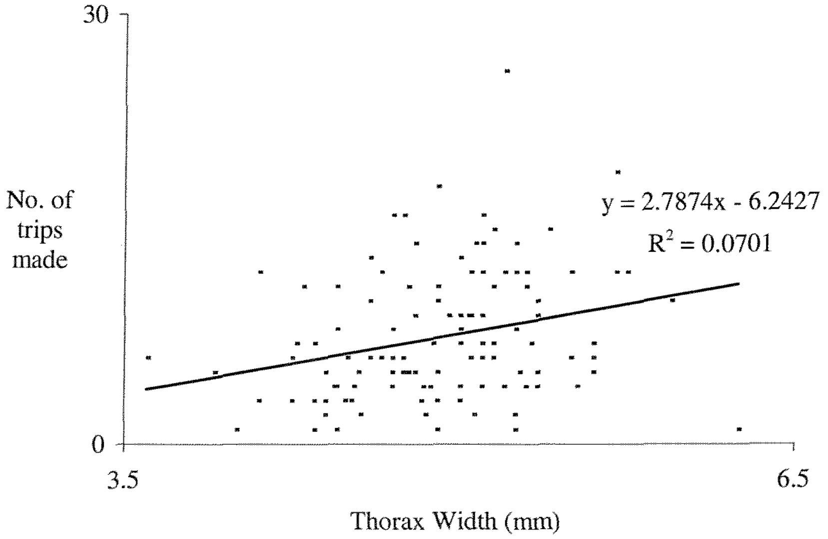

Figure 5.3 Size related pollen foraging trip frequency. When foraging for pollen, foraging frequency was positively correlated with worker size ($F_{1,122}$=9.118; p=0.003)

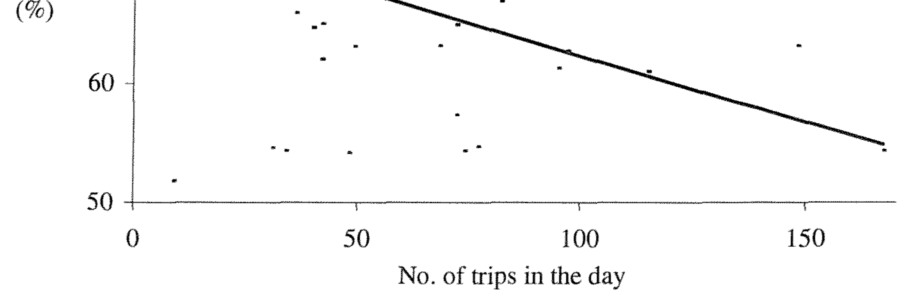

Figure 5.4 Humidity related foraging trip frequency. The number of foraging trips made per day was negatively correlated with the mean relative humidity ($F_{1,36}$=7.518; p=0.01).

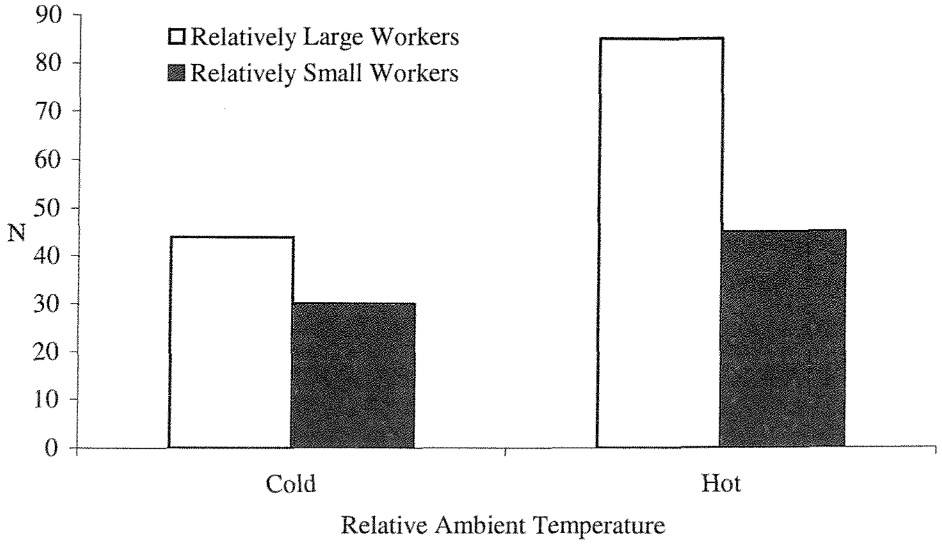

Figure 5.5 Size and temperature related foraging frequency on *Rubus fruticosus* agg. The total number of *B. terrestris* workers observed on *Rubus fruticosus* agg. was greater on hot days than old cold days ($F_{1,20}$=4.49; p<0.05) but the size of worker observed was independent of the ambient temperature ($F_{1,20}$=0.97; p>0.05).

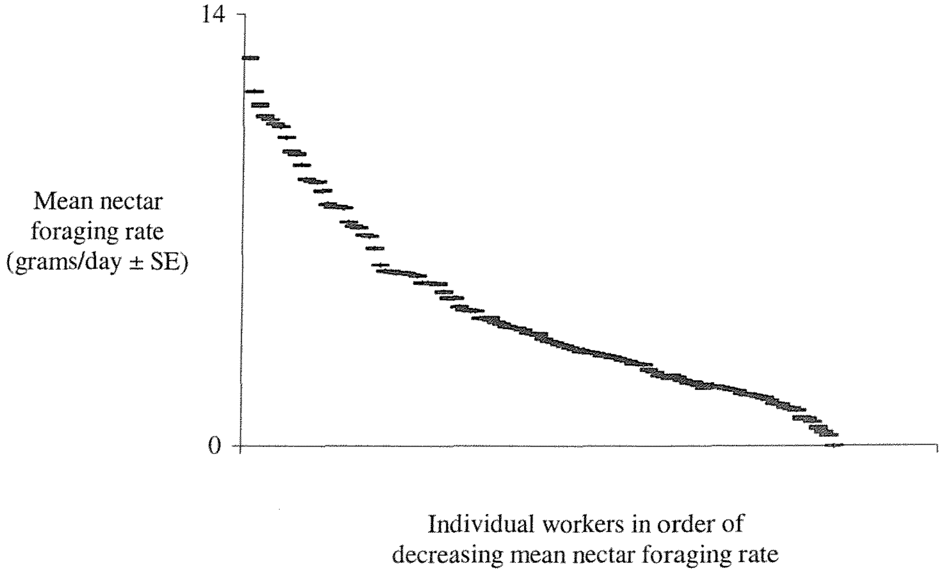

Figure 5.6 The mean nectar foraging rate per worker in order of descending efficiency. Certain workers consistently outperformed others.

5.5 Discussion

5.5.1 Summary

There was a huge variation in nectar foraging efficiency (fig. 5.6). This is partially explained by size. Larger workers collected more nectar per unit time (fig. 5.1 & 5.2; Spaethe and Weidenmuller 2002), carried greater maximum nectar loads and foraged for pollen more frequently (fig. 5.3) than smaller workers. Mean trip length was independent of size and foraging efficiency was independent of temperature and humidity (table 5.1). Foraging frequency declined with increasing humidity (fig. 5.4) and cloud cover but was independent of temperature and wind strength. When foraging for nectar, the weather conditions did not influence the size of worker. Pollen trips occurred in hotter and drier conditions than nectar trips and larger workers foraged for pollen in better weather conditions than smaller bees. During hot days, workers were observed foraging on *R. fruticosus* in greater numbers than during cold days, but the size of worker observed was independent of ambient temperature (fig. 5.5).

5.5.2 Alloethism

We already know that bumblebee workers exhibit size-polyethism, where foraging workers are, on average, larger than in-nest workers (chapter 2; Colville 1890; Cumber 1949; Free 1955; Goulson *et al.* 2002; Spaethe and Weidenmuller 2002).

It has been suggested that bigger bees are advantageous simply because they can collect more forage per trip (Alford 1975). Indeed they can carry more forage than smaller bees but only in proportion with their body mass (Goulson *et al.* 2002). Assuming the nutritional investment in different sized larvae is roughly a linear relationship, a bigger bee, per unit mass invested in its rearing, brings back the same mass of forage as a smaller bee. If all other aspects of foraging were equal then two smaller bees, with the combined mass of one bigger bee, would gather the same amount of forage in half the time. The linear relationship between mass of forager and mass of forage suggests that smaller foragers are advantageous, yet it is larger individuals that forage more frequently.

5.5.3 Size related foraging rate

In terms of nectar gathering rate, a larger worker is superior to a smaller worker (figure 5.1), a fact also discovered by Spaethe and Weidenmuller (2002). On average, foragers took the same amount of time to carry out each foraging trip regardless of size but larger foragers returned with more nectar per trip. Typically, Greek *B. terrestris* foragers range from roughly 0.1 to 0.2 grams in body mass (pers. obs.). Based on the regression equation relating mean nectar gathered per trip (y) and size of forager in terms of mass (x),

$y=0.22x+0.05$ (see results section, paragraph two, line four)

we can calculate the following:

for $x=0.1$ grams, $y=0.072$ grams and

for $x=0.2$ grams, $y=0.094$ grams.

From this calculation we can see that for a doubling in forager size, the average nectar haul is only increased by a factor of 1.31. The explanation for this is not fully understood. Bigger bees have a greater abdominal capacity. Assuming constant density among foragers, total volume should be directly proportional to mass. This enables larger bees to carry more nectar, assuming honey stomach volume varies proportionally with total bee volume, the amount being in direct proportion to their body mass. However, from a calculation identical to the above only using the equation,

$Y=0.22x+0.10$

(where y=the maximum nectar gathered in a trip and x=the size of forager in terms of mass; see results section, paragraph two, line seven) we find that for a doubling in forager size, the maximum nectar haul is only increased by a factor of 1.19, whereas we might have expected a value of roughly 2. Assuming the values of average and maximum nectar load are an accurate representation, small workers carry far more nectar per unit body mass than large workers and the discrepancy between the values 1.19 and 1.31 suggests that small workers foraging for nectar are not fulfilling their potential. It still remains that, individually, larger foragers gather nectar significantly more quickly than smaller foragers.

5.5.4 Why are larger foragers faster at harvesting nectar?

There is little evidence to show that larger bees fly faster although, in 2 out of 10 plants, size dependent differences in inter-flower search time have been found favouring larger foragers (See Chapter 6) and Pyke (1978) has shown that flight times between inflorescences decrease markedly with increasing body weight. However, his evidence was based on studies comparing workers of *B. appositus* to the smaller *B. flavifrons*, not comparing different sized individuals within the same species.

A greater choice of forage plants may be available to a bee with a greater foraging range. Although it is certain workers can forage beyond fifty metres of their nest (Dramstad 1996) and that workers of at least some species are able to navigate back to their nests from distances of up to 12 km (Hedtke 1996), it is not known for sure what their typical foraging range limits are. These are likely to differ between species (Walther-Hellwig and Frankl 2000). Modelling has predicted maximum nectar foraging ranges of several kilometres (Cresswell *et al.* 2000), but size dependent differences in foraging range have not been tested.

Maybe bigger bees are better at handling flowers. Morse (Morse 1978) found no significant difference in foraging rates between large and small *B. vagans* workers, and Stout (Stout 2000) found that small *B. terrestris* workers handled the nectarless flowers of *Cytisus scoparius* more efficiently than large workers. Plowright and Plowright (1997) found workers of short-tongued species to be more efficient at feeding on white clover, but from studies of foraging on 10 different plant species we have found that, on average, larger bees are not quicker at handling flowers than smaller bees (See Chapter 6). It is possible that larger bees obtain nectar more rapidly at each flower visit by drawing the nectar at a greater rate. Indeed, when comparing short and long-tongued species, Harder (1986) found that longer tongues drew more volume of sucrose solution per lap. He also showed that ingestion rates of sucrose were reduced at high concentrations due to viscosity. From evaporation of the water content, nectar will be more concentrated on hot, windy, dry days. If larger bees forage for nectar more quickly because it is more dilute when they forage, compared to smaller bees, they would forage in colder, damper and less windy weather, a prediction made in Chapter 3. However, in our study, the foraging frequency of different sized bees was independent of all weather variables (see table 5.1).

Spaethe and Chittka (2003) have shown that larger *B. terrestris* workers have larger eyes and larger ommatidia than smaller workers, giving them superior sensitivity and image resolution. This allows them to detect single targets, such as flowers, with greater precision. A greater optical aperture should enable larger workers to forage in a wider range of light conditions. Although we took no precise measurements of light intensity, we did measure percent cloud cover as an indication of light intensity. Overall, foraging frequency was reduced in overcast conditions, but cloud cover had no influence over size dependent foraging frequency. Also, superior eyesight may aid the memory of the physical environment, enabling larger foragers to fly directly to a known resource.

Larger bees may be more efficient forgers than smaller bees, but to what extent does this justify the additional resources ploughed into their rearing? To answer this we would need to calculate how much nectar and pollen different sized larvae consume then subtract that value from the total amount of nectar and pollen they collected during the course of their adult lives. The time and energy taken to feed them would also need to be considered. The data required for these calculations are not yet available.

With each foraging trip a worker refines her flower handling technique (Chittka and Thomson 1997) and also improves her knowledge of the geographical locations of resources. Our results suggest a weak improvement in foraging efficiency with experience, although the relationship is not significant (see table 5.1). Learning has been demonstrated previously in bumblebees through secondary nectar robbing (Olesen 1996), floral recognition associated with colour cues (Gumbert 2000), with colour and odour (Kunze and Gumbert 2001) and with rewards (Blarer *et al.* 2002; Chittka and Thomson 1997, Chittka 1998; Thivierge *et al.* 2002), olfactory learning associated with rewards (Laloi *et al.* 1999), context dependent visual learning (Colborn *et al.* 1999; Fauria *et al.* 2002), flower handling (Chittka 2002) and task alternation (Dukas 1995). Heinrich (1976) found differences between newly emerged and more experienced workers in the speed of flower visits and Manning (Manning 1956) showed that experienced workers often adopted a regular foraging route in a particular patch of flowers. Learning has also been demonstrated in moths through flower recognition (Cunningham *et al.* 1998), butterflies through flower handling (Lewis 1986), associating flower type with nectar content (Goulson and Cory 1993) and recognition of nectar guides (Kandori and Ohsaki 1998), and there is considerable evidence of learning in honeybees (Hammer and Menzel 1995) and ants (Johnson 1991). This may partially explain larger bees' superior foraging rate as they foraged more frequently than smaller bees (see table 5.1).

A likely explanation is that larger foragers, having longer glossae (Morse 1977; Harder 1982; Peat *et al.* 2004; chapter 6), ingest nectar at a faster rate (Harder 1983). Because the glossa is covered with rings of hairs, increasing it absorptive surface, a longer glossa should be capable of taking up more nectar per lap (Harder 1982). As larger bees have larger honey stomachs (see above), they are able to store this extra nectar and return to the nest with a greater amount of nectar per unit time foraging.

Can we assume that our measurement of nectar foraging efficiency is legitimate? We measured the mass change of workers during foraging trips and fairly assumed this change to accurately reflect the change in volume in the honey stomach. The concentration of nectar varies with ambient relative humidity, especially in open flowers, (Corbet, Unwin *et al.* 1979; Corbet, Willmer *et al.* 1979). The lower the humidity, the more nectar loses water though evaporation. So, if a forager is foraging in relatively hot and dry conditions she must be collecting relatively concentrated nectar. Therefore, a forager returning to the nest with 0.09 grams of nectar (the average gathered per trip) after foraging in relatively hot and dry conditions would be bringing back a more valuable load, in terms of sugar content, than a forager returning to the nest with the same load after foraging in relatively cool, humid conditions. Since temperature and humidity had no influence on the size of worker foraging (table 5.1), we can be confident that the mean concentration of nectar did not vary amongst different sized foragers as a direct result of the weather conditions. However, individual workers show flower constancy (Goulson 2003) and, moreover, different sized workers show a preference for different plant species. We cannot be sure that nectar concentration does not vary between different plant species and therefore cannot be totally confident of the legitimacy of our measure of nectar foraging efficiency.

5.5.5 Climate dependent foraging behaviour

Overall, bees foraged less when it was humid (fig. 5.3) and cloudy. This is probably because high humidity and cloud cover is a reliable indicator of rain, in which bumblebees struggle to fly. Aside from this, weather had less of an effect on foraging behaviour than anticipated. We had predicted that larger foragers would, on average, forage in cooler conditions than smaller bees as big bees lose heat more rapidly than small bees (chapter 3) and small bees have been shown to be unable to sustain flight in temperatures below 10˚C (Heinrich 1975). This was not the case. Neither ambient temperature, relative humidity, wind speed or cloud cover had any influence on the size of bee that left the nest to forage. In the field, the relative size of foragers observed on bramble was also independent of ambient temperature (fig. 5.5). How do we explain this unexpected result? It is possible that the temperature range during the summer months here in Southampton were not great enough to reveal a size and temperature-dependent difference in foraging behaviour. According to Heinrich (Heinrich 1979) bumblebees are tundra-adapted insects and are therefore equipped for colder temperatures than those we experience here in temperate southern England. If the experiment were repeated in a colder climate we might have found that temperature had an influence on the size of bee that left the nest to forage. Alternatively, it is possible that there is no link between ambient temperature and the size of foraging bumblebees.

Weather did, however, influence the collection of pollen. Foraging trips that involved pollen collection occurred at higher temperatures and lower humidities than trips that collected only nectar (see table 5.1). This is unlikely to be due to a dramatic increase in nectar viscosity, expect perhaps in open flowers, (Corbet, Unwin *et al.* 1979; Corbet, Willmer *et al.* 1979), as nectar production is monitored constantly by plants and water/sugar is added/removed as is necessary. A more likely explanation is that the switch to pollen collection reflects an increase in pollen availability through dehiscence of the anthers.

Larger foragers collected pollen more frequently than smaller workers. Why might larger foragers be specialist pollen collectors? There is no evidence relating pollen collection speed to bee size. However, given that Goulson *et al.* (2002) found pollen loads to be approximately proportional to body mass and we have found here that foraging trip duration is independent of forager size, it is possible that larger workers collect pollen more rapidly. If this is the case it may be because they have a greater surface area to which pollen can stick and longer limbs to both scrape the pollen off their back and store on their corbicula. Also, they may be superior at detecting flowers as they have larger eyes (Spaethe and Weidenmuller 2002).

5.5.6 A note on optimal foraging

According to 'optimal foraging theory', first formulated in 1966 by MacArthur and Pianka (Martin and Hine 2000), 'natural selection favours animals whose behavioural strategies maximize their net energy intake per unit time spent foraging.' If this were the case, surely we would expect a similar performance from our foraging workers, at least amongst bees of similar size? However, frequently workers returned to the nest with no net gain in nectar. Occasionally, workers came back weighing less than they did on the way out. Certain workers consistently out-performed others (fig. 5.6). Although some of this variation is explained by body size (fig. 5.1), there is still massive variation in mean efficiency amongst bees of similar size. According to our results, this variation cannot be explained by the variation in weather conditions (table 5.1). Without alternative explanations, this suggests that bumblebees don't maximize their foraging efficiency to the degree 'optimal foraging theory' predicts. What are the alternative explanations?

Foraging resources are patchy and prone to variation due to weather and competition from other bees. Bees that foraged with consistently higher efficiency may have been fortunate enough to find a nectar rich patch of flowers and exploited it regularly. Since bumblebees don't have the precise recruitment system of honeybees do, individual bees are likely to forage on independent patches. We did not measure the nectar concentration of every nectar load. It is possible that workers returning with a lighter nectar load were actually carrying a higher concentration of nectar, although, since we found no correlation between temperature and foraging efficiency, this seems unlikely. A third possibility is that some bees responded badly to the labelling method, which involved brief anaesthesis using carbon dioxide.

5.5.7 General conclusions

In summary, large bees exhibit superior nectar foraging abilities but this does not appear to be related to ambient temperature or indeed any climatic variable. Although we have shown that larger foragers collect pollen more frequently than smaller foragers, this tells us nothing of their relative pollen foraging performance. Detailed information regarding size-related pollen foraging abilities is needed. Until we can relate the amount of nectar and pollen foraged during a typical lifetime by a given sized bee to the amount of nectar and pollen required to create that bee, size-related foraging efficiency is not an adequate explanation of alloethism. Also, until we discover on a broader geographic scale what role microclimate plays in determining the size and foraging efficiency of a worker leaving the nest to forage, adaptations to microclimatic conditions can only be inferred (chapter 3).

Chapter 6:

Size related flower preferences and handling speed

To find bumblebees short and tall,
And give yourself any chance at all,
Go look on borage,
Where large workers forage,
And on clover where mean size is small.
- James Peat

6.1 Abstract

It has long been known that foraging bumblebee workers vary greatly in size, within species and within single nests, a phenomenon that has not been adequately explained. Workers of their relatives within the Apidae exhibit much less size variation. For the bumblebee *B. terrestris* we demonstrate that size, as measured by thorax width, corresponds closely with tongue length, so that larger bees are equipped to feed from deeper flowers. We show that the mean size of worker bees attracted to flowers differs between plant species, and that larger bees with longer tongues tend to visit deeper flowers. Finally, we show that the handling time depends on the match between corolla depth and tongue length: large bees are slower than small bees when handling shallow flowers, but quicker than small bees when handling deep flowers. We suggest that size variation within bumblebees is adaptive, since it enables the colony as a whole to efficiently exploit a range of different flowers. We discuss possible explanations for the marked differences in size variation exhibited by bumblebees compared to *Apis* species and stingless bees (Meliponinae).

6.2 Introduction

6.2.1 Worker Size Variation

Bumblebee workers exhibit an approximately tenfold variation in mass within species and even within single nests (Alford 1975; Goulson *et al.* 2002). In contrast, other eusocial bee species generally exhibit less than a twofold variation in worker mass within nests (Waddington *et al.* 1986; Ramalho *et al.* 1998; Roulston and Cane 2000).

What causes size variation in bumblebee workers? In species such as *Bombus terrestris*, larvae spend most of their development in individual cells, and are fed directly by the adults (Alford 1975). Therefore the size attained by each larva is probably determined by the adults (Ribeiro 1994). Why such size variation? In part the explanation may relate to division of labour according to size, known as alloethism. In a range of bumblebee species, foragers have been found to be larger, on average, than bees that remain in the nest (Colville 1890; Sladen 1912; Richards 1946; Cumber 1949; Brian 1952; Free 1955; Goulson *et al.* 2002). In honeybees, *Apis mellifera*, all workers begin life by working within the nest, and then switch to foraging. It would thus not be possible for foragers to be larger than nest bees. By contrast, in bumblebees large workers tend to switch from within-nest tasks to foraging at an earlier age than small bees, and the smallest bees may remain within the nest for their entire lives (Pouvreau 1989). Thus on average foragers are larger than nest bees.

A number of adaptive explanations (i.e. those expounding the idea that worker size variation has arisen because it confers a selective advantage to the genes of the individuals rather than by coincidence and being selectively neutral) have been proposed as to why foragers should be larger than nest bees. Large bees forage at a higher rate than small bees (Spaethe and Weidenmuller 2002). They can also carry more forage, and when collecting nectar (but not pollen) they bring back more forage per unit time (Goulson *et al.* 2002). Their greater foraging efficiency may in part be because larger bees have greater visual acuity (Spaethe and Chittka 2003), and visual acuity is likely to affect search times for flowers (Spaethe *et al.* 2001). We would also expect that large bees would be better able to maintain an adequate body temperature for activity in the sometimes inclement conditions they encounter outside the nest, although they will also be more prone to overheating in warm weather (Free and Butler 1959; Heinrich 1979). However, if we assume that the cost of rearing workers is approximately linearly related to their size, then the slightly greater foraging efficiency of large bees is probably not sufficient to offset the higher cost involved in rearing them (Goulson 2003). Rather than asking why foragers are large, Free and Butler (1959) reversed the question and discussed why nest bees are small. They suggested that there may be advantages to rearing small bees for within-nest tasks, other than the cheap cost of rearing them. Bumblebee nest structure is irregular and often crowded, and it seems likely that small bees may be better able to manoeuvre within these cramped confines.

6.2.2 Does forager size variation match flower size variation?

Whatever the explanation as to why nest bees are smaller than foragers, it is notable that even among the larger, foraging class there is still considerable size variation. In *B. terrestris*, thorax widths of all workers vary from 2.3 to 6.8 mm, with foragers varying from 3.3 to 6.8 mm (Goulson *et al.* 2002). Only the very smallest workers never forage. It has been suggested that having foragers of a range of sizes allows them each to specialise in flower types appropriate to their morphology and so improves overall foraging efficiency of the colony while minimizing intra-colony competition (Goulson 2003). It has long been known that interspecific differences in tongue length between bumblebee species explain differences in foraging preferences, with long-tongued bees tending to visit flowers with deep corollas (Pyke 1982; Inouye 1978, Inouye 1980; Barrow and Pickard 1984; Harder 1985; Johnson 1986; Graham and Jones 1996). Prys-Jones (Prys-Jones 1982) found that tongue length correlated positively with corolla length within three common UK species, *B. terrestris, B. pratorum* and *B. pascuorum.* Harder (1985) has shown that differences in glossa length are associated with the use of different plant species, although he makes no distinction between interspecific and intraspecific effects. Here we examine whether intraspecific size variation corresponds with floral choice; do bees of a single species tend to visit flowers that match their size? Harder (1983) also found a positive intraspecific relationship between glossa length, a proven predictor of body size (Morse 1977; Harder 1982), and flower visitation rate in eleven species of bumblebee on *Trifolium pratense,* and intraspecifically in *B. fervidus* and *B. griseocollis* on *Cirsium vulgare.* He showed clearly that larger workers are more suited to *Cirsium vulgare*

However, we cannot assume from this that larger bees will handle flowers more efficiently in every species of plant. Maybe smaller bees are suited to shallower flowers. Indeed, the long tongued *B. hortorum* has been shown to be relatively slow when visiting the relatively shallow flowers of red clover (Holm 1966). Here we expand on Harder's findings, testing whether the choices made by *B. terrestris* workers influence their foraging efficiency, by comparing the handling time of large versus small foragers on several plant species of differing corolla depth. This will show whether bee size and flower visit frequency are positively correlated regardless of flower species, or if this relationship is dependent on corolla depth.

6.3 Methods

All studies were carried out in southern UK during July and August of 2001, 2002 and 2003. To confirm the relationship between tongue length and bee size, the thorax width and tongue lengths (from the base of the prementum to the tip of the extended glossa) of thirty four *B. terrestris* workers were measured using Vernier callipers. These workers were sampled in wildflower meadows in Hampshire and were likely to be from up to thirty four different colonies. To examine whether bees of different size tended to visit different flowers, workers of *B. terrestris* were caught when visiting ten different plant species (mean sample size 25.7 bees per plant species). The thorax width of each bee was measured using Vernier callipers. The proportion of 'large' versus 'small' bees visiting each of eight plant species was also quantified by reference to a pinned bee of average size (4.9 mm thorax width, Goulson *et al.* 2002). The handling time of large and small bees on each of ten plant species was recorded using a stopwatch. Each bee was observed visiting at least five flowers, and the mean time per flower used in subsequent analyses. A minimum of thirty bees were observed per bee size/plant species combination. Corolla depth was defined as the distance from the bottom of the flower well to the opening of the flower. Plant species differ greatly in their flower morphology and in relatively open flowers it is not clear at which point a flower can be considered to be open. In such cases the top of the corolla was considered to be the point at which the thorax of an average sized *B. terrestris* worker (4.9 mm thorax width, Goulson *et al.* 2002) would not pass. For each flower species, corolla depth was measured for 15 flowers using Vernier callipers.

To examine whether bee size was correlated with flower depth, the ratio of large to small bees observed on each flower species was regressed against mean corolla depth. To examine how the relative handling time of large versus small bees was affected by corolla depth, the ratio of mean handling time for large bees: mean handling time for small bees was calculated and regressed against corolla depth.

I acknowledge that 'handling time' is not an absolute measure of rate of uptake of nectar. However, several studies have shown that bees leave very little behind after visiting a flower Stout *et al.* 2000, unless it is too deep for them to reach the bottom, so the rationale is that time does approximate to efficiency.

6.4 Results

In *B. terrestris* workers, tongue length was strongly and linearly related to thorax width ($r^2 = 0.876$, tongue length = 1.34 (thorax width) + 1.73), confirming that larger bees do indeed have longer tongues.

Mean thorax width of foragers differed markedly between the ten plant species ($F_{9,247} = 5.53$, $p<0.001$) (Figure 6.1). On average *Echium vulgare* and *Vicia faba* were visited by the largest workers, while *Trifolium repens* and *Melilotus officinalis* attracted the smallest bees. Similarly, the proportion of large and small bees varied significantly between the eight plant species for which this was recorded ($\chi^2_7 = 26.9$, $p<0.001$) (Table 6.1). No relationship was found between thorax width and sampling date ($F_{1,254} = 0.36$, n.s.). i.e. the average size of workers did not increase or decrease over the season.

The ratio of large to small bees visiting each plant species was significantly positively related to corolla depth ($r^2 = 0.672$, $F_{1,6} = 12.29$, $p=0.013$) (Figure 6.2). In other words, flowers with deep corollas were visited most frequently by large bees, while flowers with shallow corollas tended to be visited by small bees. The ratio of the handling time of large bees to the handling time of small bees was also significantly related to corolla depth ($r^2 = 0.445$, $F_{1,7} = 6.40$, $p=0.035$) (Figure 6.3). Large bees tended to be slower than small bees when handling flowers with short corollas, but were faster than small bees when handling flowers with deep corollas.

Table 6.1 Worker size and handling time when visiting flowers of ten different plant species. Handling times per flower are means of means (at least 5 observations per bee and 30 bees). Corolla depths are means of 30 flowers. - = data not collected

Plant species	**Mean handling time of large bees (s)**	**N**	**Mean handling time of small bees (s)**	**N**	**Corolla depth (mm)**	**Ratio of visits by large vs. small bees**	**Ratio of handling time for large vs. small bees**
Borago officinalis	2.79	30	2.93	30	1.74	1.43	0.95
Rubus fruticosus agg.	4.22	31	3.02	33	0.00	0.53	1.40
Trifolium repens	1.29	30	1.14	30	2.57	0.25	1.13
Symphytum officinale	1.49	30	1.55	30	4.47	0.92	0.96
Echium vulgare	1.32	30	1.74	31	6.73	-	0.76
Centaurea scabiosa	1.25	30	1.66	30	13.62	3.50	0.75
Melilotus officinalis	3.64	30	3.74	30	1.63	-	0.97
Onobrychis viciifolia	2.25	31	2.36	33	4.3	0.88	0.95
Knautia arvensis	1.14	30	1.13	30	5.99	3.25	1.01
Cirsium vulgare	1.00	30	1.05	30	11.21	2.67	0.96

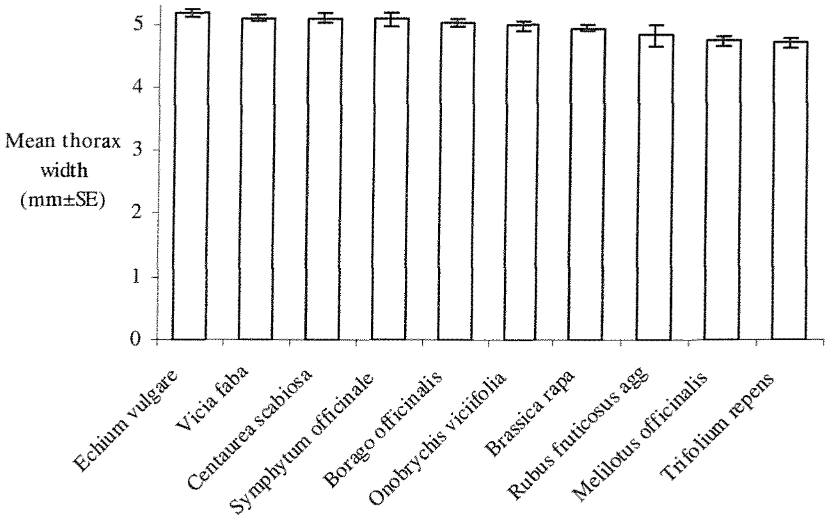

Figure 6.1 The mean thorax width of foraging *B. terrestris* workers visiting flowers of each of ten different plant species. Differences were significant ($F_{9,247} = 5.53$, $p<0.001$).

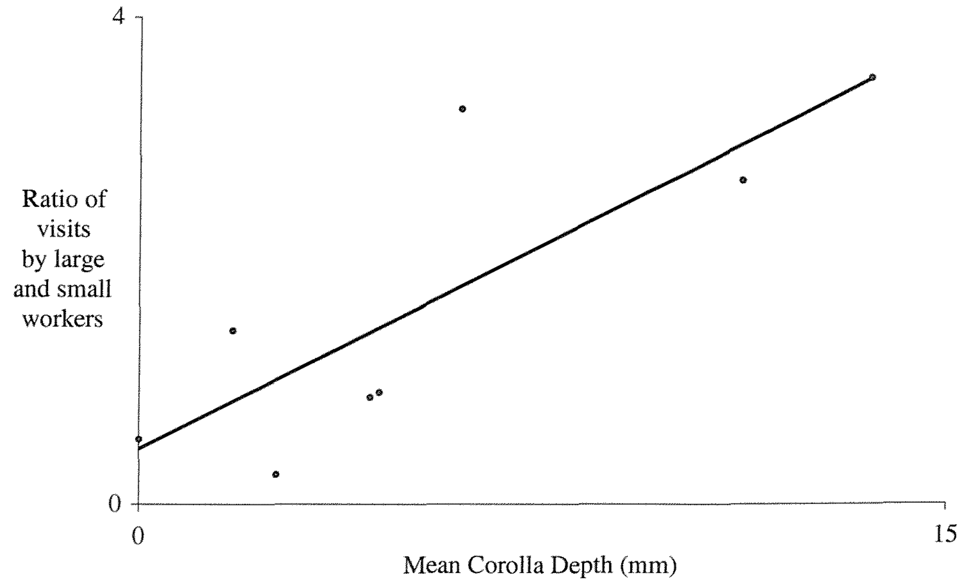

Figure 6.2 The ratio of large to small *B. terrestris* workers recorded visiting each of eight plant species, plotted against the corolla depths of the plants (linear regression, $r^2 = 0.672$, $F_{1,6} = 12.29$, p=0.013, ratio = 0.454 + 0.223 (corolla depth)).

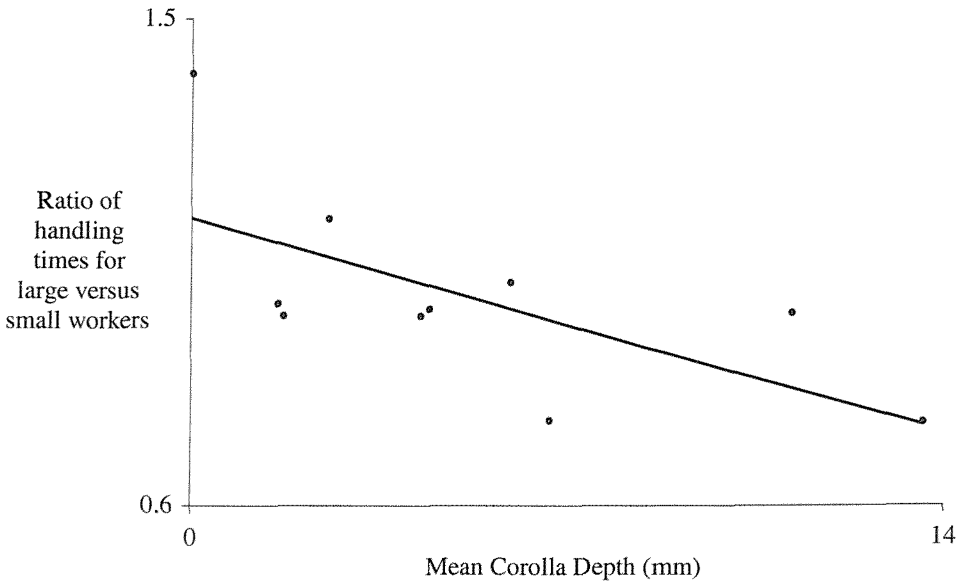

Figure 6.3 The ratio of the handling time per floret for large bees versus small bees when visiting nine different flower species, plotted against the corolla depths of the plants (linear regression, $r^2 = 0.445$, $F_{1,7} = 6.41$, p=0.035, ratio = 1.13 - 0.0282 (corolla depth)).

6.5 Discussion

6.5.1 Summary

We demonstrate that flower choices made by foraging *B. terrestris* workers are not random with respect to their size (table 6.1; fig. 6.1); large bees tend to visit different flowers to small bees (although there is considerable variation in bee size on any one plant species). Secondly, large and therefore long-tongued foragers tend to choose flowers with deeper corollas than do small foragers (fig. 6.2). Thirdly, these flower choices appear to correspond to foraging efficiency; large bees are more efficient that small bees when visiting flowers with deep corollas, and vice versa (fig. 6.3).

Previous studies have noted a tendency for workers of different sizes to visit different flowers (Cumber 1949; Morse 1978; Inouye 1980; Barrow and Pickard 1984; Johnson 1986). For example, Cumber (1949) found that large workers of *B. pascuorum* tended to visit *Lamium album*, which has a deep corolla, while the smaller workers visited *Lamiastrum galeobdolon* which has a substantially shallower corolla. However, previous studies have not demonstrated that these choices correlate with corolla depth, or that foraging efficiency (as measured by the surrogate handling time) matches the choices made by bees of different sizes.

When comparing different bumblebee species, Plowright and Plowright (1997) found that bees with long tongues fed more slowly on shallow flowers than bees with shorter tongues. Presumably a long tongue is unwieldy for drinking from a shallow flower, but has obvious advantages when drinking from a deep one. By producing foragers of a range of sizes with a range of tongue lengths a bumblebee colony is thus able to efficiently exploit a range of different flower species of varying structure and corolla depth.

6.5.2 Why do other social bees not show similar size variation?

The coefficient of variation in size of *Apis mellifera* is less than one tenth of that found in *B. terrestris* (Waddington *et al.* 1986 ; Goulson *et al.* 2002). Tropical stingless bees (Meliponinae) exhibit slightly more size variation than honeybees, but much less than bumblebees (Waddington *et al.* 1986). Honeybees and stingless bees are argued to have evolved in tropical forests feeding primarily on mass-flowering trees (Heinrich 1979). They use recruitment of nestmates to specific sites so that a large proportion of the colony is simultaneously exploiting the same large resource. In this situation size variation would not be advantageous. I have doubts over this theory as there are many different plant flowering strategies in the tropics, certainly not all mass flowering (Ollerton, pers. com. 2004). In contrast, temperate bumblebees feed on scattered, mainly herbaceous plants, and many different plant species flower at the same time. They do not recruit to particular flower patches, probably because most patches are so small that one bee can exploit them (Dornhaus and Chittka 1999). Instead, workers each specialize in visiting one or two plant species, and it seems that they choose flowers that are most suited to their own size.

6.5.3 Seasonal change in mean worker size

One criticism of our study is that the different plant species were not all studied at the same place and time. If mean worker size changes through the season, this could influence the results. Knee and Medler (1965) found an increase in worker size for three American species late in the season. Plowright and Jay (1968) found an increase in worker size as the season progressed in some species but not in others. Röseler (1970) describes an initial decline in the mean size followed by a general increase in *B. terrestris*. All agree that the change in mean size is small compared to variation found within broods. Our study was not designed to examine this, but we found no significant change in forager size over the season ($F_{1,254} = 0.36$).

6.5.4 Other factors influencing flower preference

Observation of foraging bees makes it clear that corolla depth is not the only factor that determines whether a bee is physically suited to a particular flower type. *T. repens* was visited mostly by small foragers, but occasionally by large ones. When this occurred the stalk of the inflorescence was unable to support the weight of the bee and it would collapse to the ground, making flower handling more difficult for the bee. The flowers preferred by large bees, such as *E. vulgare*, tend to have sturdy stalks. Flower constancy is a well-known characteristic of bumblebee behaviour for which there is not an entirely satisfactory explanation (reviewed in Goulson 2003). If many of the plant species available to a bee (and apparently suitable as judged by visitation from conspecifics) are poorly suited to its morphology because of its particular size relative to the flower, then this will tend to favour constancy.

Chapter 7:

Worker size frequency manipulation and its effects on the development of freely foraging colonies

When considering forager speed,
We find that the large take the lead,
But what if every bee,
Were colossal or wee?
Or is a mix really what the nests need?
- James Peat

7.1 Abstract

Within the worker caste, bumblebees exhibit a large degree of size variation. A number of adaptive explanations for this variation exist, some with strong evidence to support them. For example, larger workers forage more frequently than smaller workers and also forage for nectar with greater efficiency possibly because they have superior vision. Also, different sized workers appear to be adapted to foraging on different sized flowers and differ in their ability to sustain flight in different temperatures. However, I cannot rule out a selectively neutral, non-adaptive explanation until we show that the presence or absence of size variation amongst workers has a differential effect on colony development. Here, I show that nests with larger workers develop more rapidly than nests with smaller workers, regardless of whether the small worker workforce equals the large in terms of number or mass. I also present weak evidence suggesting that the production of small workers is maladaptive. I tentatively conclude that the production of relatively large workers is adaptive but that the production of relatively small workers remains to be explained.

7.2 Introduction

7.2.1 Worker size variation and alloethism

Bumblebees of the genus *Bombus* produce workers of varying size (chapter 2; Alford 1975; Goulson *et al.* 2002), a variation greater than that found in other eusocial bee species (Waddington *et al.* 1986; Ramalho *et al.* 1998; Roulston and Cane 2000). The proximate cause of worker size variation is the quantity of food consumed by the larvae, which are fed by either the queen or adult siblings (Ribeiro 1994). The ultimate reason is not yet clear.

Possible adaptive explanations appear, as in ants (Wilson 1980), to be related to polyethism. Bumblebees exhibit division of labour in which foraging workers are, on average, larger than in-nest workers (Colville 1890; Sladen 1912; Meidell 1934; Richards 1946; Cumber 1949; Brian 1952; Free 1955; Goulson *et al.* 2002). Maybe foragers are larger because they are more able to cope with adverse weather conditions since they cool less quickly than smaller workers (chapter 3; Heinrich 1975). Conversely, maybe smaller workers are more suited to in-nest tasks because they can manoeuvre better around small spaces, as suggested by Free and Butler (1959). Recent observations have shown large *Bombus terrestris* foragers to have a greater nectar-foraging rate than small foragers (chapter 5; Spaethe and Weidenmuller 2002), perhaps because of their superior vision (Spaethe and Chittka 2003). Additionally, we have evidence of forage plant related size-polyethism in which larger *B. terrestris* foragers (which have longer tongues) are found more commonly on plants bearing flowers with deeper corollas, and where the handling time of workers on different plant species differs according to size of worker and depth of corolla (chapter 6).

Larger workers also perform in-nest tasks. In an experiment where *B. terrestris* workers of manipulated size distributions were confined to their nests and provided with food, Cnaani and Hefetz (1994) found that workforces of large bees raised more young than workforces with an equal number of small bees. However, they did not test the difference between a workforce of large bees and a workforce with an equal mass of small bees, a fairer comparison bearing in mind that a greater number of small bees can be produced with the same amount of nutritional input.

7.2.2 Is worker size variation adaptive or not?

Is it possible that worker size variation is not adaptive, that it is an inconsequential side effect of a disorganised larval rearing system? If size variation is selectively neutral we would find that bumblebee colonies perform equally well regardless of the average size of their workers. Here we allow bumblebees to forage as well as perform in-nest tasks, to determine how nests of large workers develop in comparison to nests of small workers either when the workforce is equal in total number or equal in total mass. We also include nests of varying sized workers (reflecting a more natural distribution) equal in number to that of the large workers. If large workers are superior foragers (chapter 5; Goulson *et al.* 2002; Spaethe and Weidenmuller 2002) and superior brood carers (Cnaani and Hefetz 1994) we would expect nests of large workers to outperform nests of small workers. If the nests of large workers outperform the nests of small workers of equal total mass, this would strongly indicate that the production of large workers is adaptive. Assuming worker size variation is adaptive, we might expect the nests of varied sized workers to be the most successful as this is the closest to the natural size distribution.

7.3 Methods

All adult workers from twelve *B. terrestris* nests, bought from Koppert UK Ltd. Ltd., were removed and sorted into large and small categories. The dividing line between large and small was quantified by reference to a pinned bee of average size (4.9 mm thorax width, Goulson *et al.* 2002). Larvae were not removed as this would have risked destroying the nests. The workers were randomly mixed and distributed amongst the 12 nests in four treatments: 30 small workers ('small'), 30 large workers ('large'), small workers whose total mass (≈9 grams) equalled that of 30 large workers ('equal') and, representing a natural distribution, 30 workers of varied sizes ('natural'). Since all twelve nests were raised on the same food, it was assumed that conflict amongst the workers after mixing and reintroduction would be minimal. The nests were left for 3 days to allow the workers to acclimatise to their new home and new 'sisters', then, after the removal of the nectar bag, weighed and placed randomly at sheltered sites within the grounds of the University of Southampton's School of Biological Sciences, Basset Crescent East, Highfield, Southampton. To reduce the effects of moisture, each nest was placed on bricks and was covered with a rain resistant plastic cover (Plate 7.1). Slug pellets were regularly scattered around the nests to prevent damage from slugs. For the next three weeks the nests were weighed daily. The experiment commenced on May 29th 2002. After three weeks all nests were collected (following a 24 hour period in which one-way valves were used to allow workers to enter the nest but prevent departure) and killed and their contents analysed. It would have been preferable to allow more time to elapse however, with each passing day and each new worker produced, the differences in average size between different treatments would have reduced. Without repeated disturbance and resorting of the nests, leaving the nests out for longer would be likely to have led to less clear results. Workers present were counted and weighed, pupae, larvae and eggs were also counted and the state of the nest and presence or absence of the queen was noted. Per treatment, means of total mass difference, the number of new workers present, the number of larvae present, the total number of young present and the total increase in numbers were all analysed using Anova on StatView 5.0. Post Hoc Tests (Fisher's PLSD) were also performed on StatView 5.0.

7.4 Results

All but one nest lost mass during the trial period. Between treatments, there were no significant differences in the mass lost ($F_{3,7}$=0.153; p=0.924) or in the number of larvae present ($F_{3,7}$=2.572; p=0.137; figure 7.1). However, standard error calculations suggest that nests containing large workers outperform those of all other treatments (figure 7.1).

The total number of young ($F_{3,7}$=2.308; p=0.163), the increase in the total number of individuals present ($F_{3,7}$=3.103; p=0.098) and the number of new workers ($F_{3,7}$=3.228; p=0.091; figure 7.2) did not differ overall. However, standard error calculations suggest that nests containing large workers outperform nests containing the same number of small workers and nests containing the same mass of small workers (figure 7.2).

Before the trial period the mean size of worker (g) differed significantly among treatments ($F_{3,7}$=6.15; p=0.023). Fisher's PLSD showed that mean worker size in 'Large' nests was significantly greater than in 'Small' (p=0.013) and 'Equal' nests (p=0.011) and that mean worker size in 'Natural' nests was significantly greater than in 'Small' (p=0.038) and 'Equal' nests (p=0.029). After the trial period the mean size of worker (g) found in each treatment was the same ($F_{3,7}$=2.65; p=0.13).

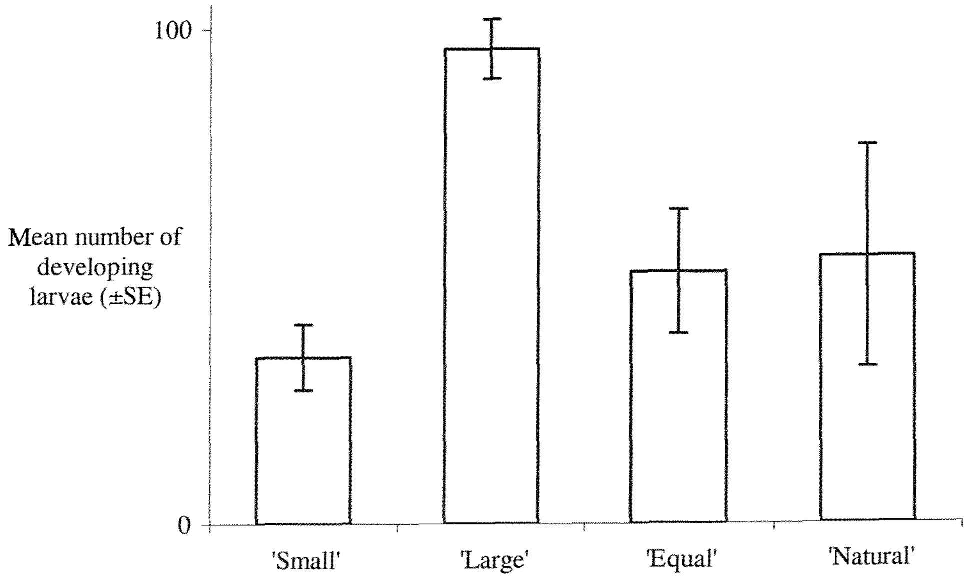

Figure 7.1 The mean number of developing larvae per treatment, post trial. ANOVA showed no significant difference between treatments ($F_{3,7}$=2.572; p>0.05).

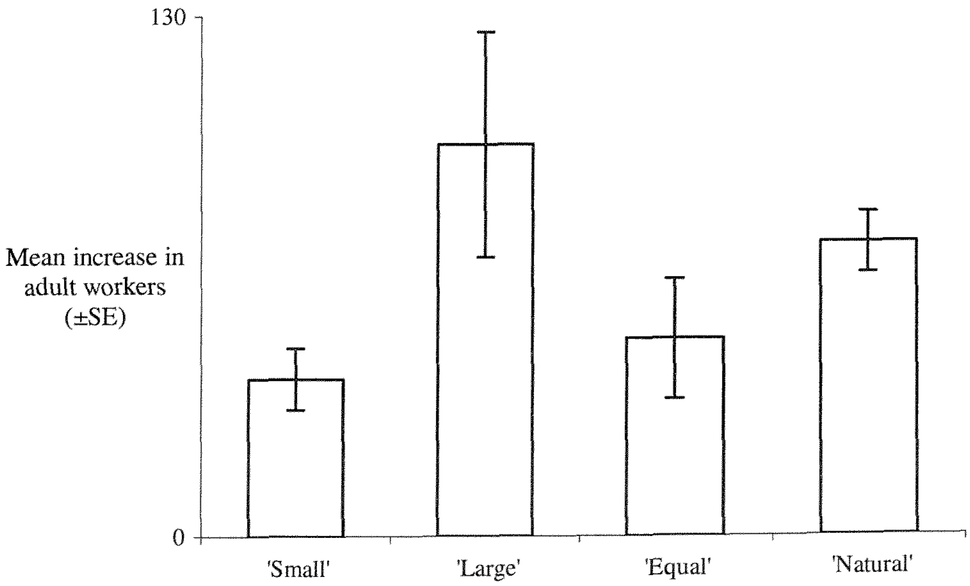

Figure 7.2 The mean increase in the number of adults per treatment over the trial period. ANOVA showed no significant differences ($F_{3,7}$=3.228; p>0.05).

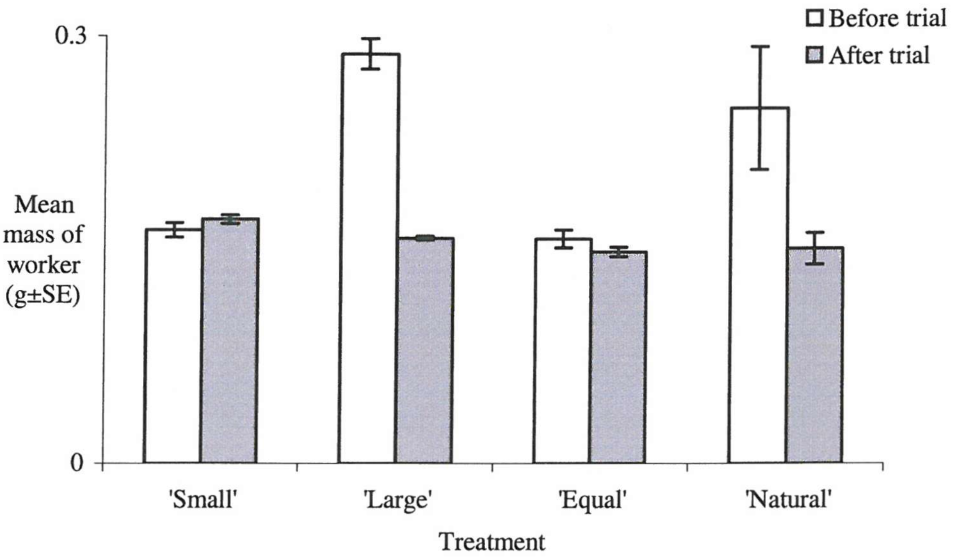

Figure 7.3 The mean mass of worker (g) per treatment before and after the trial period. Before the trial period the mean size of worker differed significantly among treatments ($F_{3,7}$=6.15; P=0.023). After the trial period the mean mass of worker found in each treatment was the same ($F_{3,7}$=2.65; P=0.13).

7.5 Discussion

7.5.1 Summary

This experiment was frustrated by unusually poor weather. Due to consistent and heavy rainfall all nests were damp to some degree and, in one (large) nest, the queen was no longer present. This nest was severely under developed and was not included in the analysis, which inevitably reduced the power of the statistical tests.

Although the colonies in our experiment began with workforces of certain sized bees, it is important to remember that the developing larvae were not removed, therefore new workers of any size were bound to emerge during the trial period. Colonies classified as having only large workers would have quickly recruited smaller workers and vice versa. Indeed, the average size of worker in each treatment was virtually the same after the trial period (figure 7.3).

Despite this, colonies with initially larger workers produced greater numbers of new individuals than colonies with the same number of smaller workers (although this wasn't statistically significant - figs. 7.1 and 7.2), probably because larger workers are superior at both foraging (chapter 5; Goulson *et al.* 2002; Spaethe and Weidenmuller 2002) and brood caring (Cnaani and Hefetz 1994).

Although inconclusive, my results also indicate that a workforce of larger bees may be superior to a workforce of smaller bees of equal total mass (figure 7.2). *i.e.* a colony will be more successful if it ploughs its resources into larger workers despite the fact that this will result in a smaller workforce numerically. Therefore, it is possible that large size is adaptive in workers of *B. terrestris*, and probably other *Bombus* species. It appears that a larger worker might be more valuable to the colony than its equal mass of smaller workers.

It was surprising that no nests gained mass during the trial period. It was anticipated that the nests would all increase in mass due to the collection of nectar and pollen, which would then be invested in growing larvae and nest structure. There are a number of reasons why this mass loss may have occurred. Prior to the experiment each nest contained a nectar bag, an effectively unlimited supply of nectar. It is possible that the bees were able to exploit this unnaturally rich source and fill nectar pots to capacity leaving the nest relatively heavy. Once the nectar bags had been removed and the nests placed outside, the bees were forced to forage for themselves. If they had a surplus of nectar in the nest this may have discouraged them from foraging for more. Nectar would have been consumed by workers to fuel pollen foraging trips and may not have been replaced. Also, it rained almost constantly during the trial period. This would have prevented the bees from foraging as effectively as they might have done and forced them to rely upon nectar stored from when they had a nectar bag. The result was a net loss in mass in all but one nests over the trial period. It is possible that there was conflict within the nests since each nest contained a mixture of workers from twelve nests. In each case, this may have resulted in relatively ineffective workforce. However, the fact that the treatments differed in the number of offspring they generated suggests that conflict, if it happened at all, did not play a great role in at least some of the nests and probably all of them.

7.5.2 Why are small workers produced?

My results also indicate that a workforce of larger bees is superior to a workforce representing the natural distribution of worker sizes (figure 7.1), although this is inconclusive. If small workers are inferior foragers and brood carers then why do they exist? Maybe smaller workers can manoeuvre more efficiently within tight spaces in the nest. Despite the fact that small individuals are inferior brood carers to large individuals (Cnaani and Hefetz 1994) we cannot be sure that small brood carers are not superior in terms of work done per gram of worker. An obvious suggestion is that they are produced early in the season when resources, collected by the queen, are limited. Rather than a small number of large workers, producing a numerous workforce of small bees would reduce the chances of the entire workforce being lost early on due to predation or severe weather. Whilst there may be some truth in this, as there is evidence showing that mean worker size increases during the season in some species (Knee and Medler 1965; Plowright and Jay 1968), production of small workers continues throughout the season (Alford 1975). Maybe small workers play an important role in nest defence. In the leaf-cutter ant *Atta capiguara* minor workers are the most active in response to a disturbance (Hughes and Goulson 2001). A worker is equipped with a sting regardless of size and small workers can be produced in relatively large numbers because they are relatively cheap to produce. For a given resource input, a colony of smaller workers will have a greater number of stings with which to defend the nest against predators.

7.5.3 General conclusions

There is evidence for why colonies produce large bumblebee workers: they are superior nectar foragers (Spaethe and Weidenmuller 2002; chapter 5) and they give colonies a fast rate of growth. There is also evidence for why size variation exists amongst the larger workers: different sized bees specialise on different sized flowers (Harder 1983; Peat *et al.* 2004; chapter 6) and may be adapted to forage in different temperatures in climates more variable than during the summer in southern England. The data in this chapter provides evidence, albeit weak, to show that the production of large workers is adaptive but leads us to question the value of smaller worker production. Repeated with greater replication, this experiment may provide more solid conclusions.

Chapter 8:

General Conclusions

I've studied the hows and the whys,
Of worker variation in size,
It's in the climatic past,
And floral contrasts,
That the answer most probably lies.
- James Peat

8.1 Summary of results

I have confirmed size variation in the worker castes of 23 *Bombus* species (Chapter 2; Alford 1975; Goulson *et al.* 2002; Heinrich 1979), that foragers are, on average, larger than in-nest bees (Chapter 2; Colville 1890; Sladen 1912; Richards 1946; Cumber 1949; Brian 1952; Free 1955; Goulson *et al.* 2002) and that workers of pocket-making species vary more than pollen-storers (Chapter 2; Alford 1975; Goulson 2003). It is believed that larger bees are suited to foraging tasks and small bees to in-nest tasks, large bees being able to collect more forage per trip (Alford 1975). I have confirmed that larger workers gather nectar more efficiently (Chapter 5; Spaethe and Weidenmuller 2002), that larger workers forage more frequently and that, in southern England, nectar foraging efficiency and frequency is independent of weather variables (Chapter 5). Workers show behavioural plasticity: smaller workers occasionally forage (Chapter 5), larger bees often perform in-nest tasks (Alford 1975) and are superior brood carers (Cnaani and Hefetz 1994). By manipulating the worker size distributions in artificial nests, I have shown that a workforce of large bees may be superior to one of small workers in terms of colony growth (Chapter 7). Larger workers are supposedly better adapted to colder conditions than smaller workers (Heinrich 1975). Here, I show that smaller workers lose heat more rapidly, have shorter thoracic setae and proportionally larger heads, legs and abdomens (Chapter 3). I show that bumblebees from cold regions have longer thoracic setae than those from hot regions (Chapter 3) and that, within species, foraging workers of races from cooler regions are often found to be larger than those from warmer regions (Chapter 4). I also show that, in southern England, ambient temperature has no influence on nectar foraging efficiency or on the size of forager, but that pollen foraging trips are made in warmer conditions (Chapter 5). Bumblebees obtain food from flowers. I show that different plant species attract foragers of different mean size, confirm that larger bees have longer tongues (Harder 1982) and handle deeper flowers more quickly (Harder 1983) and shallower flowers more slowly than smaller workers (Chapter 6).

8.2 Non-adaptive hypotheses for worker size variation

It is possible that size variation has no adaptive explanation. There is no genetic variation shown to underlie the phenomenon, suggesting that the causes of variation might be due purely to phenotypic plasticity. Worker size is mainly determined by how much they are fed as larvae, the more food consumed the larger the larva (Plowright and Jay 1968; Sutcliffe and Plowright 1988; Sutcliffe and Plowright 1990). By not storing large quantities of pollen and nectar to buffer against inclement weather, bumblebees leave themselves vulnerable to climatic fluctuations. An extended stormy period could severely limit the ability of the adults to feed larvae, resulting in a batch of small workers.

So, is it possible that size variation could simply be a result of a fluctuating input of resources? It seems unlikely. Koppert UK Ltd. rear nests using wild queens caught in Greece. They are provided with a huge nectar bag and a plentiful supply of pollen. Essentially there is no fluctuation in input of resources, yet we find very similar coefficients of variation amongst these workers as those in the wild (Chapter 2; table 2.1). If a period of bad weather results in the production of small workers we would expect the workers of nests in regions with a relatively high proportion of bad weather (Scotland, for example) to have a smaller average size. My results are not in keeping with this hypothesis. Within three of four species sampled, foragers from Scottish colonies were, on average, larger than those in southern England (Chapter 4). This may be a response to mean temperatures, producing bigger workers, on average, for their superior thermoregulatory ability in colder weather. We find size variation in bumblebees, whether they are from tundra or the tropics (Chapter 4, tables 4.1 and 4.2). If this variation is determined by variable weather conditions we would expect colonies/species from relatively stable climates to exhibit relatively less variation. However, when comparing species from temperate regions to those from tropical regions, we find no particular relationship between region and worker size variation (Chapter 4, table 4.2). All evidence suggests that worker size variation is under the control of the queen and workers and not a consequence of resource fluctuations.

When monitoring foraging behaviour of *B. terrestris* workers from three nests over a period of three months, we found no evidence that size variation has evolved to cope with fluctuating weather conditions. This casts some doubt over the adaptive argument for thermoregulation related size variation. It is possible that size variation is adaptive, through better thermoregulation of foragers, in species rich areas such as the mountains of Central Asia (Williams 1994), but is of little consequence in mild regions such as southern Britain. This begs the question, why have bumblebee workers retained their size variation in such places if it is unnecessary? Producing large workers requires more pollen (Plowright and Jay 1968; Sutcliffe and Plowright 1988, 1990). Given the extra costs they give a colony, if they are not needed then natural selection should remove them. That they are produced suggests they perform an important role. It is still not clear exactly what that role is.

8.3 Adaptive hypotheses for worker size variation

The success of a bumblebee colony relies upon the collection of food resources with which to feed growing larvae of potential workers, males and queens. Foraging workers, who exhibit a large degree of size variation, collect these food resources. Therefore, my search for an adaptive explanation of worker size variation in bumblebees has focussed on aspects broadly relating worker size to foraging behaviour and often having implications for foraging efficiency. Potentially, this opens a broad range of investigative avenues including flower choice, flower handling, thermoregulation ability in a range of temperatures, visual ability, predation and parasitism.

Although bumblebees show alloethism (Colville 1890; Sladen 1912; Richards 1946; Cumber 1949; Brian 1952; Free 1955; Goulson *et al.* 2002), the dividing line between forager and nest bee is blurred. There is a great overlap in size between the two. A foraging workforce still varies considerably in size (Goulson *et al.* 2002). I have directly measured nectar foraging efficiency in *B. terrestris* and found, like Spaethe and Weidenmuller (2002), that larger foragers collect more nectar per unit time (Chapter 4), probably because larger bees ingest nectar at a faster rate (Harder 1983) and maybe because larger bees have superior vision (Spaethe *et al.* 2001). Data relating body size to pollen foraging efficiency is scarce. Pollen load size is positively correlated with body size (Goulson *et al.* 2002) and we have shown that pollen foraging trip length is independent of body size so it is likely that pollen foraging efficiency is greater in bigger bees. This may partially explain why foragers are larger, on average, than in-nest bees. Another explanation proposed is that while large bees are superior foragers, smaller bees may be better suited to in-nest tasks (Alford 1975). Cnaani and Hefetz (1994) cast some doubt over this hypothesis by showing large workers to be superior to small workers at caring for the brood. However, since they were comparing large and small workers in terms of numbers and not mass, we cannot be sure that smaller bees aren't superior, for their mass. The same goes for foraging ability. My findings show that, although individually larger foragers collect nectar more efficiently, in terms of body mass smaller workers are superior (Chapter 5). Likewise, in the stingless bee *Melipona quadrifasciata*, smaller workers are able to carry greater amounts of pollen per unit of body mass than large workers (Ramalho 1998). So, if smaller foragers are superior nectar foragers per unit mass invested in their larval stage, why are large bees produced? There are two obvious explanations: one, that different sized bees are adapted to forage from different sized flowers; two, that larger bees are better adapted to foraging in adverse weather conditions (although we have not found this to be so in experiments in southern Britain).

Perhaps larger bees can fly further than smaller bees and therefore have a wider choice of forage resources. It is not yet known how far bumblebees typically fly to forage. Logically, we might assume that foraging as close to the nest as possible would maximise foraging efficiency (Heinrich 1979). However, if a particularly profitable resource is further away maybe it is worth spending the extra time and energy to reach it. Very little is known about bumblebee foraging ranges. Modelling by Cresswell *et al.* (2000) suggests that bumblebees may be able to forage on plants several kilometres from their nest. It is unclear whether large size would confer an ability to forage further from the nest than small bees. Although large bees can carry more fuel (nectar), they burn more fuel so the net result may well be that foraging range is independent of size. This needs testing.

Interspecific differences in tongue length explain differences in bumblebee foraging preferences, with long-tongued species tending to visit flowers with deep corollas (Pyke 1982; Inouye 1978, Inouye 1980; Barrow and Pickard 1984; Harder 1985; Johnson 1986; Graham and Jones 1996). In three *Bombus* species, Prys-Jones (1982) found intraspecific differences in flower choice dependent upon worker size. On average, workers with longer tongues fed on flowers with deeper corollas. Harder (1983) found that larger *B. fervidus* and *B. griseocollis* workers visited flowers of *Cirsium vulgare* at a faster rate than smaller workers. We also found that the average size of *B. terrestris* foragers differs between flowering plants and that this is related to corolla depth (Peat *et al.* 2004; Chapter 6). On plants where larger foragers are more commonly found, the larger foragers handle the flowers more quickly and on plants where smaller foragers are more commonly found, the smaller foragers handle the flowers more quickly, also related to corolla depth (Peat *et al.* 2004; Chapter 6). This strongly suggests that a variable sized workforce is likely to benefit a colony by being able to efficiently exploit a variable food source. Indeed, bumblebees do forage on a wide variety of plant species. Honeybees evolved in the Old World tropics where rich and clumped resources are commonly available from mass flowering trees (Heinrich 1979) and at any one time, most or all foragers would have been visiting the same flower type. This is a possible explanation for the lack of worker size variation in honeybees and why they have evolved the ability to recruit nest mates – what is good for one sister is good for another. Bumblebees, evolving in tundra where resources are more patchy and variable, have not evolved such recruitment behaviour, perhaps because what is good for a large bee is not necessarily good for a small one, and vice versa.

We know that body size influences a bee's ability to thermoregulate, probably because the rate at which a bee cools is negatively size dependent (Chapter 3). Heinrich (1975) reports that small workers are unable to fly in low temperatures and that queens and large foragers are liable to overheat in high temperatures (Heinrich 1975, 1979). We know that queens (typically larger and always earlier foragers than their daughters) have been recorded flying in temperatures approaching 0°C (Heinrich 1979). In tundra landscapes, when summer daytime temperatures may regularly fall to relatively low levels, only the larger workers will be able to forage. Without them the colony would struggle. It is likely that our experiments conducted in Southampton, southern Britain, failed to detect any size related effects of varying ambient temperature because the temperature simply did not fluctuate sufficiently to impede any of the workers concerned, regardless of their size.

Foraging increases the likelihood of mortality (Garofalo 1976; Katayama 1996; Silva-Matos and Garofalo 2000). Perhaps worker bees have a better chance of survival whilst foraging if they are large, being more able to tear themselves free of a spider's web, for example, or by showing a larger and therefore stronger aposematic signal to potential avian predators, as Forsman and Merilaita (1999) have shown with artificial butterflies. Mortality may not be entirely dependent upon predation. Parasitism may also play a part. Müller *et al.* (1996) found that bigger *B. terrestris* workers were more at risk to parasitism by conopid flies and that the larvae of these parasitoids developed more successfully in larger bumblebees. This could reflect a preference for large host size by the parasite or simply that large workers forage more frequently and are therefore more likely to come into contact with the conopids.

8.4 Future research in bumblebee worker size variation

When it comes to ecology, there is always more to be learnt and size variation in bumblebee workers is no exception. Below are some suggestions for future research on this topic.

Alloethism has only been reported in a few species, mainly because nests are so hard to find. Although we can infer that alloethism is a trait common to all bumblebee species, this needs to be confirmed. Perhaps the simplest method would be to catch queens in the spring and rear them in captivity. This is a delicate process. Guidance can be found in Prys-Jones and Corbet's handbook (Prys-Jones and Corbet 1991) and a number of papers (Beekman *et al.* 2000; Griffin *et al.* 1991; Hedtke and Schricker 1994; Manino *et al.* 1994; Plowright and Jay 1966; Röseler 1985).

I have shown that the rate at which the thorax cools is dependent upon its size (Chapter 3). We also know that there are upper and lower limits of ambient temperatures in which bumblebees can fly and that this is size dependent (Heinrich 1975). It should be possible to calculate what those limits are for a given size of worker, and species (bumblebee species differ in their insulation). Alternative methods include measuring large samples of bees across large temperature gradients, noting at what temperatures particular sized bees are excluded and encouraging bees of different sizes to fly in captivity over a range of temperatures as Heinrich (1975) did.

More work is needed on the relationship between bee size and thoracic insulation. We know that setae length increases with bee size in a roughly linear relationship (Chapter 3) but size-dependent density of setae has not been investigated. Either we might expect uniform density regardless of size or a reduction in density as size increases. Determining the density of the setae is difficult. If the follicles from which each setae emerge are evident in the cuticle density may be measured using a shaved bee under a dissecting microscope or a scanning electron microscope.

In four of the five UK (and Greek) species we sampled (Chapter 4), a correlation was found between body size and latitude. Clearly, more work is needed, as these results are not conclusive. To be certain that there is a general positive relationship between body size and latitude, samples should be spread over the entire season to avoid the effect of seasonal delay at increasing latitudes. Also, the number of species studied needs to be increased. To obtain exhaustive data relating body size to latitude, collaboration with other ecologists in Europe, or even worldwide, would be necessary. I have compared size variation between species from hot and cold regions and have found no obvious pattern. Working on a hypothesis of size variation being linked to climatic variation, it would be informative to carry out a more thorough investigation of the potential link between size variation and latitudinal and altitudinal range. The Natural History Museum, London has extensive samples of bumblebees from all over the globe, including many workers probably spanning the size range, together with details of the exact location of capture. This would be a good place to start. Maybe latitudinal size variation is genetic or maybe it occurs in direct response to the local climate. This could be determined by carrying out displacement experiments, taking care not to introduce exotics, of course.

Further research into foraging efficiency is required, particularly of pollen. Pollen is important for feeding larvae since it contains protein, the amount of which varies among plant species (Roulston *et al.* 2000). It is not known how pollen gathering efficiency varies with body size, although I have made some inferences (Chapter 5). However, in the same way the concentration of nectar cannot be ignored in measuring nectar foraging efficiency, the protein content of pollen cannot be ignored in calculating pollen foraging efficiency. An experiment similar to ours (see methods: 5.3.1) but, in addition, measuring pollen loads and their protein content by forcing the returning workers to pass through a brush would be a good way to gather data on this topic.

Repetition of our observations (Chapter 5) in a more variable climate would also be worth carrying out, or at least one in which daytime summer temperatures were lower than those in southern England. In this way we can determine if varying temperature influences the foraging behaviour of different sized workers and, if so, how much.

Adaptation to foraging efficiently on variable flora is possibly the most encouraging hypothesis we have to explain why workers vary in size (Chapter 6). There is more work to be done. Repetition of our research on both more species of plant and bumblebee would be beneficial. Prys-Jones (1982) showed intraspecific variation in flower choice depending on body size. Workers of *B. terrestris, B. pascuorum* and *B. pratorum* foraged on flowers of differing corolla depth according to their glossa length. However, apart from our data on *B. terrestris*, there are no data on handling times to accompany Prys-Jones' findings.

Whether size variation is adaptive or not is still a question not adequately answered. My experiment comparing the colony development of nests with manipulated workforces fell foul of bad weather and inadequate replication (Chapter 7). Repeated on a larger scale and with better weather it might yield interesting results. It is a very simple experiment as long as the funds are available to purchase the nests required (from personal experience I can say that rearing nests involves a lot of hard work and no guarantee of success).

It is possible that large bees, which we know forage more frequently, are less likely to be preyed upon. Size dependent longevity amongst foragers has not been quantified. This could be measured fairly simply by individually labelling all workers of a nest and monitoring their activity until the nest expired. The frequency with which each worker foraged would need to be taken into account since those that stay in the nest are more likely to live longer.

8.5 Concluding remarks

The eusocial insects fascinate us, perhaps because we are tempted to compare their societies to our own or because we are amazed at the apparent selflessness at an individual level. Obviously, it is wrong to take such a comparison too seriously as our society is far more complicated and surely very few of us match the apparent altruism of a bumblebee worker. Although simpler in bumblebee colonies, they do share with us a remarkable variation in form and division of labour. Size variation in bumblebee workers is an interesting phenomenon and is better understood with each passing year. Pollinating insects have a very intimate relationship with flowering plants both now and in the distant past. The forms and distributions of such insects and plants have been influenced by each other for millions of years. The conditions in the Central Asian mountains, where bumblebees may have originated, must have been perfect for the development of such a creature. Presumably, there has always been variation in the size of flowering plants as there are so many varied forms of pollinating insect: solitary bees, butterflies, beetles and flies. I argue that bumblebees evolved size variation in response to two factors. The temperature in the Central Asian mountains is likely to vary without the stabilising influence of a nearby ocean. Consequently, bumblebees have adapted a workforce built to cope with this range of temperatures, large foragers for cold conditions, small for hot, with both being suited to a large average temperature range. Perhaps this happened first, predisposing them to efficiently exploit a whole range of floral resources. Since their origin, bumblebees have spread around the globe and now exist in a range of climates. Many of these climates are temperate and appear not to restrain workers of any size. Perhaps worker size variation would disappear in such places were it not sustained by the variety of flowers on which bumblebees forage. Maybe, in turn, interspecific variation in floral size is partially sustained by the variation in bumblebee workers.

Many British bumblebee species are in decline (Goulson 2003) probably due to the removal of floral diversity by decades of intensive arable farming. To maintain bumblebee diversity, it is important that we maintain floral diversity. Conversely, because of the size variation of bumblebee workers and their ability to buzz pollinate, it is vital that we maintain the presence of bumblebees in order to maintain floral diversity.

GLOSSARY OF TERMS

ADAPTATION Noun. (Latin, *adaptare* = to fit to; English, *-tion* = result of an action.) The process by which an organism or lineage of organisms becomes suited to the environment (climate, habitat).

APOSEMATIC COLOURATION Warning colouration; Combination of colours or colour patterns displayed by animals and plants to indicate unpalatability to potential predators or herbivores.

BROOD Noun. (Middle English, *brood.*) Social Insects: Collectively, all immature members of a colony (eggs, larvae, pupae).

BUMBLEBEE Member of the Subfamily Bombinae (Hymenoptera: Apidae). They are relatively large bodied, robust and important pollinators. Most nest in ground, colonies are annual, and only fertilized queens overwinter. The cuckoo bumblebees (subgenus *Psithyrus*) are parasitic on other bumblebees.

CASTE Noun. (Latin, *castus* = pure.) Social Insects: Different groups of individuals of predictable morphological types or behaviour which perform specialized labour within the colony. Caste differences are permanent and not attributed to age.

COLONY Noun (Latin, *colonia* = farm.) Social Insects: A group of individuals (not a mated pair) that cooperatively construct nests or rear offspring.

COMMENSAL Noun (Latin, *cum* = with; *mensa* = table.) A participating member of a commensalistic relationship.

COMMENSALISM Noun (Latin, *cum* = with; *mensa* = table; English, *-ism* = condition.) Literally, 'common table'. A term applied to an interspecific relationship in which one species feeds on the food supply of another species without destroying or harming the owner of the supply.

COMPLETE METAMORPHOSIS Metamorphosis of Holometabola that typically involves four consecutive stages: Embryo (egg), larva, pupa and adult. Each stage is completely different in form and separated in time from other stages of the same individual.

DRONE Noun. (Anglo Saxon, *dran* = drone.) Hymenoptera: A male bee or ant.

ECTOTHERM (poikilotherm) Noun. (Greek, *ektos* = outside; *therme* = heat.) An animal that maintains it s body temperature by absorbing heat from the surrounding environment.

ENDOPTERYGOTE Adj. (Greek, *endon* = within; *pterygion* = little wing.) Pertaining to insects whose wings develop internally.

EUSOCIAL Adj. (Greek, *eu* = good; Latin, *sociare* = to associate; *-alis* = characterized by.) 1. Pertaining to insects that display social characteristics including cooperation among individuals in the care of young, contact between generations and reproductive division of labour. Eusocial insects include termites, ants, most bees, many wasps and some other insects. Eusocial insects display several levels of complexity in interaction. 2. Eusocial Bees: Colonies that are family-groups composed of two generations (mothers and daughters) and typically consist of one mother (queen) and her daughters (workers). Division of labour and development of castes are well developed in eusocial bees.

FERVID Adj. Ardent, intense.

GENUS Noun. (Latin, *genus* = race. Pl., Genera.) An assemblage of Species agreeing in one character or a series of characters. The concept is considered as arbitrary and opinionative, although some taxonomists regard the Genus as a natural assemblage.

HAEMOLYMPH Noun. (Greek, *haima* = blood; *lympha* = water.) Insects: The extracellular circulating fluid which fills the Haemocoel. Haemolymph includes plasma (fluid) and inclusions (hormones, metabolic products, cells, microorganisms and parasites).

HAPLODIPOIDY Noun. (Greek, *haploos* = simple; *diploos* = double; *eidos* = form.) A genetical condition seen in Hymenoptera, Acari and other arthropods wherein males are hemizygous and possess half the chromosomal complement of the diploid female.

HYMENOPTERA Linnaeus 1758. Noun. (Greek, *hymen* = membrane, *pteron* = wing.) Ants; bees; sawflies; wasps. A numerically large, cosmopolitan Order of endopterygote holometabolous insects. Hymenoptera are subdivided into Suborders Symphyta (sawflies) and Apocrita (bees, wasps, ants, parasitica).

INCUBATE (Latin, *incubare* = to lie on.) To brood; to cause to develop, as an egg.

MICROCLIMATE Noun. The climate of a small local area or enclosed space. (For my purposes microclimate also confers temporal climatic locality)

NECTAR Noun. (Greek, *nektar* = death overcoming; drink of gods.) Sweet, frequently scented, substances secreted by flowers, nectarines or other plant structures. Nectar provides nutrition for many insects, particularly Hymenoptera, Lepidoptera and some Diptera and serves as an attractant for pollination.

OMMATIDIA Noun. (Greek, *ommation* = little eye, *ommato* = to furnish with eyes; *-idion* = diminutive.) The basic visual elements which form the compound eye. Elements of the Ommatidium include a Lens, Cone, Rhabdom and Pigment Cells.

PETIOLE Noun. (Latin, *petiolus* = small foot.) The slender or narrow tubular segment between the thorax and abdomen.

POCKET-MAKING SPECIES Bumblebee species that construct waxen pouches or pockets near the base of the larval chamber or brood clump. Into these, returning foragers deposit their loads of pollen; the larvae feed from the resulting mass. Later, the queen (or workers) may supplement this diet by regurgitating a nectar/pollen mixture into the brood chamber, through a temporary hole in the wax envelope. The larvae of a batch remain together, sharing a common chamber and the pollen supply that goes with it.

POIKILOTHERMY Noun. (Greek, *poikilos* = various; *therme* = heat.) The passive variation in the internal body temperature of an animal, which depends on the temperature of the environment. All animals except birds and mammals exhibit poikilothermy and are described as ectotherms. Although unable to maintain a constant body temperature, they can respond to compensate for very low or very high temperatures.

POLLEN Noun. (Latin, *pollen* = fine flour.) The microscopic, multinucleate, male gametophyte generation of seed plants. Pollen transports the male gametes that are used in sexual reproduction of plants.

POLLEN-STORING SPECIES Bumblebee species that store pollen in empty pupal cocoons and specially constructed wax cells or cylinders. The queen or workers then feed it to the larvae, bit by bit, squirting a regurgitated mixture of nectar and pollen into the larval cell through a hole in the wax covering.

POLYETHISM Noun. (Greek, *polys* = many; *ethos* = custom; English, *-ism* = condition.) Social Insects: A division of labour among members of a colony.

PRIMITIVELY EUSOCIAL Social Insects: Individuals residing in a colony in which the castes are morphologically similar and food exchange is minimal or absent.

QUEEN Noun. (Anglo Saxon, *cwen* = woman.) The female member of the reproductive caste in social insects.

REPRODUCTIVES Noun. Social Insects: Any member of the colony that is capable of genetically contributing to the production of offspring. Specifically, reproductives include males, egg-laying workers and queens.

SPECIES Singular and Plural Noun. (Latin, *species* = particular kind.) An aggregation of individuals similar in appearance and structure, mating freely and producing young that themselves mate freely and bear fertile offspring resembling each other and their parents, including all varieties and races.

SUBGENUS Noun. Latin, *sub* = below; *genus* = race.) A taxonomic subdivision within a Genus, based upon a character not sufficient for generic separation.

WORKER Noun. (Middle English, *werk, weore* = work.) Hymenoptera: Individuals anatomically female which lack the capacity to reproduce or lay unfertilized eggs which produce only males. (This last point is not true in the case of bumblebees – workers are able to produce male offspring.)

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